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(54) 发明名称

蛋白质纺纱的制造方法

(57) 摘要

本发明的目的在于提供一种可以充分确保纤维彼此的缠结,进而确保稳定的强度的蛋白质纺织的制造方法。本发明的蛋白质纺纱的制造方法包括下述工序:(a)准备含有改性丝心蛋白,且包含未卷曲的人造丝心蛋白纤维的原料纺纱;及(b)使所述原料纺纱与水性介质接触,从而使所述人造丝心蛋白纤维卷曲。

1. 一种蛋白质纺纱的制造方法,包括下述工序:

- (a) 准备含有改性丝心蛋白,且包含未卷曲的人造丝心蛋白纤维的原料纺纱;及
- (b) 使所述原料纺纱与水性介质接触,从而使所述人造丝心蛋白纤维卷曲。

2. 根据权利要求1所述的蛋白质纺纱的制造方法,其中,所述人造丝心蛋白纤维的由下述式子规定的干燥收缩率超过7%,

干燥收缩率 = $\{1 - (\text{与水性介质接触之后制成干燥状态后的人造丝心蛋白纤维的长度} / \text{与水性介质接触前的人造丝心蛋白纤维的长度})\} \times 100 (\%)$ 。

3. 根据权利要求1或2所述的蛋白质纺纱的制造方法,其中,所述人造丝心蛋白纤维的由下述式子规定的湿润收缩率为2%以上,

湿润收缩率 = $\{1 - (\text{与水性介质接触之后制成干燥状态后的人造丝心蛋白纤维的长度} / \text{纺丝后且与水性介质接触前的人造丝心蛋白纤维的长度})\} \times 100 (\%)$ 。

4. 根据权利要求1~3中任一项所述的蛋白质纺纱的制造方法,其中,所述改性丝心蛋白为改性蛛丝丝心蛋白,且,所述人造丝心蛋白纤维为人造蛛丝丝心蛋白纤维。

5. 根据权利要求1~4中任一项所述的蛋白质纺纱的制造方法,其中,所述卷曲工序中使用的所述水性介质为包含水的10~230℃的液体或气体。

6. 根据权利要求1~5中任一项所述的蛋白质纺纱的制造方法,其中,所述卷曲工序包括在使所述原料纺纱与所述水性介质接触之后进一步使其干燥。

7. 根据权利要求1~6中任一项所述的蛋白质纺纱的制造方法,其中,所述卷曲工序中使用的所述水性介质包含挥发性溶剂。

蛋白质纺纱的制造方法

技术领域

[0001] 本发明涉及一种蛋白质纺纱的制造方法。

背景技术

[0002] 本发明人等提出了一种能够通过将蛋白质长丝水卷曲来高效且低成本地制造蛋白质纺纱的方法(专利文献1、未公开)。

[0003] 现有技术文献

[0004] 专利文献

[0005] 专利文献1:日本特愿2018-15588号出愿

发明内容

[0006] 发明想要解决的课题

[0007] 然而,本发明人等经过反复深入研究,结果发现,在卷曲后进行纺织的情况下,蛋白质卷曲纤维在梳理工序中被拉伸导致卷曲减弱,纤维彼此的缠结降低,其结果可能导致纺纱的强度降低。

[0008] 本发明的目的在于提供一种可以充分确保纤维彼此的缠结,进而确保稳定的强度的蛋白质纺织的制造方法。

[0009] 用于解决课题的手段

[0010] 本发明人等发现,制造蛋白质纺纱时,通过使含有改性丝心蛋白,且包含未卷曲的人造丝心蛋白纤维的原料纺纱与水性介质接触,从而使人造丝心蛋白纤维卷曲,可以充分确保纤维彼此的缠结,进而确保稳定的强度。本发明是基于该新见解的发明。

[0011] 本发明涉及例如以下的各发明。

[0012] [1]一种蛋白质纺纱的制造方法,包括下述工序:

[0013] (a)准备含有改性丝心蛋白,且包含未卷曲的人造丝心蛋白纤维的原料纺纱;及

[0014] (b)使所述原料纺纱与水性介质接触,从而使所述人造丝心蛋白纤维卷曲。

[0015] [2]根据[1]所述的蛋白质纺纱的制造方法,其中,

[0016] 所述人造丝心蛋白纤维的由下述式子定义的干燥收缩率超过7%,

[0017] 干燥收缩率 = $\{1 - (\text{与水性介质接触之后制成干燥状态后的人造丝心蛋白纤维的长度} / \text{与水性介质接触前的人造丝心蛋白纤维的长度})\} \times 100 (\%)$ 。

[0018] [3]根据[1]或[2]所述的蛋白质纺纱的制造方法,其中,

[0019] 所述人造丝心蛋白纤维的由下述式子定义的湿润收缩率为2%以上,

[0020] 湿润收缩率 = $\{1 - (\text{与水性介质接触之后制成干燥状态后的人造丝心蛋白纤维的长度} / \text{纺丝后且与水性介质接触前的人造丝心蛋白纤维的长度})\} \times 100 (\%)$ 。

[0021] [4]根据[1]~[3]中任一项所述的蛋白质纺纱的制造方法,其中,

[0022] 所述改性丝心蛋白为改性蛛丝丝心蛋白,且,所述人造丝心蛋白纤维为人造蛛丝丝心蛋白纤维。

- [0023] [5]根据[1]～[4]中任一项所述的蛋白质纺纱的制造方法,其中,
- [0024] 所述卷曲工序中使用的所述水性介质为包含水的10～230℃的液体或气体。
- [0025] [6]根据[1]～[5]中任一项所述的蛋白质纺纱的制造方法,其中,
- [0026] 所述卷曲工序包括在使所述原料纺纱与所述水性介质接触之后进一步使其干燥。
- [0027] [7]根据[1]～[6]中任一项所述的蛋白质纺纱的制造方法,其中,
- [0028] 所述卷曲工序中使用的所述水性介质包含挥发性溶剂。
- [0029] 发明效果
- [0030] 根据本发明的蛋白质纺纱的制造方法,可以提供一种可以充分确保纤维彼此的缠结,进而确保稳定的强度的蛋白质纺织的制造方法。

附图说明

- [0031] [图1]为示出改性丝心蛋白的结构域序列的一例的示意图。
- [0032] [图2]为示出天然来源的丝心蛋白的z/w(%)的值的分布的图。
- [0033] [图3]为示出来自天然来源的丝心蛋白的x/y(%)的值的分布的图。
- [0034] [图4]为示出改性丝心蛋白的结构域序列的一例的示意图。
- [0035] [图5]为示出改性丝心蛋白的结构域序列的一例的示意图。
- [0036] [图6]为简要示出用于制造人造丝心蛋白纤维的纺丝装置的一例的说明图。
- [0037] [图7]为示出通过与水性介质的接触而产生的人造丝心蛋白纤维的长度变化的例子的图。

具体实施方式

[0038] 本实施方式中的蛋白质纺纱的制造方法包括下述工序:(a)准备含有改性丝心蛋白,且包含未卷曲的人造丝心蛋白纤维的原料纺纱;及(b)使原料纺纱与水性介质接触,从而使人造丝心蛋白纤维卷曲。

[0039] [工序(a)]

[0040] (改性丝心蛋白)

[0041] 本实施方式中的改性丝心蛋白为包含式1: $[(A)_n \text{基序-REP}]_m$ 、或式2: $[(A)_n \text{基序-REP}]_m - (A)_n \text{基序}$ 所示的结构域序列的蛋白质。改性丝心蛋白的结构域序列的N端侧及C端侧中任意一者或两者上可以进一步添加有氨基酸序列(N端序列及C端序列)。N端序列及C端序列不限于于此,典型地为具有丝心蛋白中特征性的氨基酸基序的重复的区域,由约100个残基的氨基酸构成。

[0042] 改性丝心蛋白可以为其结构域序列与天然来源的丝心蛋白的氨基酸序列不同的丝心蛋白,也可以为其结构域序列与天然来源的丝心蛋白的氨基酸序列相同的丝心蛋白。另外,本说明书中所述的“天然来源的丝心蛋白”也是包含式1: $[(A)_n \text{基序-REP}]_m$ 、或式2: $[(A)_n \text{基序-REP}]_m - (A)_n \text{基序}$ 所示的结构域序列的蛋白质。

[0043] “改性丝心蛋白”可以为直接利用天然来源的丝心蛋白的氨基酸序列的蛋白,也可以为依据天然来源的丝心蛋白的氨基酸序列对该氨基酸序列进行改性而成的蛋白(例如,通过对克隆出的天然来源的丝心蛋白的基因序列进行改性而改性氨基酸序列而成的蛋白),另外,还可以为不依赖于天然来源的丝心蛋白而人工地设计及合成的蛋白(例如,通过

化学合成编码所设计的氨基酸序列的核酸而具有所需的氨基酸序列的蛋白)。

[0044] 本说明书中,“结构域序列”为产生丝心蛋白特有的结晶区域(典型地,相当于氨基酸序列的(A)_n基序)和非晶区域(典型地,相当于氨基酸序列的REP)的氨基酸序列,表示式1:[(A)_n基序-REP]_m、或式2:[(A)_n基序-REP]_m-(A)_n基序所示的氨基酸序列。在此,(A)_n基序表示以丙氨酸残基为主的氨基酸序列,氨基酸残基数为2~27。(A)_n基序的氨基酸残基数可以为2~20、4~27、4~20、8~20、10~20、4~16、8~16、或10~16的整数。另外,(A)_n基序中的丙氨酸残基数相对于氨基酸残基总数的比只要为40%以上即可,可以为60%以上、70%以上、80%以上、83%以上、85%以上、86%以上、90%以上、95%以上、或100%(表示仅由丙氨酸残基构成)。结构域序列中存在多个的(A)_n基序的至少7个可以仅由丙氨酸残基构成。REP表示由2~200个氨基酸残基构成的氨基酸序列。REP可以为由10~200个氨基酸残基构成的氨基酸序列。_m表示2~300的整数,可以为10~300的整数。存在多个的(A)_n基序可以为彼此相同的氨基酸序列,也可以为彼此不同的氨基酸序列。存在多个的REP可以为彼此相同的氨基酸序列,也可以为彼此不同的氨基酸序列。

[0045] 本实施方式中的改性丝心蛋白可以通过例如对克隆出的天然来源的丝心蛋白的基因序列进行例如相当于取代、缺失、插入及/或添加一个或多个氨基酸残基的氨基酸序列的改性而得到。氨基酸残基的取代、缺失、插入及/或添加能够通过定点诱变法等本领域技术人员周知的方法来进行。具体而言,能够依据Nucleic Acid Res.10,6487(1982), Methods in Enzymology,100,448(1983)等文献中记载的方法来进行。

[0046] 天然来源的丝心蛋白为包含式1:[(A)_n基序-REP]_m、或式2:[(A)_n基序-REP]_m-(A)_n基序所示的结构域序列的蛋白质,具体而言,可列举例如昆虫或蜘蛛类所产生的丝心蛋白。

[0047] 作为昆虫所产生的丝心蛋白,可列举例如:家蚕(*Bombyx mori*)、野蚕(*Bombyx mandarina*)、天蚕(*Antheraea yamamai*)、柞蚕(*Antheraea pernyi*)、樟蚕(*Eriogyna pyretorum*)、蓖麻蚕(*Pilosamia Cynthia ricini*)、樗蚕(*Samia cynthia*)、双黑目天蚕蛾(*Caligula japonica*)、印度柞蚕(*Antheraea mylitta*)、琥珀蚕(*Antheraea assama*)等蚕所产生的蚕丝蛋白、及雀蜂(*Vespa simillima xanthoptera*)的幼虫所吐出的蜂丝蛋白。

[0048] 作为昆虫所产生的丝心蛋白的更具体的例子,可列举例如:蚕·丝心蛋白L链(GenBank登录号M76430(碱基序列)、及AAA27840.1(氨基酸序列))。

[0049] 作为蜘蛛类所产生的丝心蛋白,可列举例如:大腹园蛛、十字园蛛、肥胖园蛛、五纹园蛛及野岛园蛛(*Araneus nojimai*)等属于园蛛属(*Araneus*属)的蜘蛛、青新园蛛、嗜水新园蛛、灌木新园蛛及类青新园蛛等属于新园蛛属(*Neoscona*属)的蜘蛛、棕类卬蛛等属于卬蛛属(*Pronus*属)的蜘蛛、蟾蜍曲腹蛛及对称曲腹蛛等属于曲腹蛛属(*Cyrtarachne*属)的蜘蛛、库氏棘腹蛛及乳突棘腹蛛等属于棘腹蛛属(*Gasteracantha*属)的蜘蛛、何氏瘤腹蛛及六刺瘤腹蛛等属于瘤腹蛛属(*Ordgarius*属)的蜘蛛、悦目金蛛、小悦目金蛛及横纹金蛛等属于金蛛属(*Argiope*属)的蜘蛛、双峰尾园蛛等属于尾园蛛属(*Arachnura*属)的蜘蛛、褐吊叶蛛等属于吊叶蛛属(*Acusilas*属)的蜘蛛、摩鹿加云斑蛛、方格云斑蛛及全色云斑蛛等属于云斑蛛属(*Cytophora*属)的蜘蛛、丑锥头蛛等属于锥头蛛属(*Polys*属)的蜘蛛、八疣尘蛛、四突尘蛛、圆腹尘蛛及黑尾尘蛛等属于尘蛛属(*Cyclosa*属)的蜘蛛、及日本壮头蛛等属于壮头蛛属(*Chorizopes*属)的蜘蛛所产生的蛛丝蛋白;以及前齿肖蛸、锥腹肖蛸、直伸肖蛸及鳞纹肖蛸等属于肖蛸属(*Tetragnatha*属)的蜘蛛、纵条银鳞蛛、肩斑银鳞蛛及小肩斑银鳞蛛等属

于银鳞蛛属 (*Leucauge* 属) 的蜘蛛、棒络新妇及斑络新妇等属于络新妇属 (*Nephila* 属) 的蜘蛛、美丽麦蛛等属于麦蛛属 (*Menosira* 属) 的蜘蛛、柔弱粗螯蛛等属于锯螯蛛属 (*Dyschiriognatha* 属) 的蜘蛛、黑寡妇蜘蛛、红背蜘蛛、几何寇蛛及间斑寇蛛等属于寇蛛属 (*Latrodectus* 属) 的蜘蛛、及属于育儿网蛛属 (*Euprosthenops* 属) 的蜘蛛等属于肖蛸科 (*Tetragnathidae* 科) 的蜘蛛所产生的蛛丝蛋白。作为蛛丝蛋白, 可列举例如: MaSp (MaSp1 及 MaSp2)、ADF (ADF3 及 ADF4) 等牵引丝蛋白、MiSp (MiSp1 及 MiSp2) 等。

[0050] 作为蜘蛛类所产生的蛛丝蛋白的更具体的例子, 可列举例如: fibroin-3 (adf-3) [来自 *Araneus diadematus*] (GenBank 登录号 AAC47010 (氨基酸序列)、U47855 (碱基序列))、fibroin-4 (adf-4) [来自 *Araneus diadematus*] (GenBank 登录号 AAC47011 (氨基酸序列)、U47856 (碱基序列))、dragline silk protein spidroin 1 [来自 *Nephila clavipes*] (GenBank 登录号 AAC04504 (氨基酸序列)、U37520 (碱基序列))、major ampullate spidroin 1 [来自 *Latrodectus hesperus*] (GenBank 登录号 ABR68856 (氨基酸序列)、EF595246 (碱基序列))、dragline silk protein spidroin 2 [来自 *Nephila clavata*] (GenBank 登录号 AAL32472 (氨基酸序列)、AF441245 (碱基序列))、major ampullate spidroin 1 [来自 *Euprosthenops australis*] (GenBank 登录号 CAJ00428 (氨基酸序列)、AJ973155 (碱基序列))、及 major ampullate spidroin 2 [*Euprosthenops australis*] (GenBank 登录号 CAM32249.1 (氨基酸序列)、AM490169 (碱基序列))、minor ampullate silk protein 1 [*Nephila clavipes*] (GenBank 登录号 AAC14589.1 (氨基酸序列))、minor ampullate silk protein 2 [*Nephila clavipes*] (GenBank 登录号 AAC14591.1 (氨基酸序列))、minor ampullate spidroin-like protein [*Nephilengys cruentata*] (GenBank 登录号 ABR37278.1 (氨基酸序列)) 等。

[0051] 作为天然来源的丝心蛋白的更具体的例子, 能够进一步列举 NCBI GenBank 中注册了序列信息的丝心蛋白。例如, 能够通过从 NCBI GenBank 中注册的序列信息中包含 INV 作为 DIVISION 的序列中提取出在 DEFINITION 记载了 spidroin、ampullate、fibroin、“silk 及 polypeptide”、或“silk 及 protein”作为关键字的序列、从 CDS 中提取出记载了特定的 product 的字符串、从 SOURCE 中提取出了在 TISSUE TYPE 中记载了特定的字符串的序列来确认。

[0052] 本实施方式中的改性丝心蛋白可以为改性蚕丝 (silk) 丝心蛋白 (对蚕所产生的丝蛋白的氨基酸序列进行改性而成的蛋白), 也可以为改性蛛丝丝心蛋白 (对蜘蛛类所产生的蛛丝蛋白的氨基酸序列进行改性而成的蛋白)。作为改性丝心蛋白, 优选改性蛛丝丝心蛋白。

[0053] 作为改性丝心蛋白的具体的例子, 可列举: 来自蜘蛛的大壶状腺产生的大吐丝管牵引丝蛋白质的改性丝心蛋白 (第一改性丝心蛋白)、具有降低了甘氨酸残基的含量的结构域序列的改性丝心蛋白 (第二改性丝心蛋白)、具有降低了 (A)_n 基序的含量的结构域序列的改性丝心蛋白 (第三改性丝心蛋白)、降低了甘氨酸残基的含量及 (A)_n 基序的含量的改性丝心蛋白 (第四改性丝心蛋白)、具有包含局部疏水性指数较大的区域的结构域序列的改性丝心蛋白 (第五改性丝心蛋白)、以及具有降低了谷氨酰胺残基的含量的结构域序列的改性丝心蛋白 (第六改性丝心蛋白)。

[0054] 作为第一改性丝心蛋白, 可列举包含式 1: [(A)_n 基序-REP]_m 所示的结构域序列的蛋

白质。在第一改性丝心蛋白中, $(A)_n$ 基序的氨基酸残基数优选3~20的整数,更优选4~20的整数,进一步优选8~20的整数,更进一步优选10~20的整数,更进一步优选4~16的整数,特别优选8~16的整数,最优选10~16的整数。作为第一改性丝心蛋白,式1中,构成REP的氨基酸残基的数量优选为10~200个残基,更优选10~150个残基,进一步优选为20~100个残基,进一步优选为20~75个残基。作为第一改性丝心蛋白,式1: $[(A)_n\text{基序-REP}]_m$ 所示的氨基酸序列中所含的甘氨酸残基、丝氨酸残基及丙氨酸残基的总残基数相对于氨基酸残基数整体优选为40%以上,更优选为60%以上,进一步优选为70%以上。

[0055] 第一改性丝心蛋白可以为包含式1: $[(A)_n\text{基序-REP}]_m$ 所示的氨基酸序列的单元,且C端序列为与序列号1~3中任一项所示的氨基酸序列或序列号1~3中任一项所示的氨基酸序列具有90%以上的同源性的氨基酸序列的多肽。

[0056] 序列号1所示的氨基酸序列与由ADF3 (GI:1263287、NCBI) 的氨基酸序列的C端的50个残基的氨基酸构成的氨基酸序列相同,序列号2所示的氨基酸序列与从序列号1所示的氨基酸序列的C端起除去20个残基后的氨基酸序列相同,序列号3所示的氨基酸序列与从序列号1所示的氨基酸序列的C端除去29个残基后的氨基酸序列相同。

[0057] 作为第一改性丝心蛋白的更具体的例子,能够列举包含(1-i)序列号4 (recombinant spider silk protein ADF3KaiLargeNRS1)所示的氨基酸序列、或与(1-ii)序列号4所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列的改性丝心蛋白。序列同一性优选为95%以上。

[0058] 序列号4所示的氨基酸序列为使在N端添加有由起始密码子、His10标签及HRV3C蛋白酶 (Human rhinovirus 3C蛋白酶) 识别位点构成的氨基酸序列 (序列号5) 的ADF3的氨基酸序列中以第1~13个重复区域增加至大致2倍,并且翻译在第1154个氨基酸残基终止的方式产生变异而成的氨基酸序列。序列号4所示的氨基酸序列的C端的氨基酸序列与序列号3所示的氨基酸序列相同。

[0059] (1-i)的改性丝心蛋白可以为由序列号4所示的氨基酸序列构成的蛋白。

[0060] 在第二改性丝心蛋白中,其结构域序列具有与天然来源的丝心蛋白相比降低了甘氨酸残基的含量的氨基酸序列。第二改性丝心蛋白可以具有与天然来源的丝心蛋白相比相当于至少REP中的一个或多个甘氨酸残基被取代为其它的氨基酸残基的氨基酸序列。

[0061] 第二改性丝心蛋白可以为下述蛋白:其结构域序列具有与天然来源的丝心蛋白相比相当于选自REP中的GGX及GPGXX (其中,G表示甘氨酸残基,P表示脯氨酸残基,X表示甘氨酸以外的氨基酸残基。) 中的至少一个基序序列中至少一个或多个该基序序列中的一个甘氨酸残基被取代为其它的氨基酸残基的氨基酸序列。

[0062] 在第二改性丝心蛋白中,上述的甘氨酸残基被取代为其它的氨基酸残基的基序序列的比例相对于总基序序列可以为10%以上。

[0063] 第二改性丝心蛋白可以具有如下氨基酸序列:包含式1: $[(A)_n\text{基序-REP}]_m$ 所示的结构域序列,将从上述结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至上述结构域序列的C端为止的序列而成的序列中的全部REP中所含的由XGX (其中,X表示甘氨酸以外的氨基酸残基。) 构成的氨基酸序列的总氨基酸残基数设为z,将从上述结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至上述结构域序列的C端为止的序列而成的序列中的总氨基酸残基数设为w时, z/w 为30%以上、40%以上、50%以上或50.9%以上。 $(A)_n$ 基序中的相对于氨基酸残基总

数的丙氨酸残基数可以为83%以上,优选为86%以上,更优选为90%以上,进一步优选为95%以上,更进一步优选为100%(表示仅由丙氨酸残基构成)。

[0064] 第二改性丝心蛋白优选为通过将GGX基序的一个甘氨酸残基取代为其它的氨基酸残基而提高了由XGX构成的氨基酸序列的含有比例的蛋白。在第二改性丝心蛋白中,结构域序列中的由GGX构成的氨基酸序列的含有比例优选为30%以下,更优选为20%以下,进一步优选为10%以下,更进一步优选为6%以下,更进一步优选为4%以下,特别优选为2%以下。结构域序列中的由GGX构成的氨基酸序列的含有比例能够通过由下述XGX构成的氨基酸序列的含有比例(z/w)的计算方法相同的方法来计算。

[0065] 下面,进一步详细说明 z/w 的计算方法。首先,在包含式1: $[(A)_n\text{基序}-\text{REP}]_m$ 所示的结构域序列的丝心蛋白(改性丝心蛋白或天然来源的丝心蛋白)中,自从结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至结构域序列的C端为止的序列而成的序列中所含的全部REP中提取出由XGX构成的氨基酸序列。构成XGX的氨基酸残基的总数为 z 。例如,在提取出50个由XGX构成的氨基酸序列的情况下(无重复), z 为 $50 \times 3 = 150$ 。另外,例如,在由XGXGX构成的氨基酸序列的情况那样存在两个XGX中所含的X(中央的X)的情况下,扣除重复量来进行计算(XGXGX的情况下为5个氨基酸残基)。 w 为从结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至结构域序列的C端为止的序列而成的序列中所含的总氨基酸残基数。例如,在图1所示的结构域序列的情况下, w 为 $4+50+4+100+4+10+4+20+4+30=230$ (除去了位于最C端侧的 $(A)_n$ 基序)。接着,通过 z 除以 w ,能够算出 $z/w(\%)$ 。

[0066] 在此,对天然来源的丝心蛋白中的 z/w 进行说明。首先,如上所述,通过例示了NCBI GenBank中注册了氨基酸序列信息的丝心蛋白的方法进行确认,结果提取出了663种丝心蛋白(其中,来自蜘蛛类的丝心蛋白为415种)。在所提取出的全部的丝心蛋白中,根据包含式1: $[(A)_n\text{基序}-\text{REP}]_m$ 所示的结构域序列,且丝心蛋白中的由GGX构成的氨基酸序列的含有比例为6%以下的天然来源的丝心蛋白的氨基酸序列,通过上述的计算方法,计算 z/w 。将其结果示于图2。图2的横轴表示 $z/w(\%)$,纵轴表示频率。如图2表明,天然来源的丝心蛋白中的 z/w 均低于50.9%(最高为50.86%)。

[0067] 在第二改性丝心蛋白中, z/w 优选为50.9%以上,更优选为56.1%以上,进一步优选为58.7%以上,更进一步优选为70%以上,更进一步优选为80%以上。 z/w 的上限没有特别限制,可以为例如95%以下。

[0068] 第二改性丝心蛋白能够通过例如下述方法进行改性而得到:从克隆出的天然来源的丝心蛋白的基因序列中取代编码甘氨酸残基的碱基序列的至少一部分以编码其它的氨基酸残基。此时,作为所改性的甘氨酸残基,可以选择GGX基序及GPGXX基序中的一个甘氨酸残基,另外,也可以进行取代以使 z/w 为50.9%以上。另外,也可以通过例如根据天然来源的丝心蛋白的氨基酸序列设计满足上述方案的氨基酸序列,并化学合成编码所设计的氨基酸序列的核酸来得到。在任意情况下,均可以在除相当于从天然来源的丝心蛋白的氨基酸序列中将REP中的甘氨酸残基取代为其它的氨基酸残基的改性之外,进一步进行相当于取代、缺失、插入及/或添加一个或多个氨基酸残基的氨基酸序列的改性。

[0069] 作为上述的其它的氨基酸残基,只要其为甘氨酸残基以外的氨基酸残基,则没有特别限制,优选缬氨酸(V)残基、亮氨酸(L)残基、异亮氨酸(I)残基、蛋氨酸(M)残基、脯氨酸(P)残基、苯丙氨酸(F)残基及色氨酸(W)残基等疏水性氨基酸残基、谷氨酰胺(Q)残基、天冬

酰胺(N)残基、丝氨酸(S)残基、赖氨酸(K)残基及谷氨酰胺(E)残基等亲水性氨基酸残基,更优选缬氨酸(V)残基、亮氨酸(L)残基、异亮氨酸(I)残基、苯丙氨酸(F)残基及谷氨酰胺(Q)残基,进一步优选谷氨酰胺(Q)残基。

[0070] 作为第二改性丝心蛋白的更具体的例子,能够列举包含(2-i)序列号6(Met-PRT380)、序列号7(Met-PRT410)、序列号8(Met-PRT525)或者序列号9(Met-PRT799)所示的氨基酸序列、或与(2-ii)序列号6、序列号7、序列号8或者序列号9所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列的改性丝心蛋白。

[0071] 下面,对(2-i)的改性丝心蛋白进行说明。序列号6所示的氨基酸序列是将相当于天然来源的丝心蛋白的序列号10(Met-PRT313)所示的氨基酸序列的REP中的全部GGX取代为GQX而成的。序列号7所示的氨基酸序列是使序列号6所示的氨基酸序列中从N端侧向C端侧使(A)_n基序每隔两个产生缺失,并进一步在C端序列的近前处插入一个[(A)_n基序-REP]而成的。序列号8所示的氨基酸序列是在序列号7所示的氨基酸序列的各(A)_n基序的C端侧插入两个丙氨酸残基,并进一步将一部分谷氨酰胺(Q)残基取代为丝氨酸(S)残基,以与序列号7的分子量大致相同的方式使C端侧的一部分氨基酸缺失而成的。序列号9所示的氨基酸序列是序列号7所示的氨基酸序列中所存在的20个结构域序列的区域(其中,该区域的C端侧的多个氨基酸残基被取代。)重复四次而成的序列的C端添加有规定的铰链序列和His标签序列而成的。

[0072] 序列号10所示的氨基酸序列(相当于天然来源的丝心蛋白)中的z/w的值为46.8%。序列号6所示的氨基酸序列、序列号7所示的氨基酸序列、序列号8所示的氨基酸序列、及序列号9所示的氨基酸序列中的z/w的值分别为58.7%、70.1%、66.1%及70.0%。另外,序列号10、序列号6、序列号7、序列号8及序列号9所示的氨基酸序列的锯齿比率(将于后文叙述)1:1.8~11.3中的x/y的值分别为15.0%、15.0%、93.4%、92.7%及89.8%。

[0073] (2-i)的改性丝心蛋白可以为由序列号6、序列号7、序列号8或序列号9所示的氨基酸序列构成的蛋白。

[0074] (2-ii)的改性丝心蛋白可以包含与序列号6、序列号7、序列号8或序列号9所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列。另外,(2-ii)的改性丝心蛋白也是包含式1:[(A)_n基序-REP]_m所示的结构域序列的蛋白质。上述序列同一性优选为95%以上。

[0075] 在(2-ii)的改性丝心蛋白中,优选地,将与序列号6、序列号7、序列号8或序列号9所示的氨基酸序列具有90%以上的序列同一性,且REP中所含的由XGX(其中,X表示甘氨酸以外的氨基酸残基。)构成的氨基酸序列的总氨基酸残基数设为z,将上述结构域序列中的REP的总氨基酸残基数设为w时,z/w为50.9%以上。

[0076] 第二改性丝心蛋白可以在N端及C端中任意一者或两者上包含标签序列。由此,可以进行改性丝心蛋白的分离、固定化、检测及可视化等。

[0077] 作为标签序列,能够列举例如:利用与其它分子的特异性亲和性(结合性、亲和力)的亲和力标签。作为亲和力标签的具体例,能够列举组氨酸标签(His标签)。His标签为4~10个左右组氨酸残基排列而成的短肽,其具有与镍等金属离子特异性结合的性质,因此,能够用于利用金属螯合层析(chelating metal chromatography)分离改性丝心蛋白。作为标签序列的具体例,可列举例如:序列号11所示的氨基酸序列(包含His标签序列及铰链序列的氨基酸序列)。

[0078] 另外,也能够利用与谷胱甘肽特异性结合的谷胱甘肽-S-转移酶(GST)、与麦芽糖特异性结合的麦芽糖结合蛋白质(MBP)等标签序列。

[0079] 此外,也能够使用利用抗原抗体反应的“表位标签”。通过将显示抗原性的肽(表位)作为标签序列添加,能够结合针对该表位的抗体。作为表位标签,能够列举:HA(流感病毒的血凝素的肽序列)标签、myc标签、FLAG标签等。通过利用表位标签,能够以高特异性容易地纯化改性丝心蛋白。

[0080] 此外,也能够使用用特定的蛋白酶切除标签序列后的蛋白。通过对经由该标签序列吸附的蛋白质进行蛋白酶处理,也能够回收切除标签序列后的改性丝心蛋白。

[0081] 作为包含标签序列的改性丝心蛋白的更具体的例子,能够列举包含(2-iii)序列号12(PRT380)、序列号13(PRT410)、序列号14(PRT525)或者序列号15(PRT799)所示的氨基酸序列、或与(2-iv)序列号12、序列号13、序列号14或者序列号15所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列的改性丝心蛋白。

[0082] 序列号16(PRT313)、序列号12、序列号13、序列号14及序列号15所示的氨基酸序列为分别在序列号10、序列号6、序列号7、序列号8及序列号9所示的氨基酸序列的N端添加有序列号11所示的氨基酸序列(包含His标签序列及铰链序列)而成的氨基酸序列。

[0083] (2-iii)的改性丝心蛋白可以为由序列号12、序列号13、序列号14或序列号15所示的氨基酸序列构成的蛋白。

[0084] (2-iv)的改性丝心蛋白包含与序列号12、序列号13、序列号14或序列号15所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列。另外,(2-iv)的改性丝心蛋白也是包含式1: $[(A)_n\text{基序-REP}]_m$ 所示的结构域序列的蛋白质。上述序列同一性优选为95%以上。

[0085] 在(2-iv)的改性丝心蛋白中,优选地,将与序列号12、序列号13、序列号14或序列号15所示的氨基酸序列具有90%以上的序列同一性,且REP中所含的由XGX(其中,X表示甘氨酸以外的氨基酸残基。)构成的氨基酸序列的总氨基酸残基数设为 z ,将上述结构域序列中的REP的总氨基酸残基数设为 w 时, z/w 为50.9%以上。

[0086] 第二改性丝心蛋白可以包含用于将重组蛋白质生产系中所生产的蛋白质释放至宿主的外部的分泌信号。分泌信号的序列能够根据宿主的种类适当地设置。

[0087] 第三改性丝心蛋白中,其结构域序列具有与天然来源的丝心蛋白相比降低了 $(A)_n$ 基序的含量的氨基酸序列。第三改性丝心蛋白的结构域序列可以具有与天然来源的丝心蛋白相比相当于缺失了至少一个或多个 $(A)_n$ 基序的氨基酸序列。

[0088] 第三改性丝心蛋白可以具有相当于从天然来源的丝心蛋白中使10~40%的 $(A)_n$ 基序缺失的氨基酸序列。

[0089] 第三改性丝心蛋白中,其结构域序列可以具有与天然来源的丝心蛋白相比相当于从N端侧向C端侧至少每1~3个 $(A)_n$ 基序中一个 $(A)_n$ 基序缺失的氨基酸序列。

[0090] 第三改性丝心蛋白中,其结构域序列可以具有与天然来源的丝心蛋白相比相当于从N端侧向C端侧至少依次重复产生了两个连续的 $(A)_n$ 基序的缺失、及一个 $(A)_n$ 基序的缺失的氨基酸序列。

[0091] 第三改性丝心蛋白中,其结构域序列可以具有相当于从N端侧向C端侧至少使 $(A)_n$ 基序每隔两个产生缺失的氨基酸序列。

[0092] 第三改性丝心蛋白可以具有如下氨基酸序列:包含式1: $[(A)_n\text{基序-REP}]_m$ 所示的结

构域序列,且,将相邻的两个[(A)_n基序-REP]单元的氨基酸残基数相加得到的合计值的最大值设为x,将结构域序列的总氨基酸残基数设为y时,x/y为20%以上、30%以上、40%以上或50%以上,其中,从N端侧向C端侧将相邻的两个[(A)_n基序-REP]单元的REP的氨基酸残基数依次进行比较后,将氨基酸残基数较少的REP的氨基酸残基数设为1时,另一个REP的氨基酸残基数的比为1.8~11.3。(A)_n基序中的相对于氨基酸残基总数的丙氨酸残基数可以为83%以上,优选为86%以上,更优选为90%以上,进一步优选为95%以上,更进一步优选为100%(表示仅由丙氨酸残基构成)。

[0093] 参考图1,对x/y的计算方法进行更详细地说明。图1中示出从改性丝心蛋白中除去N端序列及C端序列后的结构域序列。该结构域序列具有从N端侧(左侧)起为(A)_n基序-第一REP(50个氨基酸残基)-(A)_n基序-第二REP(100个氨基酸残基)-(A)_n基序-第三REP(10个氨基酸残基)-(A)_n基序-第四REP(20个氨基酸残基)-(A)_n基序-第五REP(30个氨基酸残基)-(A)_n基序这样的序列。

[0094] 从N端侧向C端侧依次选择相邻的两个[(A)_n基序-REP]单元,并使其不重复。此时,可以存在未被选择的[(A)_n基序-REP]单元。图1中示出了模式1(第一REP与第二REP的比较、及第三REP与第四REP的比较)、模式2(第一REP与第二REP的比较、及第四REP与第五REP的比较)、模式3(第二REP与第三REP的比较、及第四REP与第五REP的比较)、模式4(第一REP与第二REP的比较)。需要说明的是,此外还存在其它选择方法。

[0095] 接着,针对各模式,将所选择的相邻的两个[(A)_n基序-REP]单元中的各REP的氨基酸残基数进行比较。通过求出将氨基酸残基数更少的一者设为1时另一者的氨基酸残基数的比来进行比较。例如,在第一REP(50个氨基酸残基)与第二REP(100个氨基酸残基)比较的情况下,将氨基酸残基数更少的第一REP设为1时,第二REP的氨基酸残基数的比为100/50=2。同样地,在第四REP(20个氨基酸残基)与第五REP(30个氨基酸残基)比较的情况下,将氨基酸残基数更少的第四REP设为1时,第五REP的氨基酸残基数的比为30/20=1.5。

[0096] 图1中,用实线示出了将氨基酸残基数更少的一者设为1时,另一者的氨基酸残基数的比为1.8~11.3的[(A)_n基序-REP]单元的组。本说明书中,将该比称为锯齿比率。用虚线示出了将氨基酸残基数更少的一者设为1时,另一者的氨基酸残基数的比低于1.8或超过11.3的[(A)_n基序-REP]单元的组。

[0097] 在各模式中,将用实线表示的相邻的两个[(A)_n基序-REP]单元的全部氨基酸残基数相加(不仅是REP,还有(A)_n基序的氨基酸残基数。),并且,对相加得到的合计值进行比较,将该合计值最大的模式的合计值(合计值的最大值)设为x。在图1所示的例子中,模式1的合计值最大。

[0098] 接着,通过x除以结构域序列的总氨基酸残基数y,能够算出x/y(%)。

[0099] 第三改性丝心蛋白中,x/y优选为50%以上,更优选为60%以上,进一步优选为65%以上,更进一步优选为70%以上,更进一步优选为75%以上,特别优选为80%以上。x/y的上限没有特别限制,例如可以为100%以下。在锯齿比率为1:1.9~11.3的情况下,x/y优选为89.6%以上,在锯齿比率为1:1.8~3.4的情况下,x/y优选为77.1%以上,在锯齿比率为1:1.9~8.4的情况下,x/y优选为75.9%以上,在锯齿比率为1:1.9~4.1的情况下,x/y优选为64.2%以上。

[0100] 在第三改性丝心蛋白为结构域序列中存在多个的(A)_n基序的至少7个仅由丙氨酸

残基构成的改性丝心蛋白的情况下, x/y 优选为46.4%以上, 更优选为50%以上, 进一步优选为55%以上, 更进一步优选为60%以上, 更进一步优选为70%以上, 特别优选为80%以上。 x/y 的上限没有特别限制, 只要为100%以下即可。

[0101] 在此, 对天然来源的丝心蛋白中的 x/y 进行说明。首先, 如上所述, 利用例示出NCBI GenBank注册了氨基酸序列信息的丝心蛋白的方法进行确认后, 结果提取出663种丝心蛋白(其中, 来自蜘蛛类的丝心蛋白为415种)。在所提取出的全部丝心蛋白中, 根据式1: $[(A)_n \text{基序-REP}]_m$ 所示的结构域序列构成的天然来源的丝心蛋白的氨基酸序列, 利用上述的计算方法计算 x/y 。将锯齿比率为1:1.9~4.1的情况的结果示于图3。

[0102] 图3的横轴表示 x/y (%), 纵轴表示频率。如图3明确所示, 天然来源的丝心蛋白中的 x/y 均低于64.2% (最高为64.14%)。

[0103] 第三改性丝心蛋白例如可以通过从克隆出的天然来源的丝心蛋白的基因序列中使编码 $(A)_n$ 基序的序列的一个或多个产生了缺失以使 x/y 为64.2%以上而得到的。另外, 也可以通过例如设计相当于从天然来源的丝心蛋白的氨基酸序列中使一个或多个 $(A)_n$ 基序产生了缺失以使 x/y 为64.2%以上的氨基酸序列, 并化学合成编码所设计的氨基酸序列的核酸而得到。在任意情况下, 均可以除相当于从天然来源的丝心蛋白的氨基酸序列中使 $(A)_n$ 基序缺失的改性之外, 进一步进行相当于取代、缺失、插入及/或添加一个或多个氨基酸残基的氨基酸序列的改性。

[0104] 作为第三改性丝心蛋白的更具体的例子, 能够列举包含 (3-i) 序列号17 (Met-PRT399)、序列号7 (Met-PRT410)、序列号8 (Met-PRT525) 或者序列号9 (Met-PRT799) 所示的氨基酸序列、或与 (3-ii) 序列号17、序列号7、序列号8或者序列号9所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列的改性丝心蛋白。

[0105] 下面, 对 (3-i) 的改性丝心蛋白进行说明。序列号17所示的氨基酸序列为从相当于天然来源的丝心蛋白的序列号10 (Met-PRT313) 所示的氨基酸序列中使从N端侧向C端侧使 $(A)_n$ 基序每隔两个产生缺失, 并进一步在C端序列的近前处插入一个 $[(A)_n \text{基序-REP}]$ 而成的氨基酸序列。序列号7、序列号8或序列号9所示的氨基酸序列如第二改性丝心蛋白中所说明那样。

[0106] 序列号10所示的氨基酸序列(相当于天然来源的丝心蛋白)的锯齿比率1:1.8~11.3中的 x/y 的值为15.0%。序列号17所示的氨基酸序列、及序列号7所示的氨基酸序列中的 x/y 的值均为93.4%。序列号8所示的氨基酸序列中的 x/y 的值为92.7%。序列号9所示的氨基酸序列中的 x/y 的值为89.8%。序列号10、序列号17、序列号7、序列号8及序列号9所示的氨基酸序列中的 z/w 的值分别为46.8%、56.2%、70.1%、66.1%及70.0%。

[0107] (3-i) 的改性丝心蛋白可以为由序列号17、序列号7、序列号8或序列号9所示的氨基酸序列构成的蛋白。

[0108] (3-ii) 的改性丝心蛋白包含与序列号17、序列号7、序列号8或序列号9所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列。另外, (3-ii) 的改性丝心蛋白也是包含式1: $[(A)_n \text{基序-REP}]_m$ 所示的结构域序列的蛋白质。上述序列同一性优选为95%以上。

[0109] 在 (3-ii) 的改性丝心蛋白中, 优选地, 与序列号17、序列号7、序列号8或序列号9所示的氨基酸序列具有90%以上的序列同一性, 且相邻的两个 $[(A)_n \text{基序-REP}]$ 单元的氨基酸残基数相加得到的合计值的最大值设为 x , 将结构域序列的总氨基酸残基数设为 y 时, x/y 为

64.2%以上,其中,从N端侧向C端侧将相邻的两个 $[(A)_n\text{基序-REP}]$ 单元的REP的氨基酸残基数依次进行比较后,将氨基酸残基数较少的REP的氨基酸残基数设为1时,另一个REP的氨基酸残基数的比为1.8~11.3(锯齿比率为1:1.8~11.3)。

[0110] 第三改性丝心蛋白可以在N端及C端中任意一者或两者上包含上述的标签序列。

[0111] 作为包含标签序列的改性丝心蛋白的更具体的例子,能够列举包含(3-iii)序列号18(PRT399)、序列号13(PRT410)、序列号14(PRT525)或者序列号15(PRT799)所示的氨基酸序列、或与(3-iv)序列号18、序列号13、序列号14或者序列号15所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列的改性丝心蛋白。

[0112] 序列号18、序列号13、序列号14及序列号15所示的氨基酸序列为分别在序列号17、序列号7、序列号8及序列号9所示的氨基酸序列的N端添加有序列号11所示的氨基酸序列(包含His标签序列及铰链序列)而成的氨基酸序列。

[0113] (3-iii)的改性丝心蛋白可以为由序列号18、序列号13、序列号14或序列号15所示的氨基酸序列构成的蛋白。

[0114] (3-iv)的改性丝心蛋白包含与序列号18、序列号13、序列号14或序列号15所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列。另外,(3-iv)的改性丝心蛋白也是包含式1: $[(A)_n\text{基序-REP}]_m$ 所示的结构域序列的蛋白质。上述序列同一性优选为95%以上。

[0115] 在(3-iv)的改性丝心蛋白中,优选地,将与序列号18、序列号13、序列号14或序列号15所示的氨基酸序列具有90%以上的序列同一性,且相邻的两个 $[(A)_n\text{基序-REP}]$ 单元的氨基酸残基数相加的合计值的最大值设为 x ,将结构域序列的总氨基酸残基数设为 y 时, x/y 为64.2%以上,其中,从N端侧向C端侧将相邻的两个 $[(A)_n\text{基序-REP}]$ 单元的REP的氨基酸残基数依次进行比较后,将氨基酸残基数较少的REP的氨基酸残基数设为1时,另一个REP的氨基酸残基数的比为1.8~11.3。

[0116] 第三改性丝心蛋白可以包含用于将重组蛋白质生产系中所生产的蛋白质释放至宿主的外部的分泌信号。分泌信号的序列能够根据宿主的种类适当地设置。

[0117] 第四改性丝心蛋白中,其结构域序列具有与天然来源的丝心蛋白相比除降低了 $(A)_n$ 基序的含量之外,还降低了甘氨酸残基的含量的氨基酸序列。第四改性丝心蛋白的结构域序列可以具有相当于与天然来源的丝心蛋白相比除缺失了至少一个或多个 $(A)_n$ 基序之外,进一步将REP中的至少一个或多个甘氨酸残基取代为其它的氨基酸残基的氨基酸序列。即,第四改性丝心蛋白为兼具上述的第二改性丝心蛋白和第三改性丝心蛋白的特征的改性丝心蛋白。具体的方案等如第二改性丝心蛋白、及第三改性丝心蛋白中所述。

[0118] 作为第四改性丝心蛋白的更具体的例子,能够列举包含(4-i)序列号7(Met-PRT410)、序列号8(Met-PRT525)、序列号9(Met-PRT799)、序列号13(PRT410)、序列号14(PRT525)或者序列号15(PRT799)所示的氨基酸序列、或与(4-ii)序列号7、序列号8、序列号9、序列号13、序列号14或者序列号15所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列的改性丝心蛋白。包含序列号7、序列号8、序列号9、序列号13、序列号14或序列号15所示的氨基酸序列的改性丝心蛋白的具体方案如上所述。

[0119] 第五改性丝心蛋白可以为如下蛋白:其结构域序列具有包含局部疏水性指数较大的区域的氨基酸序列,该氨基酸序列相当于与天然来源的丝心蛋白相比,REP中的一个或多个氨基酸残基被取代为疏水性指数较大的氨基酸残基、及/或REP中插入有一个或多个疏水

性指数较大的氨基酸残基。

[0120] 局部疏水性指数较大的区域优选由连续的2~4个氨基酸残基构成。

[0121] 上述的疏水性指数较大的氨基酸残基优选为选自异亮氨酸(I)、缬氨酸(V)、亮氨酸(L)、苯丙氨酸(F)、半胱氨酸(C)、蛋氨酸(M)及丙氨酸(A)的氨基酸残基。

[0122] 在第五改性丝心蛋白中,除相当于与天然来源的丝心蛋白相比,REP中的一个或多个氨基酸残基被取代为疏水性指数较大的氨基酸残基、及/或REP中插入有一个或多个疏水性指数较大的氨基酸残基的改性之外,可以进一步具有相当于与天然来源的丝心蛋白相比取代、缺失、插入及/或添加一个或多个氨基酸残基的氨基酸序列的改性。

[0123] 第五改性丝心蛋白能够通过例如从克隆出的天然来源的丝心蛋白的基因序列中将REP中的一个或多个亲水性氨基酸残基(例如,疏水性指数为负的氨基酸残基)取代为疏水性氨基酸残基(例如,疏水性指数为正的氨基酸残基)、及/或在REP中插入一个或多个疏水性氨基酸残基而得到。另外,也能够通过例如设计相当于从天然来源的丝心蛋白的氨基酸序列中将REP中的一个或多个亲水性氨基酸残基取代为疏水性氨基酸残基、及/或REP中插入有一个或多个疏水性氨基酸残基的氨基酸序列,并化学合成编码所设计的氨基酸序列的核酸来得到。在任意情况下,均可以在除相当于从天然来源的丝心蛋白的氨基酸序列中将REP中的一个或多个亲水性氨基酸残基取代为疏水性氨基酸残基、及/或在REP中插入有一个或多个疏水性氨基酸残基的改性之外,进一步进行相当于取代、缺失、插入及/或添加一个或多个氨基酸残基的氨基酸序列的改性。

[0124] 第五改性丝心蛋白可以具有下述氨基酸序列:在包含式1: $[(A)_n \text{基序}-REP]_m$ 所示的结构域序列,且从上述结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至上述结构域序列的C端为止的序列而成的序列中所含的全部REP中,将连续4个氨基酸残基的疏水性指数的平均值为2.6以上的区域中所含的氨基酸残基的总数设为p,将从上述结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至上述结构域序列的C端为止的序列而成的序列中所含的氨基酸残基的总数设为q时, p/q 为6.2%以上。

[0125] 关于氨基酸残基的疏水性指数,使用公知的指数(Hydropathy index:Kyte J,& Doolittle R(1982) "A simple method for displaying the hydropathic character of a protein", J.Mol.Biol.,157,pp.105-132)。具体而言,各氨基酸的疏水性指数(hydropathy index,以下,也记作“HI”)如下述表1所示。

[0126] [表1]

[0127]

氨基酸	HI	氨基酸	HI
异亮氨酸(Ile)	4.5	色氨酸(Trp)	-0.9
缬氨酸(Val)	4.2	酪氨酸(Tyr)	-1.3
亮氨酸(Leu)	3.8	脯氨酸(Pro)	-1.6
苯丙氨酸(Phe)	2.8	组氨酸(His)	-3.2
半胱氨酸(Cys)	2.5	天冬酰胺(Asn)	-3.5
蛋氨酸(Met)	1.9	天冬氨酸(Asp)	-3.5
丙氨酸(Ala)	1.8	谷氨酰胺(Gln)	-3.5
甘氨酸(Gly)	-0.4	谷氨酸(Glu)	-3.5
苏氨酸(Thr)	-0.7	赖氨酸(Lys)	-3.9

丝氨酸(Ser)	-0.8	精氨酸(Arg)	-4.5
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[0128] 下面,进一步详细说明 p/q 的计算方法。计算中使用从式1: $[(A)_n \text{基序}-\text{REP}]_m$ 所示的结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至结构域序列的C端为止的序列而成的序列(以下,称为“序列A”)。首先,在序列A中所含的全部REP中,计算连续的4个氨基酸残基的疏水性指数的平均值。疏水性指数的平均值通过连续的4个氨基酸残基中所含的各氨基酸残基的HI的总和除以4(氨基酸残基数)而求得。疏水性指数的平均值针对全部连续的4个氨基酸残基而求得(各氨基酸残基用于计算1~4个平均值。)。接着,确定连续的4个氨基酸残基的疏水性指数的平均值为2.6以上的区域。即使在某个氨基酸残基相当于多个“疏水性指数的平均值为2.6以上的连续的4个氨基酸残基”的情况下,该某个氨基酸残基也作为1个氨基酸残基包含于区域中。并且,该区域中所含的氨基酸残基的总数为 p 。另外,序列A中所含的氨基酸残基的总数为 q 。

[0129] 例如,在“疏水性指数的平均值为2.6以上的连续的4个氨基酸残基”提取了20处的情况下(无重复),在连续的4个氨基酸残基的疏水性指数的平均值为2.6以上的区域中包含20个连续的4个氨基酸残基(无重复), p 为 $20 \times 4 = 80$ 。另外,例如,在两个“疏水性指数的平均值为2.6以上的连续的4个氨基酸残基”重复存在一个氨基酸残基的情况下,在连续的4个氨基酸残基的疏水性指数的平均值为2.6以上的区域中包含7个氨基酸残基($p = 2 \times 4 - 1 = 7$ 。“-1”表示扣除重复量。)。例如,在图4所示的结构域序列的情况下,“疏水性指数的平均值为2.6以上的连续的4个氨基酸残基”无重复地存在7个,因此, p 为 $7 \times 4 = 28$ 。另外,例如,在图4所示的结构域序列的情况下, q 为 $4 + 50 + 4 + 40 + 4 + 10 + 4 + 20 + 4 + 30 = 170$ (不包含存在于C端侧的最后的 $(A)_n$ 基序)。接着,用 p 除以 q ,由此能够算出 p/q (%)。在图4的情况下, $28/170 = 16.47\%$ 。

[0130] 在第五改性丝心蛋白中, p/q 优选为6.2%以上,更优选为7%以上,进一步优选为10%以上,更进一步优选为20%以上,更进一步优选为30%以上。 p/q 的上限不受特别限制,可以为例如45%以下。

[0131] 第五改性丝心蛋白能够通过例如以满足上述的 p/q 的条件的方式,将REP中的一个或多个亲水性氨基酸残基(例如,疏水性指数为负的氨基酸残基)取代为疏水性氨基酸残基(例如,疏水性指数为正的氨基酸残基)、及/或在REP中插入一个或多个疏水性氨基酸残基,将克隆出的天然来源的丝心蛋白的氨基酸序列改性于包含局部疏水性指数较大的区域的氨基酸序列来得到。另外,还可以通过例如根据天然来源的丝心蛋白的氨基酸序列设计满足上述的 p/q 的条件的氨基酸序列,并化学合成编码所设计的氨基酸序列的核酸而得到。在任意情况下,均可以在除相当于与天然来源的丝心蛋白相比REP中的一个或多个氨基酸残基被取代为疏水性指数较大的氨基酸残基、及/或REP中插入有一个或多个疏水性指数较大的氨基酸残基的改性之外,进一步进行相当于取代、缺失、插入及/或添加一个或多个氨基酸残基的改性。

[0132] 作为疏水性指数较大的氨基酸残基,没有特别限制,优选异亮氨酸(I)、缬氨酸(V)、亮氨酸(L)、苯丙氨酸(F)、半胱氨酸(C)、蛋氨酸(M)及丙氨酸(A),更优选缬氨酸(V)、亮氨酸(L)及异亮氨酸(I)。

[0133] 作为第五改性丝心蛋白的更具体的例子,能够列举包含(5-i)序列号19(Met-PRT720)、序列号20(Met-PRT665)或者序列号21(Met-PRT666)所示的氨基酸序列、或与(5-

ii) 序列号19、序列号20或者序列号21所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列的改性丝心蛋白。

[0134] 下面,对(5-i)的改性丝心蛋白进行说明。序列号19所示的氨基酸序列是对于序列号7 (Met-PRT410) 所示的氨基酸序列除去C端侧的末端的结构域序列,每隔一个REP分别插入两处由3个氨基酸残基构成的氨基酸序列(VLI),并进一步将一部分谷氨酰胺(Q) 残基取代为丝氨酸(S) 残基,且使C端侧的一部分氨基酸缺失而成的。序列号20所示的氨基酸序列是对于序列号8 (Met-PRT525) 所示的氨基酸序列每隔一个REP分别插入一个由3个氨基酸残基构成的氨基酸序列(VLI) 而成的。序列号21所示的氨基酸序列是对于序列号8所示的氨基酸序列每隔一个REP分别插入两处由3个氨基酸残基构成的氨基酸序列(VLI) 而成的。

[0135] (5-i)的改性丝心蛋白可以为由序列号19、序列号20或序列号21所示的氨基酸序列构成的蛋白。

[0136] (5-ii)的改性丝心蛋白包含与序列号19、序列号20或序列号21所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列。另外,(5-ii)的改性丝心蛋白也是包含式1:
[(A)_n基序-REP]_m所示的结构域序列的蛋白质。上述序列同一性优选为95%以上。

[0137] 在(5-ii)的改性丝心蛋白中,优选地,在具有与序列号19、序列号20或序列号21所示的氨基酸序列具有90%以上的序列同一性,且从结构域序列中除去从位于最C端侧的(A)_n基序至结构域序列的C端为止的序列而成的序列中所含的全部REP中,在将连续的4个氨基酸残基的疏水性指数的平均值为2.6以上的区域中所含的氨基酸残基的总数设为p,将从结构域序列中除去从位于最C端侧的(A)_n基序至结构域序列的C端为止的序列而成的序列中所含的氨基酸残基的总数设为q时,p/q为6.2%以上。

[0138] 第五改性丝心蛋白可以在N端及C端中任意一者或两者上包含标签序列。

[0139] 作为包含标签序列的改性丝心蛋白的更具体的例子,能够列举包含(5-iii) 序列号22 (PRT720)、序列号23 (PRT665) 或者序列号24 (PRT666) 所示的氨基酸序列、或与(5-iv) 序列号22、序列号23或者序列号24所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列的改性丝心蛋白。

[0140] 序列号22、序列号23及序列号24所示的氨基酸序列为分别在序列号19、序列号20及序列号21所示的氨基酸序列的N端添加有序列号11所示的氨基酸序列(包含His标签序列及铰链序列) 而成的氨基酸序列。

[0141] (5-iii)的改性丝心蛋白可以为由序列号22、序列号23或序列号24所示的氨基酸序列构成的蛋白。

[0142] (5-iv)的改性丝心蛋白包含与序列号22、序列号23或序列号24所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列。另外,(5-iv)的改性丝心蛋白也是包含式1:
[(A)_n基序-REP]_m所示的结构域序列的蛋白质。上述序列同一性优选为95%以上。

[0143] 在(5-iv)的改性丝心蛋白中,优选地,在与序列号22、序列号23或序列号24所示的氨基酸序列具有90%以上的序列同一性,且从结构域序列中除去从位于最C端侧的(A)_n基序至结构域序列的C端为止的序列而成的序列中所含的全部REP中,将连续的4个氨基酸残基的疏水性指数的平均值为2.6以上的区域中所含的氨基酸残基的总数设为p,将从结构域序列中除去从位于最C端侧的(A)_n基序至结构域序列的C端为止的序列而成的序列中所含的氨基酸残基的总数设为q时,p/q优选为6.2%以上。

[0144] 第五改性丝心蛋白可以包含用于将重组蛋白质生产系中所生产的蛋白质释放至宿主的外部的分泌信号。分泌信号的序列能够根据宿主的种类适当地设置。

[0145] 第六改性丝心蛋白具有与天然来源的丝心蛋白相比降低了谷氨酰胺残基的含量的氨基酸序列。

[0146] 第六改性丝心蛋白优选在REP的氨基酸序列中包含选自GGX基序及GPGXX基序中的至少一个基序。

[0147] 在第六改性丝心蛋白的REP中包含GPGXX基序的情况下，GPGXX基序含有率通常为1%以上，可以为5%以上，优选为10%以上。GPGXX基序含有率的上限没有特别限制，只要为50%以下即可，可以为30%以下。

[0148] 本说明书中，“GPGXX基序含有率”为通过以下的方法而算出的值。

[0149] 在包含式1： $[(A)_n\text{基序}-\text{REP}]_m$ 、或式2： $[(A)_n\text{基序}-\text{REP}]_m - (A)_n\text{基序}$ 所示的结构域序列的丝心蛋白(改性丝心蛋白或天然来源的丝心蛋白)中，在从结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至结构域序列的C端为止的序列而成的序列中所含的全部REP中，将该区域中所含的GPGXX基序的个数的总数乘以3得到的数(即，相当于GPGXX基序中的G及P的总数)设为s，将从结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至结构域序列的C端为止的序列，并进一步除去 $(A)_n$ 基序后的全部REP的氨基酸残基的总数设为t，此时， s/t 计算GPGXX基序含有率。

[0150] 计算GPGXX基序含有率时，“从位于最C端侧的 $(A)_n$ 基序至结构域序列的C端为止的序列”(相当于REP的序列)中具有与丝心蛋白的特征性的序列的相关性较低的序列，在m较小的情况下(即，结构域序列较短的情况下)，会影响GPGXX基序含有率的计算结果，因此，为了排除该影响，将“从结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至结构域序列的C端为止的序列而成的序列”作为对象。需要说明的是，在“GPGXX基序”位于REP的C端的情况下，即使在“XX”为例如“AA”的情况下，也作为“GPGXX基序”处理。

[0151] 图5为示出改性丝心蛋白的结构域序列的示意图。参考图5对GPGXX基序含有率的计算方法进行具体地说明。首先，在图5所示的改性丝心蛋白的结构域序列(“ $[(A)_n\text{基序}-\text{REP}]_m - (A)_n\text{基序}$ ”类型。)中，全部的REP包含于“从结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至结构域序列的C端为止的序列而成的序列”(图5中，由“区域A”所示的序列。)中，因此，用于计算s的GPGXX基序的个数为7，s为 $7 \times 3 = 21$ 。同理，全部的REP包含于“从结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至结构域序列的C端为止的序列而成的序列”(图5中，由“区域A”所示的序列。)中，因此，从该序列中进一步除去 $(A)_n$ 基序后的全部REP的氨基酸残基的总数t为 $50 + 40 + 10 + 20 + 30 = 150$ 。接着，通过s除以t，能够算出 $s/t(\%)$ ，在图5的改性丝心蛋白的情况下， $21/150 = 14.0\%$ 。

[0152] 在第六改性丝心蛋白中，谷氨酰胺残基含有率优选为9%以下，更优选为7%以下，进一步优选为4%以下，特别优选为0%。

[0153] 本说明书中，“谷氨酰胺残基含有率”为通过以下的方法而算出的值。

[0154] 在包含式1： $[(A)_n\text{基序}-\text{REP}]_m$ 、或式2： $[(A)_n\text{基序}-\text{REP}]_m - (A)_n\text{基序}$ 所示的结构域序列的丝心蛋白(改性丝心蛋白或天然来源的丝心蛋白)中，在从结构域序列中除去从位于最C端侧的 $(A)_n$ 基序至结构域序列的C端为止的序列而成的序列(相当于图5的“区域A”的序列。)中所含的全部REP中，将该区域中所含的谷氨酰胺残基的总数设为u，将从结构域序列

中除去从位于最C端侧的(A)_n基序至结构域序列的C端为止的序列,并进一步除去(A)_n基序后的全部REP的氨基酸残基的总数设为t,此时,作为u/t计算谷氨酰胺残基含有率。计算谷氨酰胺残基含有率时,将“从结构域序列中除去从位于最C端侧的(A)_n基序至结构域序列的C端为止的序列而成的序列”作为对象的理由与上述的理由相同。

[0155] 在第六改性丝心蛋白中,其结构域序列可以具有相当于与天然来源的丝心蛋白相比缺失了REP中的一个或多个谷氨酰胺残基、或将其取代为其它的氨基酸残基的氨基酸序列。

[0156] “其它的氨基酸残基”只要为谷氨酰胺残基以外的氨基酸残基即可,优选为疏水性指数大于谷氨酰胺残基的氨基酸残基。氨基酸残基的疏水性指数如表1所示。

[0157] 如表1所示,作为疏水性指数大于谷氨酰胺残基的氨基酸残基,能够列举选自异亮氨酸(I)、缬氨酸(V)、亮氨酸(L)、苯丙氨酸(F)、半胱氨酸(C)、蛋氨酸(M)丙氨酸(A)、甘氨酸(G)、苏氨酸(T)、丝氨酸(S)、色氨酸(W)、酪氨酸(Y)、脯氨酸(P)及组氨酸(H)的氨基酸残基。其中,更优选为选自异亮氨酸(I)、缬氨酸(V)、亮氨酸(L)、苯丙氨酸(F)、半胱氨酸(C)、蛋氨酸(M)及丙氨酸(A)的氨基酸残基,进一步优选为选自异亮氨酸(I)、缬氨酸(V)、亮氨酸(L)及苯丙氨酸(F)的氨基酸残基。

[0158] 在第六改性丝心蛋白中,REP的疏水性度优选为-0.8以上,更优选为-0.7以上,进一步优选为0以上,更进一步优选为0.3以上,特别优选为0.4以上。REP的疏水性度的上限没有特别限制,只要为1.0以下即可,可以为0.7以下。

[0159] 本说明书中,“REP的疏水性度”为通过以下的方法而算出的值。

[0160] 在包含式1:[(A)_n基序-REP]_m、或式2:[(A)_n基序-REP]_m-(A)_n基序所示的结构域序列的丝心蛋白(改性丝心蛋白或天然来源的丝心蛋白)中,在从结构域序列中除去从位于最C端侧的(A)_n基序至结构域序列的C端为止的序列而成的序列(相当于图5的“区域A”的序列。)中所含的全部REP中,在将该区域的各氨基酸残基的疏水性指数的总和设为v,将从结构域序列中除去从位于最C端侧的(A)_n基序至结构域序列的C端为止的序列,并进一步除去(A)_n基序后的全部REP的氨基酸残基的总数设为t,此时,作为v/t计算REP的疏水性度。计算REP的疏水性度时,将“从结构域序列中除去从位于最C端侧的(A)_n基序至结构域序列的C端为止的序列而成的序列”作为对象的理由与上述的理由相同。

[0161] 在第六改性丝心蛋白中,其结构域序列除相当于与天然来源的丝心蛋白相比缺失了REP中的一个或多个谷氨酰胺残基、及/或将REP中的一个或多个谷氨酰胺残基取代为其它氨基酸残基的改性之外,还可以进一步具有相当于取代、缺失、插入及/或添加一个或多个氨基酸残基的氨基酸序列的改性。

[0162] 第六改性丝心蛋白通过例如能够从克隆出的天然来源的丝心蛋白的基因序列中使REP中的一个或多个谷氨酰胺残基缺失、及/或将REP中的一个或多个谷氨酰胺残基取代为其它氨基酸残基而得到。另外,也能够通过例如设计相当于从天然来源的丝心蛋白的氨基酸序列中缺失REP中的一个或多个谷氨酰胺残基、及/或将REP中的一个或多个谷氨酰胺残基取代为其它的氨基酸残基的氨基酸序列,并化学合成编码所设计的氨基酸序列的核酸而得到。

[0163] 作为第六改性丝心蛋白的更具体的例子,能够列举包含(6-i)序列号25(Met-PRT888)、序列号26(Met-PRT965)、序列号27(Met-PRT889)、序列号28(Met-PRT916)、序列号

29 (Met-PRT918)、序列号30 (Met-PRT699)、序列号31 (Met-PRT698)、序列号32 (Met-PRT966)、序列号41 (Met-PRT917) 或者序列号42 (Met-PRT1028) 所示的氨基酸序列的改性丝心蛋白、或包含与 (6-ii) 序列号25、序列号26、序列号27、序列号28、序列号29、序列号30、序列号31、序列号32、序列号41或者序列号42所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列的改性丝心蛋白。

[0164] 下面,对 (6-i) 的改性丝心蛋白进行说明。序列号25所示的氨基酸序列是将序列号7所示的氨基酸序列 (Met-PRT410) 中的QQ全部取代为VL而成的。序列号26所示的氨基酸序列是将序列号7所示的氨基酸序列中的QQ全部取代为TS,且将剩余的Q取代为A而成的。序列号27所示的氨基酸序列是将序列号7所示的氨基酸序列中的QQ全部取代为VL,且将剩余的Q取代为I而成的。序列号28所示的氨基酸序列是将序列号7所示的氨基酸序列中的QQ全部取代为VI,且将剩余的Q取代为L而成的。序列号29所示的氨基酸序列是将序列号7所示的氨基酸序列中的QQ全部取代为VF,且将剩余的Q取代为I而成的。

[0165] 序列号30所示的氨基酸序列是将序列号8所示的氨基酸序列 (Met-PRT525) 中的QQ全部取代为VL而成的。序列号31所示的氨基酸序列是将序列号8所示的氨基酸序列中的QQ全部取代为VL,且将剩余的Q取代为I而成的。

[0166] 序列号32所示的氨基酸序列是将序列号7所示的氨基酸序列 (Met-PRT410) 中存在的20个结构域序列的区域重复两次而成的序列中的QQ全部取代为VF,且将剩余的Q取代为I而成的。

[0167] 序列号41所示的氨基酸序列 (Met-PRT917) 是将序列号7所示的氨基酸序列中的QQ全部取代为LI,且将剩余的Q取代为V而成的。序列号42所示的氨基酸序列 (Met-PRT1028) 是将序列号7所示的氨基酸序列中的QQ全部取代为IF,且将剩余的Q取代为T而成的。

[0168] 序列号25、序列号26、序列号27、序列号28、序列号29、序列号30、序列号31、序列号32、序列号41及序列号42所示的氨基酸序列的谷氨酰胺残基含有率均为9%以下(表2)。

[0169] [表2]

[0170]	改性丝心蛋白	谷氨酰胺残基含有率	GPGXX 基序含有率	REP 的疏水性度
	Met-PRT410 (序列号 7)	17.7%	27.9%	-1.52
	Met-PRT888 (序列号 25)	6.3%	27.9%	0.07
	Met-PRT965 (序列号 26)	0.0%	27.9%	-0.65
	Met-PRT889 (序列号 27)	0.0%	27.9%	0.35
	Met-PRT916 (序列号 28)	0.0%	27.9%	0.47
	Met-PRT918 (序列号 29)	0.0%	27.9%	0.45
	Met-PRT699 (序列号 30)	3.6%	26.4%	-0.78
	Met-PRT698 (序列号 31)	0.0%	26.4%	-0.03
	Met-PRT966 (序列号 32)	0.0%	28.0%	0.35
	Met-PRT917 (序列号 41)	0.0%	27.9%	0.46
	Met-PRT1028 (序列号 42)	0.0%	28.1%	0.05

[0171] (6-i) 的改性丝心蛋白可以为由序列号25、序列号26、序列号27、序列号28、序列号29、序列号30、序列号31、序列号32、序列号41或序列号42所示的氨基酸序列构成的蛋白。

[0172] (6-ii) 的改性丝心蛋白可以包含与序列号25、序列号26、序列号27、序列号28、序列号29、序列号30、序列号31、序列号32、序列号41或序列号42所示的氨基酸序列具有90%

以上的序列同一性的氨基酸序列。另外, (6-ii) 的改性丝心蛋白也是包含式1: $[(A)_n \text{基序-REP}]_m$ 、或式2: $[(A)_n \text{基序-REP}]_m - (A)_n \text{基序}$ 所示的结构域序列的蛋白质。上述序列同一性优选为95%以上。

[0173] (6-ii) 的改性丝心蛋白的谷氨酰胺残基含有率优选为9%以下。另外, (6-ii) 的改性丝心蛋白的GPGXX基序含有率优选为10%以上。

[0174] 第六改性丝心蛋白可以在N端及C端中任意一者或两者上包含标签序列。由此, 可以进行改性丝心蛋白的分离、固定化、检测及可视化等。

[0175] 作为包含标签序列的改性丝心蛋白的更具体的例子, 能够列举包含与 (6-iii) 序列号33 (PRT888)、序列号34 (PRT965)、序列号35 (PRT889)、序列号36 (PRT916)、序列号37 (PRT918)、序列号38 (PRT699)、序列号39 (PRT698)、序列号40 (PRT966)、序列号43 (PRT917) 或者序列号44 (PRT1028) 所示的氨基酸序列的改性丝心蛋白、或包含与 (6-iv) 序列号33、序列号34、序列号35、序列号36、序列号37、序列号38、序列号39、序列号40、序列号43或者序列号44所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列的改性丝心蛋白。

[0176] 序列号33、序列号34、序列号35、序列号36、序列号37、序列号38、序列号39、序列号40、序列号43及序列号44所示的氨基酸序列为分别在序列号25、序列号26、序列号27、序列号28、序列号29、序列号30、序列号31、序列号32、序列号41及序列号42所示的氨基酸序列的N端添加有序列号11所示的氨基酸序列 (包含His标签序列及铰链序列) 而成的氨基酸序列。由于仅在N端添加有标签序列, 因此, 谷氨酰胺残基含有率没有变化, 序列号33、序列号34、序列号35、序列号36、序列号37、序列号38、序列号39、序列号40、序列号43及序列号44所示的氨基酸序列的谷氨酰胺残基含有率均为9%以下 (表3)。

[0177] [表3]

[0178]	改性丝心蛋白	谷氨酰胺残基含有率	GPGXX 基序含有率	REP 的疏水性度
	PRT888 (序列号 33)	6.3%	27.9%	-0.07
	PRT965 (序列号 34)	0.0%	27.9%	-0.65
	PRT889 (序列号 35)	0.0%	27.9%	0.35
	PRT916 (序列号 36)	0.0%	27.9%	0.47
	PRT918 (序列号 37)	0.0%	27.9%	0.45
	PRT699 (序列号 38)	3.6%	26.4%	-0.78
	PRT698 (序列号 39)	0.0%	26.4%	-0.03
	PRT966 (序列号 40)	0.0%	28.0%	0.35
	PRT917 (序列号 43)	0.0%	27.9%	0.46
	PRT1028 (序列号 44)	0.0%	28.1%	0.05

[0179] (6-iii) 的改性丝心蛋白可以为由序列号33、序列号34、序列号35、序列号36、序列号37、序列号38、序列号39、序列号40、序列号43或序列号44所示的氨基酸序列构成的蛋白。

[0180] (6-iv) 的改性丝心蛋白包含与序列号33、序列号34、序列号35、序列号36、序列号37、序列号38、序列号39、序列号40、序列号43或序列号44所示的氨基酸序列具有90%以上的序列同一性的氨基酸序列。另外, (6-iv) 的改性丝心蛋白也是包含式1: $[(A)_n \text{基序-REP}]_m$ 、或式2: $[(A)_n \text{基序-REP}]_m - (A)_n \text{基序}$ 所示的结构域序列的蛋白质。上述序列同一性优选为95%以上。

[0181] (6-iv)的改性丝心蛋白的谷氨酰胺残基含有率优选为9%以下。另外,(6-iv)的改性丝心蛋白的GPGXX基序含有率优选为10%以上。

[0182] 第六改性丝心蛋白可以包含用于将重组蛋白质生产系中生产的蛋白质释放至宿主的外部的分泌信号。分泌信号的序列能够根据宿主的种类适当地设置。

[0183] 改性丝心蛋白可以为兼备第一改性丝心蛋白、第二改性丝心蛋白、第三改性丝心蛋白、第四改性丝心蛋白、第五改性丝心蛋白、及第六改性丝心蛋白所具有的特征中的至少两个以上特征的改性丝心蛋白。

[0184] 改性丝心蛋白可以为亲水性改性丝心蛋白,也可以为疏水性改性丝心蛋白。本说明书中,“疏水性改性丝心蛋白”是求得构成改性丝心蛋白的全部氨基酸残基的疏水性指数(HI)的总和,接着用该总和除以氨基酸残基总数而得到的值(平均HI)超过0的改性丝心蛋白。疏水性指数如表1所示。另外,“亲水性改性丝心蛋白”是指平均HI为0以下的改性丝心蛋白。作为改性丝心蛋白,从耐燃性优异的观点出发,优选亲水性改性丝心蛋白,从吸湿发热性优异的观点出发,优选疏水性改性丝心蛋白。

[0185] 作为疏水性改性丝心蛋白,可列举例如:包含序列号27、序列号28、序列号29、序列号30、序列号31、序列号32、序列号33或序列号43所示的氨基酸序列,序列号35、序列号37、序列号38、序列号39、序列号40、序列号41或序列号44所示的氨基酸序列的改性丝心蛋白。

[0186] 作为亲水性改性丝心蛋白,可列举例如:包含序列号4所示的氨基酸序列、序列号6、序列号7、序列号8或序列号9所示的氨基酸序列、序列号13、序列号11、序列号14或序列号15所示的氨基酸序列、序列号18、序列号7、序列号8或序列号9所示的氨基酸序列、序列号17、序列号11、序列号14或序列号15所示的氨基酸序列、序列号19、序列号20或序列号21所示的氨基酸序列的改性丝心蛋白。

[0187] 本实施方式中的人造丝心蛋白纤维可以单独含有一种改性丝心蛋白,也可以组合含有两种以上改性丝心蛋白。

[0188] (改性丝心蛋白的制造方法)

[0189] 上述任意的实施方式中的改性丝心蛋白也可以通过例如利用宿主表达该核酸来生产,其中,该宿主是被具有编码该改性丝心蛋白的核酸序列和可操作地连接于该核酸序列的一个或多个调控序列的表达载体转化而成的。

[0190] 编码改性丝心蛋白的核酸的制造方法不受特别限制。能够通过例如利用编码天然的丝心蛋白的基因,通过聚合酶链式反应(PCR)等进行扩增并克隆,并通过基因工程手法进行改性的方法、或化学合成的方法来制造该核酸。核酸的化学合成方法也不受特别限制,例如,能够基于从NCBI的网络数据库等中获得的丝心蛋白的氨基酸序列信息,将由AKTA oligopilot plus 10/100(GE HEALTHCARE JAPAN株式会社)等自动合成的寡核苷酸用PCR等连接的方法来化学合成基因。此时,为了方便改性丝心蛋白的纯化及/或确认,也可以合成由在上述的氨基酸序列的N端添加有编码改性丝心蛋白的核酸,该核酸由起始密码子及His10标签构成的氨基酸序列的氨基酸序列构成。

[0191] 调控序列为控制宿主中的改性丝心蛋白的表达的序列(例如,启动子、增强子、核糖体结合序列、转录终止序列等),能够根据宿主的种类适当地选择。作为启动子,可以使用在宿主细胞中其作用,并可以诱导表达改性丝心蛋白的诱导型启动子。诱导型启动子是能够通过存在诱导物质(表达诱导剂)、不存在阻遏分子、或升高或者降低温度、渗透压或者pH

值等物理因素来控制转录的启动子。

[0192] 表达载体的种类能够根据质粒载体、病毒载体、粘粒载体、fosmid载体、人工染色体载体等、宿主的种类适当地选择。作为表达载体,优选使用可以在宿主细胞中独立复制、或可以向宿主的染色体中整合,并在能够转录编码改性丝心蛋白的核酸的位置处含有启动子的载体。

[0193] 作为宿主,原核生物、以及酵母、丝状真菌、昆虫细胞、动物细胞及植物细胞等真核生物均能够优选使用。

[0194] 作为原核生物的宿主的优选例子,能够列举:属于埃希氏菌属、短芽孢杆菌属、沙雷氏菌属、芽孢杆菌属、微杆菌属、短杆菌属、棒杆菌属及假单胞菌属等的细菌。作为属于埃希氏菌属的微生物,能够列举例如大肠杆菌等。作为属于短芽孢杆菌属的微生物,能够列举例如土壤短芽孢杆菌等。作为属于沙雷氏菌属的微生物,能够列举例如液化沙雷氏菌等。作为属于芽孢杆菌属的微生物,能够列举例如草芽孢杆菌等。作为属于微杆菌属的微生物,能够列举例如嗜氨微杆菌等。作为属于短杆菌属的微生物,能够列举例如叉开短杆菌等。作为属于棒杆菌属的微生物,能够列举例如产氨棒杆菌等。作为属于假单胞菌(Pseudomonas)属的微生物,能够列举例如恶臭假单胞菌等。

[0195] 在将原核生物作为宿主的情况下,作为导入编码改性丝心蛋白的核酸的载体,能够列举例如:pBTrp2(Boehringer Mannheim公司制造)、pGEX(Pharmacia公司制造)、pUC18、pBluescriptII、pSupex、pET22b、pCold、pUB110、pNC02(日本特开2002-238569号公报)等。

[0196] 作为真核生物的宿主,能够列举例如酵母及丝状真菌(霉菌等)。作为酵母,能够列举例如:属于酵母属、毕赤酵母属、裂殖酵母属等的酵母。作为丝状真菌,能够列举例如:属于曲霉属、青霉属、木霉(Trichoderma)属等的丝状真菌。

[0197] 在将真核生物作为宿主的情况下,作为导入编码改性丝心蛋白的核酸的载体,能够列举例如:YEP13(ATCC37115)、YEp24(ATCC37051)等。作为向上述宿主细胞导入表达载体的方法,只要为向上述宿主细胞导入DNA的方法,则均能够使用。能够列举例如:使用钙离子的方法(Proc.Natl.Acad.Sci.USA,69,2110(1972))、电穿孔法、原生质球法、原生质体法、乙酸锂法、感受态法等。

[0198] 作为利用被表达载体转化的宿主表达核酸的方法,除直接表达之外,还能够依据分子克隆第2版中记载的方法等,进行分泌生产、融合蛋白质表达等。

[0199] 改性丝心蛋白能够通过例如将被表达载体转化后的宿主在培养用培养基中培养,使该改性丝心蛋白在培养用培养基中生成及积蓄,并从该培养用培养基中采集该改性丝心蛋白而制造。在培养用培养基中培养宿主的方法能够按照培养宿主时通常所使用的方法来进行。

[0200] 在宿主为大肠杆菌等原核生物或酵母等真核生物的情况下,作为培养用培养基,只要为含有宿主能够同化的碳源、氮源及无机盐类等,并能够高效地培养宿主的培养基,则可以使用任意的天然培养基及合成培养基。

[0201] 作为碳源,只要为上述转化微生物能够同化的物质即可,能够使用例如:葡萄糖、果糖、蔗糖、及含有这些的糖蜜、淀粉及淀粉水解物等碳水化合物、乙酸及丙酸等有机酸、以及乙醇及丙醇等醇类。作为氮源,能够使用例如:氨、氯化铵、硫酸铵、乙酸铵及磷酸铵等无机酸或有机酸的铵盐、其它含氮化合物、以及蛋白胨、肉提取物、酵母提取物、玉米浆、酪蛋

白水解物、豆粕及豆粕水解物、各种发酵菌体及其消化物。作为无机盐类,能够使用例如:磷酸二氢钾、磷酸氢二钾、磷酸镁、硫酸镁、氯化钠、硫酸亚铁、硫酸锰、硫酸铜及碳酸钙。

[0202] 大肠杆菌等原核生物或酵母等真核生物的培养能够在例如振荡培养或深部通气搅拌培养等有氧条件下进行。培养温度为例如15~40℃。培养时间通常为16小时~7天。培养中的培养用培养基的pH优选保持为3.0~9.0。培养用培养基的pH的调节能够使用无机酸、有机酸、碱溶液、尿素、碳酸钙及氨等来进行。

[0203] 另外,培养期间,可以根据需要向培养用培养基中添加氨苄青霉素及四环素等抗生素。在培养被使用诱导型启动子作为启动子的表达载体转化后的微生物时,也可以根据需要向培养基中添加诱导剂。例如,在培养被使用lac启动子的表达载体转化后的微生物时,可以向培养基中添加异丙基-β-D-硫代吡喃半乳糖苷等,在培养被使用trp启动子的表达载体转化后的微生物时,可以向培养基中添加吡啶丙烯酸等。

[0204] 表达后的改性丝心蛋白的分离及纯化能够通过通常所使用的方法来进行。例如,在该改性丝心蛋白在细胞内以溶解状态表达的情况下,培养结束后,通过离心分离回收宿主细胞,并将其悬浮于水系缓冲液之后,通过超声波破碎机、超声波破碎机、manton-gaulin匀浆器及戴诺研磨机(dyno-mill)等将宿主细胞破碎,得到无细胞提取液。通过将该无细胞提取液离心分离得到上清,单独或组合使用通常用于蛋白质的分离纯化的方法、即溶剂提取法、利用硫酸铵等的盐析法、脱盐法、利用有机溶剂的沉淀法、二乙基氨基乙基(DEAE)-琼脂糖、使用DIAION HPA-75(三菱化成公司制造)等树脂的阴离子交换层析法、使用S-Sepharose FF(Pharmacia公司制造)等树脂的阳离子交换层析法、使用丁基琼脂糖、苯基琼脂糖等树脂的疏水性层析法、使用分子筛的凝胶过滤法、亲和层析法、聚焦层析法、等电聚焦电泳等电泳法等方法,能够从上清中得到纯化标准品,。

[0205] 另外,在改性丝心蛋白在细胞内形成不溶体而表达的情况下,同样在回收宿主细胞之后,将其破碎并进行离心分离,由此作为沉淀级分回收改性丝心蛋白的不溶体。回收的改性丝心蛋白的不溶体能够被蛋白质改性剂可溶化。该操作之后,能够通过与上述相同的分离纯化法得到改性丝心蛋白的纯化标准品。在该改性丝心蛋白被分泌至细胞外的情况下,能够从培养上清中回收该改性丝心蛋白。即,能够通过利用离心分离等手法处理培养物而取得培养上清,并通过使用与上述相同的分离纯化法从该培养上清中得到纯化标准品。

[0206] (人造丝心蛋白纤维)

[0207] 本实施方式中的人造丝心蛋白纤维(以下,有时也称为“人造丝心蛋白未卷曲纤维”)含有改性丝心蛋白,且未卷曲。人造丝心蛋白未卷曲纤维优选为含有改性蛛丝丝心蛋白的人造蛛丝丝心蛋白纤维。人造丝心蛋白未卷曲纤维是将上述的改性丝心蛋白纺丝而成的,其包含上述的改性丝心蛋白作为主成分。本实施方式中的人造丝心蛋白未卷曲纤维为纺丝后且与水性介质接触前的纤维。

[0208] 本实施方式中的人造丝心蛋白未卷曲纤维能够通过公知的纺丝方法来制造。即,例如,首先,将依据上述的方法制造的改性丝心蛋白与作为溶解促进剂的无机盐共同添加于二甲基亚砷(DMSO)、N,N-二甲基甲酰胺(DMF)、或六氟异丙醇(HFIP)等溶剂中并溶解,从而制作纺丝原液。接着,使用该纺丝原液,通过湿法纺丝、干法纺丝、干湿法纺丝或熔融纺丝等公知的纺丝方法纺丝,能够得到目标人造丝心蛋白未卷曲纤维。作为优选的纺丝方法,能够列举湿法纺丝或干湿法纺丝。

[0209] 图6为简要示出用于制造人造丝心蛋白未卷曲纤维的纺丝装置的一例的说明图。图6所示的纺丝装置10为用于干湿法纺丝的纺丝装置的一例,其具有挤压装置1、未拉伸纱制造装置2、湿热拉伸装置3、及干燥装置4。

[0210] 下面,对使用纺丝装置10的纺丝方法进行说明。首先,将储存于储槽7的纺丝原液6通过齿轮泵8从管头9挤出。在实验室规模下,也可以将纺丝原液填充于料筒,并使用注射泵从喷嘴中挤出。接着,将被挤出的纺丝原液6经过气隙19供料至凝固液槽20的凝固液11内,除去溶剂,改性丝心蛋白凝固,从而形成纤维状凝固体。接着,将纤维状凝固体被供料至拉伸浴槽21内的温水12中并拉伸。拉伸倍率根据供料夹送辊13与拾取夹送辊14的速度比来确定。然后,将被拉伸后的纤维状凝固体供料至干燥装置4,在纱路22内干燥,作为卷丝体5得到人造丝心蛋白未卷曲纤维。18a~18g为导丝器。

[0211] 作为凝固液11,只要其为能够脱溶剂的溶剂即可,能够列举例如:甲醇、乙醇及2-丙醇等碳原子数1~5的低级醇、以及丙酮等。凝固液11可以适当地包含水。凝固液11的温度优选为0~30℃。在使用具有直径0.1~0.6mm的喷嘴的注射泵作为管头9的情况下,挤压速度优选每孔0.2~6.0ml/小时,更优选为1.4~4.0ml/小时。作为凝固后的蛋白质通过凝固液11中的距离(实质上为从导丝器18a至导丝器18b的距离),只要具有能够高效地脱溶剂的长度即可,例如为200~500mm。未拉伸纱的拾取速度为例如1~20m/分钟即可,优选为1~3m/分钟。凝固液11中的停留时间为例如0.01~3分钟即可,优选为0.05~0.15分钟。另外,可以在凝固液11中进行拉伸(预拉伸)。凝固液槽20可以多段设置,另外,拉伸可以根据需要在每段或特定的段进行。

[0212] 需要说明的是,作为得到人造丝心蛋白未卷曲纤维时所实施的拉伸,例如,除上述在凝固液槽20内进行预拉伸、及在拉伸浴槽21内进行湿热拉伸之外,也可以采用干热拉伸。

[0213] 湿热拉伸能够在温水中、向温水中加入有机溶剂等后的溶液中、或蒸气加热中进行。温度只要为例如50~90℃即可,优选为75~85℃。湿热拉伸中,能够将未拉伸纱(或预拉伸纱)拉伸例如1~10倍,优选拉伸2~8倍。

[0214] 干热拉伸能够使用管式电热炉、干热板等来进行。温度只要为例如140℃~270℃即可,优选160℃~230℃。干热拉伸中,能够将未拉伸纱(或预拉伸纱)拉伸例如0.5~8倍,优选拉伸1~4倍。

[0215] 湿热拉伸及干热拉伸可以分别单独进行,另外,也可以将它们分多段或组合进行。即,能够适当地组合湿热拉伸及干热拉伸后进行,例如,通过湿热拉伸进行第一段拉伸,通过干热拉伸进行第二段拉伸,或通过湿热拉伸进行第一段拉伸,通过湿热拉伸进行第二段拉伸,再通过干热拉伸进行第三步拉伸等。

[0216] 作为最终的拉伸倍率,其下限值相对于未拉伸纱(或预拉伸纱)优选为超过1倍、2倍以上、3倍以上、4倍以上、5倍以上、6倍以上、7倍以上、8倍以上、9倍以上中的任意之一,上限值优选为40倍以下、30倍以下、20倍以下、15倍以下、14倍以下、13倍以下、12倍以下、11倍以下、10倍以下。若人造丝心蛋白未卷曲纤维为以2倍以上的拉伸倍率纺丝后的纤维,则使人造丝心蛋白未卷曲纤维与水性介质接触之后制成干燥状态的时的收缩率进一步提高。

[0217] (原料纺纱)

[0218] 本实施方式中的原料纺纱包含上述人造丝心蛋白未卷曲纤维。原料纺纱可以为单纱,也可以为双股纱等混纺纱。作为原料纺纱的种类,其可以为仅由人造丝心蛋白未卷曲纤

维构成的纺纱(改性丝心蛋白100%的纺纱),也可以为人造丝心蛋白未卷曲纤维(改性丝心蛋白100%的纤维)与其它纤维的混纺纱,其中,所述其它纤维为例如选自羊毛等卷曲纤维、及丝绸或者合成纤维等非卷曲纤维构成的纤维中的至少一种。

[0219] 在原料纺纱仅由人造丝心蛋白未卷曲纤维构成的情况下,原料纺纱能够通过包括下述工序的方法而得到:将人造丝心蛋白未卷曲纤维(长丝)裁切为适当的长度,从而得到改性丝心蛋白短丝的裁切工序;及将得到的改性丝心蛋白短丝进行纺织的纺织工序。

[0220] 裁切工序能够使用能够裁切改性丝心蛋白纤维的任意的装置来进行。作为这样的装置,可列举例如台式纤维裁切机(s/N0.IT-160201-NP-300)。

[0221] 改性丝心蛋白短丝的长度不受特别限定,例如为20mm以上,可以为20~140mm,也可以为70~140mm,还可以为20~70mm。

[0222] 纺织工序能够通过公知的纺织方法来实施。作为纺织方法,可列举例如:棉纺式、梳毛式及纺毛式等方法。用于这些纺织方法的装置不受特别限定,能够使用通常使用的装置。另外,纺织工序中,也可以首先通过开毛机(Opener)或解纤机(Breaker)等将改性丝心蛋白短丝开毛或解纤。

[0223] 纺织工序能够通过例如下述方式来进行:对裁切工序中得到的改性丝心蛋白短丝的集合体进行梳理(梳理工序),制成纤维片之后,由纤维片制作纤维条,然后对纤维条加捻制得纺纱(纺毛式)、或者由纤维片制作定长纤维纱条,然后将定长纤维纱条对齐制得纺纱(梳毛式)。

[0224] 在原料纺纱除人造丝心蛋白未卷曲纤维之外还包含非卷曲纤维(丝绸等)的情况下,原料纺纱能够通过包括下述工序的方法而得到:将人造丝心蛋白未卷曲纤维(长丝)及其它的非卷曲纤维分别裁切为适当的长度,从而分别得到改性丝心蛋白短丝及其它的非卷曲纤维的短丝的裁切工序;及将得到的短丝混合并纺织的纺织工序。纺织之前,可以对其它非卷曲纤维的短丝进行机械卷曲等制成卷曲纤维,然后再进行纺织。纺织工序如上所述。

[0225] 在原料纺纱除人造丝心蛋白未卷曲纤维之外还包含卷曲纤维(羊毛等)的情况下,优选包括下述工序:将人造丝心蛋白未卷曲纤维(长丝)及卷曲纤维分别裁切为适当的长度,从而分别得到改性丝心蛋白短丝及卷曲纤维的短丝的裁切工序;及将得到的短丝混合并通过纺毛式进行纺织的工序,在该情况下,能够利用羊毛等卷曲纤维的卷曲将人造丝心蛋白未卷曲纤维与羊毛交缠。

[0226] 为了容易开解人造丝心蛋白未卷曲纤维及其它纤维,可以在纺织工序之前预先附着油剂。能够在制造工序中的任意阶段附着油剂。例如,可以在裁切工序之前、与裁切工序同时、或裁切工序后附着油剂。油剂不受特别限定,只要为用于防止静电、降低摩擦、赋予柔软性、或赋予拒水性等工序通过性或赋予功能等普通的目的而使用的公知的油剂,则均可以使用。

[0227] [工序(b)]

[0228] 工序(b)为使原料纺纱与水性介质接触,从而使上述人造丝心蛋白未卷曲纤维(以下,有时称为“人造丝心蛋白纤维”)卷曲的(以下,有时称为“水卷曲”)工序。需要说明的是,该水卷曲工序除包括使原料纺纱直接与水性介质接触之外,也包含使用原料纺纱制作以针织布为主的各种结构物及成型物等物品之后,使这些物品与水性介质接触,由此使原料纺纱卷曲。

[0229] 水性介质是指包含水(包括水蒸气。)的液体或气体(蒸气)的介质。水性介质可以为水,也可以为水和亲水性介质的混合液。另外,作为亲水性介质,也可以使用例如乙醇及甲醇等挥发性溶剂或其蒸气。水性介质只要为水和乙醇、甲醇等挥发性溶剂的混合液体即可,优选为水或水和乙醇的混合液体。通过使用包含挥发性溶剂或其蒸气的水性介质,能够提高水卷曲后的干燥速度,进而可能能够赋予最终得到的蛋白质纺纱柔和的质地。水与挥发性溶剂或其蒸气的比率不受特别限定,例如,水:挥发性溶剂或其蒸气以质量比计可以为10:90~90:10。水的比例优选为30质量%以上,可以为40质量%或50质量%以上。在水性介质为液体的情况下,优选在水性介质中分散油剂。在该情况下,能够同时进行水卷曲及油剂附着。需要说明的是,作为油剂,只要为例如用于防止静电、降低摩擦、赋予柔软性、或赋予拒水性等工序通过性或赋予功能等普通的目的而使用的公知的油剂,则均可以使用。需要说明的是,油剂的量不受特别限定,例如相对于油剂和水性介质的总量可以为1~10质量%,或者可以为2~5质量%。

[0230] 水性介质优选为包含水(包括水蒸气)的10~230℃的液体或气体。水性介质的温度只要为10℃以上、25℃以上、40℃以上、60℃以上、或100℃以上,可以为230℃以下、120℃以下或100℃以下。更具体而言,在水性介质为气体(蒸气)的情况下,水性介质的温度优选100~230℃,更优选100~120℃。若水性介质的蒸气为230℃以下,则能够防止蛋白质长丝的热变性。在水性介质为液体的情况下,从高效地赋予卷曲的观点出发,水性介质的温度优选10℃以上、25℃以上或40℃以上,从较高地保持蛋白质长丝的纤维强度的观点出发,优选60℃以下。

[0231] 与水性介质接触的时间不受特别限制,只要为30秒以上即可,可以为1分钟以上或2分钟以上,从生产性的观点出发,优选为10分钟以下。另外,在蒸气的情况下,一般认为与液体相比以较短时间得到较大收缩率。与水性介质的接触可以在常压下进行,也可以在减压下(例如,真空)进行。

[0232] 作为与水性介质接触的方法,可列举:将原料纺纱浸渍于水性介质的方法、对原料纺纱喷射水性介质的蒸气的方法、将原料纺纱暴露于充满水性介质的蒸气的环境的方法等。在水性介质为蒸气的情况下,水性介质与原料纺纱的接触能够使用普通的蒸气定型装置来进行。作为蒸气定型装置的具体例,能够列举产品名:FMSA型蒸气定型器(福伸工业株式会社制造)、产品名:EPS-400(辻井染机工业株式会社制造)等装置。作为通过水性介质的蒸气卷曲人造丝心蛋白纤维的方法的具体例,可列举:将原料纺纱收容在规定的收容室内,另一方面,向收容室内导入水性介质的蒸气,以将收容室内的温度调节为上述规定温度(例如,100℃~230℃),同时使原料纺纱与蒸气接触。

[0233] 需要说明的是,通过与水性介质接触所进行的卷曲工序优选在不对原料纺纱施加任何拉力(丝毫不沿纤维轴方向拉紧)的状态、或者施加规定大小拉力(沿纤维轴方向拉紧规定量)的状态下实施。此时,可以通过调节施加于原料纺纱的拉力来控制卷曲的程度。作为施加于原料纺纱的拉力的制备方法,可列举例如如下方法等:在原料纺纱上垂吊各种重量的砝码等,从而调节对这些原料纺纱所负载的荷重;在使原料纺纱松弛的状态将其两个末端固定,并且不断变更其的松弛量;及将原料纺纱卷绕在纸管或筒管等被卷绕体上,并且适当地变更此时的卷绕力(向纸管或筒管的收紧力)。

[0234] 卷曲工序还可以包含在使原料纺纱与水性介质接触之后进一步使其干燥。干燥方

法不受特别限定,干燥可以为自然干燥,也可以通过热风或热辊进行干燥。干燥温度不受特别限定,可以为例如20~150℃,优选为40~120℃,更优选为60~100℃。

[0235] (人造丝心蛋白纤维的收缩率)

[0236] 通过使人造丝心蛋白纤维(纺丝后且与水性介质接触前的纤维)与水性介质接触,能够使人造丝心蛋白纤维不可逆地收缩。另外,能够通过与水性介质接触后使其干燥来使之进一步收缩。

[0237] 图7为示出通过与水性介质接触而产生的人造丝心蛋白纤维的长度变化的例子的图。本实施方式中的人造丝心蛋白纤维具有通过与水性介质接触(湿润)而不可逆地收缩(图7中,由“一次收缩”所示的长度变化)的特性。一次收缩后,一旦使其干燥,则进一步收缩(图7中,由“二次收缩”所示的长度变化)。经过一次收缩或二次收缩得到的人造丝心蛋白纤维与水性介质并形成湿润状态后,伸长至与二次收缩前相同或与之近似的长度,然后反复进行干燥和湿润之后,按照与二次收缩相同程度的宽度(图7中,由“伸缩率”所示的宽度)反复进行收缩和伸长。

[0238] 一般认为,人造丝心蛋白纤维的不可逆收缩(图7中的“一次收缩”)因例如以下的理由而产生。即,一般认为,其中的一个理由起因于由于人造丝心蛋白纤维的二次结构或三次结构,另外,另一个理由因在由于例如制造工序中的拉伸等而具有残留应力的人造丝心蛋白纤维中通过使水性介质进入纤维之间或纤维内而缓和残留应力而产生。因此,一般认为,收缩工序中的人造丝心蛋白纤维的收缩率也能够根据例如上述的人造丝心蛋白纤维的制造过程中的拉伸倍率的大小而任意控制。

[0239] 本实施方式中的人造丝心蛋白纤维的由下述式子规定的干燥收缩率可以超过7%。

[0240] 干燥收缩率 = $\{1 - (\text{与水性介质接触之后制成干燥状态后的人造丝心蛋白纤维的长度} / \text{与水性介质接触前的人造丝心蛋白纤维的长度})\} \times 100 (\%)$

[0241] 本实施方式中的人造丝心蛋白纤维的干燥收缩率可以为8%以上、10%以上、15%以上、20%以上、25%以上、30%以上、35%以上、37%以上、38%以上、或39%以上。干燥收缩率的上限不受特别限定,可以为80%以下、70%以下、60%以下、50%以下、或40%以下。

[0242] 本实施方式中的人造丝心蛋白纤维的由下述式子规定的湿润收缩率可以为2%以上。

[0243] 湿润收缩率 = $\{1 - (\text{与水性介质接触之后制成干燥状态后的人造丝心蛋白纤维的长度} / \text{纺丝后且与水性介质接触前的人造丝心蛋白纤维的长度})\} \times 100 (\%)$

[0244] 本实施方式中的人造丝心蛋白纤维的湿润收缩率可以为2.5%以上、3%以上、3.5%以上、4%以上、4.5%以上、5%以上、5.5%以上、或6%以上。湿润收缩率的上限不受特别限定,可以为80%以下、60%以下、40%以下、20%以下、10%以下、7%以下、6%以下、5%以下、4%以下、或3%以下。

[0245] 在本发明的制造方法中,在梳理工序等纺丝工序之后进行卷曲,因而,可以提供一种可以充分确保纤维彼此的缠结,进而确保稳定的强度的蛋白质纺织,而不会使卷曲后的人造丝心蛋白纤维被拉伸导致卷曲减弱。

[0246] 通过本发明的制造方法得到的蛋白质纺纱通过水卷曲呈现出较为柔软的感触。另外,由于该蛋白质纺纱是与水分(水性介质)接触后的纺纱,因而,能够抑制纺纱制造后保存

期间及使用纺纱的产品(针织布等)的制造工序中因吸湿而导致的纺纱的尺寸变化(收缩)。

[0247] 通过本发明的制造方法得到的蛋白质纺纱有望用于衣服、医疗卫生用品、室内装饰用品、床上用品、饰物、包、配饰、杂货、车辆零件、与树脂等其它材料的复合品等。

[0248] [实施例]

[0249] 以下,基于实施例对本发明进行更具体的说明。但是,本发明不限定于以下的实施例。

[0250] <人造蛛丝蛋白质(人造蛛丝丝心蛋白)长丝的制造例>

[0251] (1) 质粒表达株的制作

[0252] 基于来自络新妇蛛(*Nephila clavipes*)的丝心蛋白(GenBank登录号:P46804.1, GI:1174415)的碱基序列及氨基酸序列,设计基于序列号13所示的氨基酸序列的改性丝心蛋白(以下,也称为“PRT799”)。需要说明的是,序列号13所示的氨基酸序列具有以提高生产性为目的对来自络新妇蛛的丝心蛋白的氨基酸序列实施了氨基酸残基的取代、插入及缺失而成的氨基酸序列,此外,在N端添加有序列号5所示的氨基酸序列(标签序列及铰链序列)。

[0253] 接着,合成编码PRT799的核酸。该核酸中,在5'端于NdeI位点及终止密码子下游添加有EcoRI位点。将该核酸克隆至克隆载体(pUC118)。然后,用NdeI及EcoRI对该核酸进行酶切处理将其切除后,重组至蛋白质表达载体pET-22b(+),从而得到表达载体。

[0254] (2) 蛋白质的表达

[0255] 用包含核酸的pET22b(+)表达载体转化大肠杆菌BLR(DE3),其中,该核酸编码具有序列号13所示的氨基酸序列的蛋白质。将该转化大肠杆菌用包含氨苄青霉素的2mL LB培养基培养15小时。将该培养液添加至包含氨苄青霉素的100mL种子培养用培养基(表4),使 OD_{600} 达到0.005。将培养液温度保持为30℃,进行摇瓶培养直至 OD_{600} 达到5(约15小时),得到种子培养液。

[0256] [表4]

[0257] 种子培养用培养基

[0258]	试剂	浓度(g/L)
	葡萄糖	5.0
	KH ₂ PO ₄	4.0
	K ₂ HPO ₄	9.3
	Yeast Extract	6.0
	氨苄青霉素	0.1

[0259] 将该种子培养液添加至添加有500mL生产培养基(表5)的发酵罐中,使 OD_{600} 达到0.05。将培养液温度保持为37℃,将pH稳定控制至6.9进行培养。另外,将培养液中的溶解氧浓度保持为溶解氧饱和浓度的20%。

[0260] [表5]

[0261] 生产培养基

[0262]	试剂	浓度(g/L)
	葡萄糖	12.0
	KH ₂ PO ₄	9.0
	MgSO ₄ ·7H ₂ O	2.4
	Yeast Extract	15
	FeSO ₄ ·7H ₂ O	0.04
	MnSO ₄ ·5H ₂ O	0.04
	CaCl ₂ ·2H ₂ O	0.04
	GD-113 (消泡剂)	0.1 (mL/L)

[0263] 生产培养基中的葡萄糖被完全消耗完之后,立刻以1mL/分钟的速度添加料液(葡萄糖455g/1L、Yeast Extract 120g/1L)。将培养液温度保持为37℃,将pH稳定控制至6.9进行培养。另外,将培养液中的溶解氧浓度保持为溶解氧饱和浓度的20%,培养20小时。然后,以相对于培养液使异丙基-β-硫代吡喃半乳糖苷最终浓度达到1mM的方式添加1M的异丙基-β-硫代吡喃半乳糖苷(IPTG),从而诱导改性丝心蛋白表达。在添加IPTG后经过了20小时时,离心分离培养液,回收菌体。使用由添加IPTG前和添加IPTG后的培养液所制备的菌体进行SDS-PAGE,通过依赖于添加IPTG而出现的目标改性丝心蛋白尺寸的条带,确认目标改性丝心蛋白的表达。

[0264] (3) 蛋白质的纯化

[0265] 将添加IPTG2小时后所回收的菌体用20mM Tris-HCl buffer (pH7.4) 清洗。将清洗后的菌体悬浮于包含约1mM的PMSF的20mMTris-HCl缓冲液 (pH7.4) 中,通过高压均质机(GEA Niro Soavi公司制造)破碎细胞。离心分离破碎后的细胞,得到沉淀物。将得到的沉淀物用20mMTris-HCl缓冲液 (pH7.4) 清洗至高纯度。将清洗后的沉淀物用8M胍缓冲液(8M胍盐酸盐、10mM磷酸二氢钠、20mMNaCl、1mMTris-HCl、pH7.0) 悬浮使其达到100mg/mL的浓度,在60℃下用搅拌器搅拌30分钟使其溶解。溶解后,使用透析管(三光纯药株式会社制造的纤维素管36/32) 用水进行透析。通过离心分离回收透析后得到的白色的凝聚蛋白质,用冻干机除去水分,回收冻干粉末,由此得到改性蛛丝丝心蛋白“PRT799”。

[0266] (4) 蛋白质长丝的制造

[0267] 向DMSO中添加上述的改性丝心蛋白(PRT799),使其浓度达到24质量%,然后,作为溶解促进剂添加LiCl,使其浓度达到4.0质量%。然后,使用摇床用时3小时使改性丝心蛋白溶解,得到DMSO溶液。除去得到的DMSO溶液中的垃圾及泡沫,制得纺丝原液。纺丝原液的溶液粘度在90℃下为5000cP(厘泊)。

[0268] 使用如上所述那样得到的纺丝原液和图6所示的纺丝装置10进行公知的干湿法纺丝,将人造蛛丝丝心蛋白纤维卷绕于筒管。需要说明的是,在此,干湿法纺丝在下述的条件下进行。

[0269] 凝固液(甲醇)的温度:5~10℃

[0270] 拉伸倍率:4.52倍

[0271] 干燥温度:80℃

[0272] <实施例1>

[0273] 将人造蛛丝蛋白质的制造例中得到的卷绕于筒管的人造蛛丝长丝多根捆束,并用台式纤维裁切机裁切为平均50mm的长度,从而制作人造蛛丝蛋白质短丝。将制成的人造蛛丝蛋白质短丝用公知的开毛机械开解,同时进行混合以将打乱方向性,然后,用解毛机械纾

解以形成单一纤维状(均匀的解毛状态)。接着,用于四峰纺毛纺织梳理机械,从第一峰向第二峰、从第二峰向第三峰、从第三峰向第四峰过渡时分别将丝网方向改变90度。将从第四峰取出的丝网分为7~12mm的带状,并在冷凝器橡胶状态下揉搓团固为纤维条的状态。然后,通过走锭纺纱机施加牵伸,并按照约350的捻数加Z捻,从而得到纺纱。

[0274] 将上述未卷曲纺纱在40℃的水中浸渍1分钟使其收缩,从而使上述未卷曲纺纱卷曲,然后,在40℃下干燥18小时。由此,得到具有充分的卷曲的纺纱。

[0275] 符号说明

[0276] 1…挤压装置、2…未拉伸纱制造装置、3…湿热拉伸装置、4…干燥装置、6…纺丝原液、10…纺丝装置、20…凝固液槽、21…拉伸浴槽、36…人造丝心蛋白纤维。

序列表

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丝芭博株式会社

<120>蛋白质纺纱的制造方法

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Gly	Gln	Gln	Gly	Pro	Ser	Ala	Ser	Ala	Ala	Ala	Ala	Ala	Gly	Pro	Gly	
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Ser	Gly	Gln	Gln	Gly	Pro	Gly	Ala	Ser	Gly	Gln	Tyr	Gly	Pro	Gly	Gln	
			85				90				95					
Gln	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Gly	Ser	Ser	Ala	Ala	Ala	Ala	Ala	
			100				105				110					
Gly	Gln	Tyr	Gly	Ser	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Tyr	Gly	Ser	Ala	
			115				120				125					
Ala	Ala	Ala	Ala	Gly	Pro	Gly	Ser	Gly	Gln	Tyr	Gly	Gln	Gly	Pro	Tyr	
130							135				140					
Gly	Pro	Gly	Ala	Ser	Gly	Pro	Gly	Gln	Tyr	Gly	Pro	Gly	Gln	Gln	Gly	

145	150	155	160
Pro Ser Ala Ser	Ala Ala Ala Ala Gly	Ser Gly Gln Gln Gly	Pro
	165	170	175
Gly Gln Tyr Gly	Pro Tyr Ala Ser	Ala Ala Ala Ala Gly	Gln Tyr
	180	185	190
Gly Ser Gly Pro	Gly Gln Gln Gly	Pro Tyr Gly Pro	Gly Gln Ser Gly
	195	200	205
Ser Gly Gln Gln	Gly Pro Gly Gln	Gln Gly Pro Tyr	Ala Ser Ala Ala
	210	215	220
Ala Ala Ala Gly	Pro Gly Gln Gln	Gly Pro Tyr Gly	Pro Gly Ser Ser
225	230	235	240
Ala Ala Ala Ala	Ala Gly Gln Tyr	Gly Tyr Gly Pro	Gly Gln Gln Gly
	245	250	255
Pro Tyr Gly Pro	Gly Ala Ser Gly	Gln Asn Gly Pro	Gly Ser Gly Gln
	260	265	270
Tyr Gly Pro Gly	Gln Gln Gly Pro	Gly Gln Ser Ala	Ala Ala Ala Ala
	275	280	285
Gly Pro Gly Gln	Gln Gly Pro Tyr	Gly Pro Gly Ala	Ser Ala Ala Ala
	290	295	300
Ala Ala Gly Gln	Tyr Gly Pro Gly	Gln Gln Gly Pro	Gly Gln Tyr Gly
305	310	315	320
Pro Gly Ser Ser	Gly Pro Gly Gln	Gln Gly Pro Tyr	Gly Pro Gly Ser
	325	330	335
Ser Ala Ala Ala	Ala Ala Gly Gln	Tyr Gly Pro Gly	Gln Gln Gly Pro
	340	345	350
Tyr Gly Pro Gly	Gln Ser Ala Ala	Ala Ala Gly Gln	Tyr Gln Gln
	355	360	365
Gly Pro Gly Gln	Gln Gly Pro Tyr	Gly Pro Gly Ala	Ser Gly Pro Gly
	370	375	380
Gln Gln Gly Pro	Tyr Gly Pro Gly	Ala Ser Ala Ala	Ala Ala Ala Gly
385	390	395	400
Pro Gly Gln Tyr	Gly Pro Gly Gln	Gln Gly Pro Ser	Ala Ser Ala Ala
	405	410	415
Ala Ala Ala Gly	Gln Tyr Gly Ser	Gly Pro Gly Gln	Tyr Gly Pro Tyr
	420	425	430
Gly Pro Gly Gln	Ser Gly Pro Gly	Ser Gly Gln	Gln Gly Pro
	435	440	445
Tyr Gly Pro Gly	Ala Ser Ala Ala	Ala Ala Gly Gln	Tyr Gly Pro
	450	455	460

Gly	Gln	Gln	Gly	Pro	Tyr	Gly	Pro	Gly	Gln	Ser	Ala	Ala	Ala	Ala	Ala
465					470					475					480
Gly	Pro	Gly	Ser	Gly	Gln	Tyr	Gly	Pro	Gly	Ala	Ser	Gly	Gln	Asn	Gly
			485						490					495	
Pro	Gly	Ser	Gly	Gln	Tyr	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Gly	Gln	Ser
			500					505					510		
Ala	Ala	Ala	Ala	Ala	Gly	Gln	Tyr	Gln	Gln	Gly	Pro	Gly	Gln	Gln	Gly
			515					520					525		
Pro	Tyr	Gly	Pro	Gly	Ala	Ser	Ala	Ala	Ala	Ala	Ala	Gly	Gln	Tyr	Gly
			530				535					540			
Ser	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Tyr	Gly	Pro	Gly	Gln	Ser	Gly	Ser
545					550					555					560
Gly	Gln	Gln	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Tyr	Ala	Ser	Ala	Ala	Ala
				565					570						575
Ala	Ala	Gly	Pro	Gly	Ser	Gly	Gln	Gln	Gly	Pro	Gly	Ala	Ser		
			580						585				590		

<210> 8

<211> 565

<212> PRT

<213>人工序列

<220>

<223> Met-PRT525

<400> 8

Met	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Tyr	Gly	Pro	Gly	Ala	Ser	Ala	Ala
1				5					10					15	
Ala	Ala	Ala	Ala	Ala	Gly	Ser	Asn	Gly	Pro	Gly	Ser	Gly	Gln	Gln	Gly
				20				25					30		
Pro	Gly	Gln	Ser	Gly	Gln	Tyr	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Gly	Gln
			35				40					45			
Gln	Gly	Pro	Gly	Ser	Ser	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Gly	Pro	Gly
			50			55					60				
Gln	Tyr	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Ser	Ala	Ser	Ala	Ala	Ala	Ala
65					70					75					80
Ala	Ala	Ala	Gly	Pro	Gly	Ser	Gly	Gln	Gln	Gly	Pro	Gly	Ala	Ser	Gly
				85					90					95	
Gln	Tyr	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Gly	Ser
			100						105					110	
Ser	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Gly	Ser	Tyr	Gly	Ser	Gly	Pro	Gly
			115						120						125

Gln Gln Gly Pro Tyr Gly Ser Ala Ala Ala Ala Ala Ala Gly Pro		
130	135	140
Gly Ser Gly Gln Tyr Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Gly		
145	150	155 160
Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala		
165	170	175
Ala Ala Ala Ala Ala Gly Ser Gly Gln Gln Gly Pro Gly Gln Tyr Gly		
180	185	190
Pro Tyr Ala Ser Ala Ala Ala Ala Ala Ala Gly Ser Tyr Gly Ser		
195	200	205
Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Gly Ser Gly		
210	215	220
Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr Ala Ser Ala Ala Ala Ala		
225	230	235 240
Ala Ala Ala Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ser Ser		
245	250	255
Ala Ala Ala Ala Ala Ala Ala Gly Ser Tyr Gly Tyr Gly Pro Gly Gln		
260	265	270
Gln Gly Pro Tyr Gly Pro Gly Ala Ser Gly Gln Asn Gly Pro Gly Ser		
275	280	285
Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Pro Ser Ala Ala Ala		
290	295	300
Ala Ala Ala Ala Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala		
305	310	315 320
Ser Ala Ala Ala Ala Ala Ala Ala Gly Ser Tyr Gly Pro Gly Gln Gln		
325	330	335
Gly Pro Gly Gln Tyr Gly Pro Gly Ser Ser Gly Pro Gly Gln Gln Gly		
340	345	350
Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala Ala Ala Gly Ser		
355	360	365
Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Pro Ser Ala Ala		
370	375	380
Ala Ala Ala Ala Ala Gly Ser Tyr Gln Gln Gly Pro Gly Gln Gln Gly		
385	390	395 400
Pro Tyr Gly Pro Gly Ala Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly		
405	410	415
Pro Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Pro Gly Gln Tyr		
420	425	430
Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala Ala Ala Ala		

435	440	445
Ala Gly Ser Tyr Gly Ser Gly Pro Gly Gln Tyr Gly Pro Tyr Gly Pro		
450	455	460
Gly Gln Ser Gly Pro Gly Ser Gly Gln Gln Gly Gln Gly Pro Tyr Gly		
465	470	475
Pro Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Ser Tyr Gly Pro		
485	490	495
Gly Gln Gln Gly Pro Tyr Gly Pro Gly Pro Ser Ala Ala Ala Ala Ala		
500	505	510
Ala Ala Gly Pro Gly Ser Gly Gln Tyr Gly Pro Gly Ala Ser Gly Gln		
515	520	525
Asn Gly Pro Gly Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly		
530	535	540
Pro Ser Ala Ala Ala Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln Gln		
545	550	555
Gly Pro Gly Ala Ser		
565		

<210> 9

<211> 2364

<212> PRT

<213>人工序列

<220>

<223> Met-PRT799

<400> 9

Met Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala		
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Ala Ala Ala Gly Gln Asn Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly		
20	25	30
Gln Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Gly		
35	40	45
Pro Gly Ser Ser Ala Ala Ala Ala Gly Pro Gly Gln Tyr Gly Pro		
50	55	60
Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala Ala Ala Gly Pro Gly		
65	70	75
Ser Gly Gln Gln Gly Pro Gly Ala Ser Gly Gln Tyr Gly Pro Gly Gln		
85	90	95
Gln Gly Pro Gly Gln Gln Gly Pro Gly Ser Ser Ala Ala Ala Ala Ala		
100	105	110
Gly Gln Tyr Gly Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly Ser Ala		

115	120	125
Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln Tyr Gly Gln Gly Pro Tyr		
130	135	140
Gly Pro Gly Ala Ser Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly		
145	150	155
Pro Ser Ala Ser Ala Ala Ala Ala Gly Ser Gly Gln Gln Gly Pro		
165	170	175
Gly Gln Tyr Gly Pro Tyr Ala Ser Ala Ala Ala Ala Gly Gln Tyr		
180	185	190
Gly Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Gly		
195	200	205
Ser Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr Ala Ser Ala Ala		
210	215	220
Ala Ala Ala Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ser Ser		
225	230	235
Ala Ala Ala Ala Ala Gly Gln Tyr Gly Tyr Gly Pro Gly Gln Gln Gly		
245	250	255
Pro Tyr Gly Pro Gly Ala Ser Gly Gln Asn Gly Pro Gly Ser Gly Gln		
260	265	270
Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Ser Ala Ala Ala Ala Ala		
275	280	285
Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala		
290	295	300
Ala Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Tyr Gly		
305	310	315
Pro Gly Ser Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ser		
325	330	335
Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro		
340	345	350
Tyr Gly Pro Gly Gln Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gln Gln		
355	360	365
Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser Gly Pro Gly		
370	375	380
Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala Gly		
385	390	395
Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala		
405	410	415
Ala Ala Ala Gly Gln Tyr Gly Ser Gly Pro Gly Gln Tyr Gly Pro Tyr		
420	425	430

Gly Pro Gly Gln Ser Gly Pro Gly Ser Gly Gln Gln Gly Gln Gly Pro		
435	440	445
Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Gln Tyr Gly Pro		
450	455	460
Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Ala Ala Ala Ala Ala		
465	470	475
Gly Pro Gly Ser Gly Gln Tyr Gly Pro Gly Ala Ser Gly Gln Asn Gly		
	485	490
Pro Gly Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Ser		
	500	505
Ala Ala Ala Ala Ala Gly Gln Tyr Gln Gln Gly Pro Gly Gln Gln Gly		
	515	520
Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Gln Tyr Gly		
	530	535
Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Gly Ser		
545	550	555
Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr Ala Ser Ala Ala Ala		
	565	570
Ala Ala Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Ala Ser Gly Gln		
	580	585
Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Gln		
	595	600
Asn Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Ser Gly Gln Tyr		
610	615	620
Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly Ser Ser Ala		
625	630	635
Ala Ala Ala Ala Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro		
	645	650
Ser Ala Ser Ala Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln Gln Gly		
	660	665
Pro Gly Ala Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln		
	675	680
Gln Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly Gln Tyr Gly Ser		
	695	700
Gly Pro Gly Gln Gln Gly Pro Tyr Gly Ser Ala Ala Ala Ala Gly		
705	710	715
Pro Gly Ser Gly Gln Tyr Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser		
	725	730
Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala		

740	745	750
Ala Ala Ala Ala Gly Ser Gly Gln Gln Gly Pro Gly Gln Tyr Gly Pro		
755	760	765
Tyr Ala Ser Ala Ala Ala Ala Gly Gln Tyr Gly Ser Gly Pro Gly		
770	775	780
Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Gly Ser Gly Gln Gln Gly		
785	790	795
Pro Gly Gln Gln Gly Pro Tyr Ala Ser Ala Ala Ala Ala Gly Pro		800
805	810	815
Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala Ala		
820	825	830
Gly Gln Tyr Gly Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly		
835	840	845
Ala Ser Gly Gln Asn Gly Pro Gly Ser Gly Gln Tyr Gly Pro Gly Gln		
850	855	860
Gln Gly Pro Gly Gln Ser Ala Ala Ala Ala Gly Pro Gly Gln Gln		
865	870	875
Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Gln Tyr		
885	890	895
Gly Pro Gly Gln Gln Gly Pro Gly Gln Tyr Gly Pro Gly Ser Ser Gly		
900	905	910
Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala		
915	920	925
Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln		
930	935	940
Ser Ala Ala Ala Ala Gly Gln Tyr Gln Gln Gly Pro Gly Gln Gln		
945	950	955
Gly Pro Tyr Gly Pro Gly Ala Ser Gly Pro Gly Gln Gln Gly Pro Tyr		
965	970	975
Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Pro Gly Gln Tyr Gly		
980	985	990
Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala Ala Ala Gly Gln		
995	1000	1005
Tyr Gly Ser Gly Pro Gly Gln Tyr Gly Pro Tyr Gly Pro Gly Gln		
1010	1015	1020
Ser Gly Pro Gly Ser Gly Gln Gln Gly Gln Gly Pro Tyr Gly Pro		
1025	1030	1035
Gly Ala Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Pro Gly Gln		
1040	1045	1050

Gln Gly Pro Tyr Gly Pro Gly Gln Ser Ala Ala Ala Ala Gly		
1055	1060	1065
Pro Gly Ser Gly Gln Tyr Gly Pro Gly Ala Ser Gly Gln Asn Gly		
1070	1075	1080
Pro Gly Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln		
1085	1090	1095
Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gln Gln Gly Pro Gly Gln		
1100	1105	1110
Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly		
1115	1120	1125
Gln Tyr Gly Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly		
1130	1135	1140
Gln Ser Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr		
1145	1150	1155
Ala Ser Ala Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln Gln Gly		
1160	1165	1170
Pro Gly Ala Ser Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser		
1175	1180	1185
Ala Ala Ala Ala Ala Gly Gln Asn Gly Pro Gly Ser Gly Gln Gln		
1190	1195	1200
Gly Pro Gly Gln Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro		
1205	1210	1215
Gly Gln Gln Gly Pro Gly Ser Ser Ala Ala Ala Ala Ala Gly Pro		
1220	1225	1230
Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala		
1235	1240	1245
Ala Ala Ala Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Ala Ser		
1250	1255	1260
Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro		
1265	1270	1275
Gly Ser Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Ser Gly Pro		
1280	1285	1290
Gly Gln Gln Gly Pro Tyr Gly Ser Ala Ala Ala Ala Ala Gly Pro		
1295	1300	1305
Gly Ser Gly Gln Tyr Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser		
1310	1315	1320
Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser		
1325	1330	1335
Ala Ala Ala Ala Ala Gly Ser Gly Gln Gln Gly Pro Gly Gln Tyr		

1340	1345	1350
Gly Pro Tyr Ala Ser Ala	Ala Ala Ala Ala Gly	Gln Tyr Gly Ser
1355	1360	1365
Gly Pro Gly Gln Gln Gly	Pro Tyr Gly Pro Gly	Gln Ser Gly Ser
1370	1375	1380
Gly Gln Gln Gly Pro Gly	Gln Gln Gly Pro Tyr	Ala Ser Ala Ala
1385	1390	1395
Ala Ala Ala Gly Pro Gly	Gln Gln Gly Pro Tyr	Gly Pro Gly Ser
1400	1405	1410
Ser Ala Ala Ala Ala Ala	Gly Gln Tyr Gly Tyr	Gly Pro Gly Gln
1415	1420	1425
Gln Gly Pro Tyr Gly Pro	Gly Ala Ser Gly Gln	Asn Gly Pro Gly
1430	1435	1440
Ser Gly Gln Tyr Gly Pro	Gly Gln Gln Gly Pro	Gly Gln Ser Ala
1445	1450	1455
Ala Ala Ala Ala Gly Pro	Gly Gln Gln Gly Pro	Tyr Gly Pro Gly
1460	1465	1470
Ala Ser Ala Ala Ala Ala	Ala Gly Gln Tyr Gly	Pro Gly Gln Gln
1475	1480	1485
Gly Pro Gly Gln Tyr Gly	Pro Gly Ser Ser Gly	Pro Gly Gln Gln
1490	1495	1500
Gly Pro Tyr Gly Pro Gly	Ser Ser Ala Ala Ala	Ala Ala Gly Gln
1505	1510	1515
Tyr Gly Pro Gly Gln Gln	Gly Pro Tyr Gly Pro	Gly Gln Ser Ala
1520	1525	1530
Ala Ala Ala Ala Gly Gln	Tyr Gln Gln Gly Pro	Gly Gln Gln Gly
1535	1540	1545
Pro Tyr Gly Pro Gly Ala	Ser Gly Pro Gly Gln	Gln Gly Pro Tyr
1550	1555	1560
Gly Pro Gly Ala Ser Ala	Ala Ala Ala Ala Gly	Pro Gly Gln Tyr
1565	1570	1575
Gly Pro Gly Gln Gln Gly	Pro Ser Ala Ser Ala	Ala Ala Ala Ala
1580	1585	1590
Gly Gln Tyr Gly Ser Gly	Pro Gly Gln Tyr Gly	Pro Tyr Gly Pro
1595	1600	1605
Gly Gln Ser Gly Pro Gly	Ser Gly Gln Gln Gly	Gln Gly Pro Tyr
1610	1615	1620
Gly Pro Gly Ala Ser Ala	Ala Ala Ala Ala Gly	Gln Tyr Gly Pro
1625	1630	1635

Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Ala Ala Ala Ala		
1640	1645	1650
Ala Gly Pro Gly Ser Gly Gln Tyr Gly Pro Gly Ala Ser Gly Gln		
1655	1660	1665
Asn Gly Pro Gly Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro		
1670	1675	1680
Gly Gln Ser Ala Ala Ala Ala Gly Gln Tyr Gln Gln Gly Pro		
1685	1690	1695
Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala		
1700	1705	1710
Ala Gly Gln Tyr Gly Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly		
1715	1720	1725
Pro Gly Gln Ser Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln Gly		
1730	1735	1740
Pro Tyr Ala Ser Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln		
1745	1750	1755
Gln Gly Pro Gly Ala Ser Gly Gln Gln Gly Pro Tyr Gly Pro Gly		
1760	1765	1770
Ala Ser Ala Ala Ala Ala Ala Gly Gln Asn Gly Pro Gly Ser Gly		
1775	1780	1785
Gln Gln Gly Pro Gly Gln Ser Gly Gln Tyr Gly Pro Gly Gln Gln		
1790	1795	1800
Gly Pro Gly Gln Gln Gly Pro Gly Ser Ser Ala Ala Ala Ala Ala		
1805	1810	1815
Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser		
1820	1825	1830
Ala Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly		
1835	1840	1845
Ala Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln		
1850	1855	1860
Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly Gln Tyr Gly Ser		
1865	1870	1875
Gly Pro Gly Gln Gln Gly Pro Tyr Gly Ser Ala Ala Ala Ala Ala		
1880	1885	1890
Gly Pro Gly Ser Gly Gln Tyr Gly Gln Gly Pro Tyr Gly Pro Gly		
1895	1900	1905
Ala Ser Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser		
1910	1915	1920
Ala Ser Ala Ala Ala Ala Ala Gly Ser Gly Gln Gln Gly Pro Gly		

1925	1930	1935
Gln Tyr Gly Pro Tyr Ala	Ser Ala Ala Ala Ala	Ala Gly Gln Tyr
1940	1945	1950
Gly Ser Gly Pro Gly Gln	Gln Gly Pro Tyr Gly	Pro Gly Gln Ser
1955	1960	1965
Gly Ser Gly Gln Gln Gly	Pro Gly Gln Gln Gly	Pro Tyr Ala Ser
1970	1975	1980
Ala Ala Ala Ala Ala Gly	Pro Gly Gln Gln Gly	Pro Tyr Gly Pro
1985	1990	1995
Gly Ser Ser Ala Ala Ala	Ala Ala Gly Gln Tyr	Gly Tyr Gly Pro
2000	2005	2010
Gly Gln Gln Gly Pro Tyr	Gly Pro Gly Ala Ser	Gly Gln Asn Gly
2015	2020	2025
Pro Gly Ser Gly Gln Tyr	Gly Pro Gly Gln Gln	Gly Pro Gly Gln
2030	2035	2040
Ser Ala Ala Ala Ala Ala	Gly Pro Gly Gln Gln	Gly Pro Tyr Gly
2045	2050	2055
Pro Gly Ala Ser Ala Ala	Ala Ala Ala Gly Gln	Tyr Gly Pro Gly
2060	2065	2070
Gln Gln Gly Pro Gly Gln	Tyr Gly Pro Gly Ser	Ser Gly Pro Gly
2075	2080	2085
Gln Gln Gly Pro Tyr Gly	Pro Gly Ser Ser Ala	Ala Ala Ala Ala
2090	2095	2100
Gly Gln Tyr Gly Pro Gly	Gln Gln Gly Pro Tyr	Gly Pro Gly Gln
2105	2110	2115
Ser Ala Ala Ala Ala Ala	Gly Gln Tyr Gln Gln	Gly Pro Gly Gln
2120	2125	2130
Gln Gly Pro Tyr Gly Pro	Gly Ala Ser Gly Pro	Gly Gln Gln Gly
2135	2140	2145
Pro Tyr Gly Pro Gly Ala	Ser Ala Ala Ala Ala	Ala Gly Pro Gly
2150	2155	2160
Gln Tyr Gly Pro Gly Gln	Gln Gly Pro Ser Ala	Ser Ala Ala Ala
2165	2170	2175
Ala Ala Gly Gln Tyr Gly	Ser Gly Pro Gly Gln	Tyr Gly Pro Tyr
2180	2185	2190
Gly Pro Gly Gln Ser Gly	Pro Gly Ser Gly Gln	Gln Gly Gln Gly
2195	2200	2205
Pro Tyr Gly Pro Gly Ala	Ser Ala Ala Ala Ala	Ala Gly Gln Tyr
2210	2215	2220

Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Ala Ala		
2225	2230	2235
Ala Ala Ala Gly Pro Gly Ser Gly Gln Tyr Gly Pro Gly Ala Ser		
2240	2245	2250
Gly Gln Asn Gly Pro Gly Ser Gly Gln Tyr Gly Pro Gly Gln Gln		
2255	2260	2265
Gly Pro Gly Gln Ser Ala Ala Ala Ala Gly Gln Tyr Gln Gln		
2270	2275	2280
Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala		
2285	2290	2295
Ala Ala Ala Gly Gln Tyr Gly Ser Gly Pro Gly Gln Gln Gly Pro		
2300	2305	2310
Tyr Gly Pro Gly Gln Ser Gly Ser Gly Gln Gln Gly Pro Gly Gln		
2315	2320	2325
Gln Gly Pro Tyr Ala Ser Ala Ala Ala Ala Gly Pro Gly Ser		
2330	2335	2340
Gly Gln Gln Gly Ser Ser Val Asp Lys Leu Ala Ala Ala Leu Glu		
2345	2350	2355
His His His His His His		
2360		

<210> 10

<211> 597

<212> PRT

<213>人工序列

<220>

<223> Met-PRT313

<400> 10

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Ala Ala Ala Gly Gly Asn Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly		
20	25	30
Gly Ser Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Gly Gln Gly		
35	40	45
Pro Gly Gln Gln Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly Pro		
50	55	60
Gly Gly Tyr Gly Pro Gly Gly Gln Gly Pro Ser Ala Ser Ala Ala Ala		
65	70	75
Ala Ala Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Ala Ser Ala Ala		
85	90	95

Ala Ala Ala Gly Gly Tyr Gly Pro Gly Gly Gln Gly Pro Gly Gln Gln		
100	105	110
Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly Gly Tyr Gly Ser Gly		
115	120	125
Pro Gly Gln Gln Gly Pro Tyr Gly Ser Ala Ala Ala Ala Gly Pro		
130	135	140
Gly Ser Gly Gly Tyr Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala		
145	150	155
Ala Ala Ala Ala Gly Pro Gly Gly Tyr Gly Pro Gly Gly Gln Gly Pro		
165	170	175
Ser Ala Ser Ala Ala Ala Ala Ala Gly Ser Gly Gln Gln Gly Pro Gly		
180	185	190
Gly Tyr Gly Pro Tyr Ala Ser Ala Ala Ala Ala Gly Gly Tyr Gly		
195	200	205
Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gly Ser Ala Ala		
210	215	220
Ala Ala Ala Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr		
225	230	235
Ala Ser Ala Ala Ala Ala Ala Gly Pro Gly Gly Gln Gly Pro Tyr Gly		
245	250	255
Pro Gly Ser Ser Ala Ala Ala Ala Gly Gly Tyr Gly Tyr Gly Pro		
260	265	270
Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala		
275	280	285
Gly Gly Asn Gly Pro Gly Ser Gly Gly Tyr Gly Pro Gly Gln Gln Gly		
290	295	300
Pro Gly Gly Ser Ala Ala Ala Ala Gly Pro Gly Gly Gln Gly Pro		
305	310	315
Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Gly Tyr Gly Pro		
325	330	335
Gly Gly Gln Gly Pro Gly Gly Tyr Gly Pro Gly Ser Ser Ala Ala Ala		
340	345	350
Ala Ala Gly Pro Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ser Ser Ala		
355	360	365
Ala Ala Ala Ala Gly Gly Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly		
370	375	380
Pro Gly Gly Ser Ala Ala Ala Ala Gly Gly Tyr Gln Gln Gly Pro		
385	390	395
Gly Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala		

405	410	415
Gly Pro Gly Gly Gln Gly Pro Tyr	Gly Pro Gly Ala Ser Ala Ala Ala	
420	425	430
Ala Ala Gly Pro Gly Gly Tyr Gly Pro Gly Gly Gln Gly Pro Ser Ala		
435	440	445
Ser Ala Ala Ala Ala Ala Gly Gly Tyr Gly Ser Gly Pro Gly Gly Tyr		
450	455	460
Gly Pro Tyr Gly Pro Gly Gly Ser Ala Ala Ala Ala Gly Pro Gly		
465	470	475
Ser Gly Gln Gln Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala		
485	490	495
Ala Ala Ala Gly Gly Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro		
500	505	510
Gly Gly Ser Ala Ala Ala Ala Ala Gly Pro Gly Ser Gly Gly Tyr Gly		
515	520	525
Pro Gly Ala Ser Ala Ala Ala Ala Ala Gly Gly Asn Gly Pro Gly Ser		
530	535	540
Gly Gly Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gly Ser Ala Ala Ala		
545	550	555
Ala Ala Gly Gly Tyr Gln Gln Gly Pro Gly Gly Gln Gly Pro Tyr Gly		
565	570	575
Pro Gly Ala Ser Ala Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln Gln		
580	585	590
Gly Pro Gly Ala Ser		
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<211> 608

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<213>人工序列

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<223> PRT380

<400> 12

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Met His His His His His His Ser Ser Gly Ser Ser Gly Pro Gly Gln
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          20           25           30
Asn Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Ser Ala Ala Ala
          35           40           45
Ala Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Gly
          50           55           60
Pro Gly Ser Ser Ala Ala Ala Ala Gly Pro Gly Gln Tyr Gly Pro
65           70           75           80
Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala Ala Ala Gly Pro Gly
          85           90           95
Ser Gly Gln Gln Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Gln
          100          105          110
Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly Ser Ser
          115          120          125
Ala Ala Ala Ala Ala Gly Gln Tyr Gly Ser Gly Pro Gly Gln Gln Gly
          130          135          140
Pro Tyr Gly Ser Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln Tyr
145          150          155          160
Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly
          165          170          175
Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala
          180          185          190
Ala Ala Ala Gly Ser Gly Gln Gln Gly Pro Gly Gln Tyr Gly Pro Tyr
          195          200          205
Ala Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Ser Gly Pro Gly Gln
          210          215          220
Gln Gly Pro Tyr Gly Pro Gly Gln Ser Ala Ala Ala Ala Gly Ser
225          230          235          240
Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr Ala Ser Ala Ala Ala
          245          250          255
Ala Ala Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ser Ser Ala
          260          265          270
Ala Ala Ala Ala Gly Gln Tyr Gly Tyr Gly Pro Gly Gln Gln Gly Pro
          275          280          285
Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Gln Asn Gly Pro

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290	295	300
Gly Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Ser Ala		
305	310	315
Ala Ala Ala Ala Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala		320
325	330	335
Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro		
340	345	350
Gly Gln Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly Pro Gly		
355	360	365
Gln Gln Gly Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly		
370	375	380
Gln Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Ala		
385	390	395
Ala Ala Ala Ala Gly Gln Tyr Gln Gln Gly Pro Gly Gln Gln Gly Pro		400
405	410	415
Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Pro Gly Gln Gln		
420	425	430
Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Pro Gly		
435	440	445
Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala Ala Ala		
450	455	460
Ala Gly Gln Tyr Gly Ser Gly Pro Gly Gln Tyr Gly Pro Tyr Gly Pro		
465	470	475
Gly Gln Ser Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln Gln Gly		
485	490	495
Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Gln		
500	505	510
Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Ala Ala		
515	520	525
Ala Ala Ala Gly Pro Gly Ser Gly Gln Tyr Gly Pro Gly Ala Ser Ala		
530	535	540
Ala Ala Ala Ala Gly Gln Asn Gly Pro Gly Ser Gly Gln Tyr Gly Pro		
545	550	555
Gly Gln Gln Gly Pro Gly Gln Ser Ala Ala Ala Ala Gly Gln Tyr		
565	570	575
Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala		
580	585	590
Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Ala Ser		
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Asn Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Ser Gly Gln Tyr
          35          40          45
Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly Ser Ser Ala
          50          55          60
Ala Ala Ala Ala Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro
65          70          75          80
Ser Ala Ser Ala Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln Gln Gly
          85          90          95
Pro Gly Ala Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln
          100          105          110
Gln Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly Gln Tyr Gly Ser
          115          120          125
Gly Pro Gly Gln Gln Gly Pro Tyr Gly Ser Ala Ala Ala Ala Ala Gly
          130          135          140
Pro Gly Ser Gly Gln Tyr Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser
145          150          155          160
Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala
          165          170          175
Ala Ala Ala Ala Gly Ser Gly Gln Gln Gly Pro Gly Gln Tyr Gly Pro
          180          185          190
Tyr Ala Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Ser Gly Pro Gly
          195          200          205
Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Gly Ser Gly Gln Gln Gly
          210          215          220
Pro Gly Gln Gln Gly Pro Tyr Ala Ser Ala Ala Ala Ala Ala Gly Pro
225          230          235          240
Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala Ala
          245          250          255

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Gly Gln Tyr Gly Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly			
260	265	270	
Ala Ser Gly Gln Asn Gly Pro Gly Ser Gly Gln Tyr Gly Pro Gly Gln			
275	280	285	
Gln Gly Pro Gly Gln Ser Ala Ala Ala Ala Gly Pro Gly Gln Gln			
290	295	300	
Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Gln Tyr			
305	310	315	320
Gly Pro Gly Gln Gln Gly Pro Gly Gln Tyr Gly Pro Gly Ser Ser Gly			
325	330	335	
Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala			
340	345	350	
Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln			
355	360	365	
Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gln Gln Gly Pro Gly Gln Gln			
370	375	380	
Gly Pro Tyr Gly Pro Gly Ala Ser Gly Pro Gly Gln Gln Gly Pro Tyr			
385	390	395	400
Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Pro Gly Gln Tyr Gly			
405	410	415	
Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala Ala Ala Gly Gln			
420	425	430	
Tyr Gly Ser Gly Pro Gly Gln Tyr Gly Pro Tyr Gly Pro Gly Gln Ser			
435	440	445	
Gly Pro Gly Ser Gly Gln Gln Gly Gln Gly Pro Tyr Gly Pro Gly Ala			
450	455	460	
Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro			
465	470	475	480
Tyr Gly Pro Gly Gln Ser Ala Ala Ala Ala Gly Pro Gly Ser Gly			
485	490	495	
Gln Tyr Gly Pro Gly Ala Ser Gly Gln Asn Gly Pro Gly Ser Gly Gln			
500	505	510	
Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Ser Ala Ala Ala Ala Ala			
515	520	525	
Gly Gln Tyr Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly			
530	535	540	
Ala Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Ser Gly Pro Gly Gln			
545	550	555	560
Gln Gly Pro Tyr Gly Pro Gly Gln Ser Gly Ser Gly Gln Gln Gly Pro			

				565				570				575			
Gly	Gln	Gln	Gly	Pro	Tyr	Ala	Ser	Ala	Ala	Ala	Ala	Ala	Gly	Pro	Gly
				580				585				590			
Ser	Gly	Gln	Gln	Gly	Pro	Gly	Ala	Ser							
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Gln	Gly	Pro	Tyr	Gly	Pro	Gly	Ala	Ser	Ala	Ala	Ala	Ala	Ala	Ala	Ala
				20				25				30			
Gly	Ser	Asn	Gly	Pro	Gly	Ser	Gly	Gln	Gln	Gly	Pro	Gly	Gln	Ser	Gly
				35				40				45			
Gln	Tyr	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Gly	Ser
50								55				60			
Ser	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Gly	Pro	Gly	Gln	Tyr	Gly	Pro	Gly
65				70				75				80			
Gln	Gln	Gly	Pro	Ser	Ala	Ser	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Gly	Pro
				85				90				95			
Gly	Ser	Gly	Gln	Gln	Gly	Pro	Gly	Ala	Ser	Gly	Gln	Tyr	Gly	Pro	Gly
				100				105				110			
Gln	Gln	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Gly	Ser	Ser	Ala	Ala	Ala	Ala
				115				120				125			
Ala	Ala	Ala	Gly	Ser	Tyr	Gly	Ser	Gly	Pro	Gly	Gln	Gln	Gly	Pro	Tyr
130								135				140			
Gly	Ser	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Gly	Pro	Gly	Ser	Gly	Gln	Tyr
145				150				155				160			
Gly	Gln	Gly	Pro	Tyr	Gly	Pro	Gly	Ala	Ser	Gly	Pro	Gly	Gln	Tyr	Gly
				165				170				175			
Pro	Gly	Gln	Gln	Gly	Pro	Ser	Ala	Ser	Ala	Ala	Ala	Ala	Ala	Ala	Ala
				180				185				190			
Gly	Ser	Gly	Gln	Gln	Gly	Pro	Gly	Gln	Tyr	Gly	Pro	Tyr	Ala	Ser	Ala
				195				200				205			
Ala	Ala	Ala	Ala	Ala	Ala	Gly	Ser	Tyr	Gly	Ser	Gly	Pro	Gly	Gln	Gln

210	215	220
Gly Pro Tyr Gly Pro Gly Gln Ser Gly Ser Gly Gln Gln Gly Pro Gly		
225	230	235
Gln Gln Gly Pro Tyr Ala Ser Ala Ala Ala Ala Ala Ala Ala Gly Pro		240
	245	250
Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala Ala		255
	260	265
Ala Ala Gly Ser Tyr Gly Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly		270
	275	280
Pro Gly Ala Ser Gly Gln Asn Gly Pro Gly Ser Gly Gln Tyr Gly Pro		285
290	295	300
Gly Gln Gln Gly Pro Gly Pro Ser Ala Ala Ala Ala Ala Ala Ala Gly		
305	310	315
Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala		320
	325	330
Ala Ala Ala Gly Ser Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Tyr		335
	340	345
Gly Pro Gly Ser Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly		350
	355	360
Ser Ser Ala Ala Ala Ala Ala Ala Ala Gly Ser Tyr Gly Pro Gly Gln		365
370	375	380
Gln Gly Pro Tyr Gly Pro Gly Pro Ser Ala Ala Ala Ala Ala Ala Ala		
385	390	395
Gly Ser Tyr Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly		400
	405	410
Ala Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala		415
	420	425
Ala Ala Ala Ala Ala Ala Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln		430
	435	440
Gly Pro Ser Ala Ser Ala Ala Ala Ala Ala Ala Gly Ser Tyr Gly		445
	450	455
Ser Gly Pro Gly Gln Tyr Gly Pro Tyr Gly Pro Gly Gln Ser Gly Pro		460
465	470	475
Gly Ser Gly Gln Gln Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala		480
	485	490
Ala Ala Ala Ala Ala Ala Gly Ser Tyr Gly Pro Gly Gln Gln Gly Pro		495
	500	505
Tyr Gly Pro Gly Pro Ser Ala Ala Ala Ala Ala Ala Ala Gly Pro Gly		510
	515	520
		525

Ser Gly Gln Tyr Gly Pro Gly Ala Ser Gly Gln Asn Gly Pro Gly Ser
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 Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala Gly Gln
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 Asn Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Gln Ser Gly Gln Tyr
 35 40 45
 Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly Ser Ser Ala
 50 55 60
 Ala Ala Ala Ala Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro
 65 70 75 80
 Ser Ala Ser Ala Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln Gln Gly
 85 90 95
 Pro Gly Ala Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln
 100 105 110
 Gln Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly Gln Tyr Gly Ser
 115 120 125
 Gly Pro Gly Gln Gln Gly Pro Tyr Gly Ser Ala Ala Ala Ala Ala Gly
 130 135 140
 Pro Gly Ser Gly Gln Tyr Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser
 145 150 155 160
 Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala
 165 170 175
 Ala Ala Ala Ala Gly Ser Gly Gln Gln Gly Pro Gly Gln Tyr Gly Pro
 180 185 190
 Tyr Ala Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Ser Gly Pro Gly
 195 200 205

Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Gly Ser Gly Gln Gln Gly			
210	215	220	
Pro Gly Gln Gln Gly Pro Tyr Ala Ser Ala Ala Ala Ala Gly Pro			
225	230	235	240
Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala			
245	250	255	
Gly Gln Tyr Gly Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly			
260	265	270	
Ala Ser Gly Gln Asn Gly Pro Gly Ser Gly Gln Tyr Gly Pro Gly Gln			
275	280	285	
Gln Gly Pro Gly Gln Ser Ala Ala Ala Ala Gly Pro Gly Gln Gln			
290	295	300	
Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Gln Tyr			
305	310	315	320
Gly Pro Gly Gln Gln Gly Pro Gly Gln Tyr Gly Pro Gly Ser Ser Gly			
325	330	335	
Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala			
340	345	350	
Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln			
355	360	365	
Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gln Gln Gly Pro Gly Gln Gln			
370	375	380	
Gly Pro Tyr Gly Pro Gly Ala Ser Gly Pro Gly Gln Gln Gly Pro Tyr			
385	390	395	400
Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Pro Gly Gln Tyr Gly			
405	410	415	
Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala Ala Ala Gly Gln			
420	425	430	
Tyr Gly Ser Gly Pro Gly Gln Tyr Gly Pro Tyr Gly Pro Gly Gln Ser			
435	440	445	
Gly Pro Gly Ser Gly Gln Gln Gly Gln Gly Pro Tyr Gly Pro Gly Ala			
450	455	460	
Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro			
465	470	475	480
Tyr Gly Pro Gly Gln Ser Ala Ala Ala Ala Gly Pro Gly Ser Gly			
485	490	495	
Gln Tyr Gly Pro Gly Ala Ser Gly Gln Asn Gly Pro Gly Ser Gly Gln			
500	505	510	
Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Ser Ala Ala Ala Ala Ala			

515	520	525
Gly Gln Tyr Gln Gln Gly Pro	Gly Gln Gln Gly Pro Tyr Gly Pro Gly	
530	535	540
Ala Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Ser Gly Pro Gly Gln		
545	550	555
Gln Gly Pro Tyr Gly Pro Gly Gln Ser Gly Ser Gly Gln Gln Gly Pro		
565	570	575
Gly Gln Gln Gly Pro Tyr Ala Ser Ala Ala Ala Ala Ala Gly Pro Gly		
580	585	590
Ser Gly Gln Gln Gly Pro Gly Ala Ser Gly Gln Gln Gly Pro Tyr Gly		
595	600	605
Pro Gly Ala Ser Ala Ala Ala Ala Ala Gly Gln Asn Gly Pro Gly Ser		
610	615	620
Gly Gln Gln Gly Pro Gly Gln Ser Gly Gln Tyr Gly Pro Gly Gln Gln		
625	630	635
Gly Pro Gly Gln Gln Gly Pro Gly Ser Ser Ala Ala Ala Ala Ala Gly		
645	650	655
Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala		
660	665	670
Ala Ala Ala Gly Pro Gly Ser Gly Gln Gln Gly Pro Gly Ala Ser Gly		
675	680	685
Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Gln Gly Pro Gly Ser		
690	695	700
Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Ser Gly Pro Gly Gln Gln		
705	710	715
Gly Pro Tyr Gly Ser Ala Ala Ala Ala Ala Gly Pro Gly Ser Gly Gln		
725	730	735
Tyr Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Gly Pro Gly Gln Tyr		
740	745	750
Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala Ala Ala Ala Gly		
755	760	765
Ser Gly Gln Gln Gly Pro Gly Gln Tyr Gly Pro Tyr Ala Ser Ala Ala		
770	775	780
Ala Ala Ala Gly Gln Tyr Gly Ser Gly Pro Gly Gln Gln Gly Pro Tyr		
785	790	795
Gly Pro Gly Gln Ser Gly Ser Gly Gln Gln Gly Pro Gly Gln Gln Gly		
805	810	815
Pro Tyr Ala Ser Ala Ala Ala Ala Ala Gly Pro Gly Gln Gln Gly Pro		
820	825	830

Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly Gln Tyr Gly Tyr		
835	840	845
Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser Gly Gln Asn		
850	855	860
Gly Pro Gly Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln		
865	870	875
Ser Ala Ala Ala Ala Ala Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro		
885	890	895
Gly Ala Ser Ala Ala Ala Ala Gly Gln Tyr Gly Pro Gly Gln Gln		
900	905	910
Gly Pro Gly Gln Tyr Gly Pro Gly Ser Ser Gly Pro Gly Gln Gln Gly		
915	920	925
Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly Gln Tyr Gly		
930	935	940
Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Ala Ala Ala Ala		
945	950	955
Ala Gly Gln Tyr Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro		
965	970	975
Gly Ala Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser		
980	985	990
Ala Ala Ala Ala Ala Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly		
995	1000	1005
Pro Ser Ala Ser Ala Ala Ala Ala Gly Gln Tyr Gly Ser Gly		
1010	1015	1020
Pro Gly Gln Tyr Gly Pro Tyr Gly Pro Gly Gln Ser Gly Pro Gly		
1025	1030	1035
Ser Gly Gln Gln Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala		
1040	1045	1050
Ala Ala Ala Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Tyr		
1055	1060	1065
Gly Pro Gly Gln Ser Ala Ala Ala Ala Gly Pro Gly Ser Gly		
1070	1075	1080
Gln Tyr Gly Pro Gly Ala Ser Gly Gln Asn Gly Pro Gly Ser Gly		
1085	1090	1095
Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Ser Ala Ala Ala		
1100	1105	1110
Ala Ala Gly Gln Tyr Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr		
1115	1120	1125
Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Gln Tyr Gly Ser		

1130	1135	1140
Gly Pro Gly Gln Gln Gly	Pro Tyr Gly Pro Gly	Gln Ser Gly Ser
1145	1150	1155
Gly Gln Gln Gly Pro Gly	Gln Gln Gly Pro Tyr	Ala Ser Ala Ala
1160	1165	1170
Ala Ala Ala Gly Pro Gly	Ser Gly Gln Gln Gly	Pro Gly Ala Ser
1175	1180	1185
Gly Gln Gln Gly Pro Tyr	Gly Pro Gly Ala Ser	Ala Ala Ala Ala
1190	1195	1200
Ala Gly Gln Asn Gly Pro	Gly Ser Gly Gln Gln	Gly Pro Gly Gln
1205	1210	1215
Ser Gly Gln Tyr Gly Pro	Gly Gln Gln Gly Pro	Gly Gln Gln Gly
1220	1225	1230
Pro Gly Ser Ser Ala Ala	Ala Ala Ala Gly Pro	Gly Gln Tyr Gly
1235	1240	1245
Pro Gly Gln Gln Gly Pro	Ser Ala Ser Ala Ala	Ala Ala Ala Gly
1250	1255	1260
Pro Gly Ser Gly Gln Gln	Gly Pro Gly Ala Ser	Gly Gln Tyr Gly
1265	1270	1275
Pro Gly Gln Gln Gly Pro	Gly Gln Gln Gly Pro	Gly Ser Ser Ala
1280	1285	1290
Ala Ala Ala Ala Gly Gln	Tyr Gly Ser Gly Pro	Gly Gln Gln Gly
1295	1300	1305
Pro Tyr Gly Ser Ala Ala	Ala Ala Ala Gly Pro	Gly Ser Gly Gln
1310	1315	1320
Tyr Gly Gln Gly Pro Tyr	Gly Pro Gly Ala Ser	Gly Pro Gly Gln
1325	1330	1335
Tyr Gly Pro Gly Gln Gln	Gly Pro Ser Ala Ser	Ala Ala Ala Ala
1340	1345	1350
Ala Gly Ser Gly Gln Gln	Gly Pro Gly Gln Tyr	Gly Pro Tyr Ala
1355	1360	1365
Ser Ala Ala Ala Ala Ala	Gly Gln Tyr Gly Ser	Gly Pro Gly Gln
1370	1375	1380
Gln Gly Pro Tyr Gly Pro	Gly Gln Ser Gly Ser	Gly Gln Gln Gly
1385	1390	1395
Pro Gly Gln Gln Gly Pro	Tyr Ala Ser Ala Ala	Ala Ala Ala Gly
1400	1405	1410
Pro Gly Gln Gln Gly Pro	Tyr Gly Pro Gly Ser	Ser Ala Ala Ala
1415	1420	1425

Ala Ala Gly Gln Tyr Gly Tyr Gly Pro Gly Gln Gln Gly Pro Tyr		
1430	1435	1440
Gly Pro Gly Ala Ser Gly Gln Asn Gly Pro Gly Ser Gly Gln Tyr		
1445	1450	1455
Gly Pro Gly Gln Gln Gly Pro Gly Gln Ser Ala Ala Ala Ala Ala		
1460	1465	1470
Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala		
1475	1480	1485
Ala Ala Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln		
1490	1495	1500
Tyr Gly Pro Gly Ser Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly		
1505	1510	1515
Pro Gly Ser Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Pro Gly		
1520	1525	1530
Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Ala Ala Ala Ala Ala		
1535	1540	1545
Gly Gln Tyr Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro		
1550	1555	1560
Gly Ala Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala		
1565	1570	1575
Ser Ala Ala Ala Ala Ala Gly Pro Gly Gln Tyr Gly Pro Gly Gln		
1580	1585	1590
Gln Gly Pro Ser Ala Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly		
1595	1600	1605
Ser Gly Pro Gly Gln Tyr Gly Pro Tyr Gly Pro Gly Gln Ser Gly		
1610	1615	1620
Pro Gly Ser Gly Gln Gln Gly Gln Gly Pro Tyr Gly Pro Gly Ala		
1625	1630	1635
Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly		
1640	1645	1650
Pro Tyr Gly Pro Gly Gln Ser Ala Ala Ala Ala Ala Gly Pro Gly		
1655	1660	1665
Ser Gly Gln Tyr Gly Pro Gly Ala Ser Gly Gln Asn Gly Pro Gly		
1670	1675	1680
Ser Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Ser Ala		
1685	1690	1695
Ala Ala Ala Ala Gly Gln Tyr Gln Gln Gly Pro Gly Gln Gln Gly		
1700	1705	1710
Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala Gly Gln Tyr		

1715	1720	1725
Gly Ser Gly Pro Gly Gln	Gln Gly Pro Tyr Gly	Pro Gly Gln Ser
1730	1735	1740
Gly Ser Gly Gln Gln Gly	Pro Gly Gln Gln Gly	Pro Tyr Ala Ser
1745	1750	1755
Ala Ala Ala Ala Ala Gly	Pro Gly Ser Gly Gln	Gln Gly Pro Gly
1760	1765	1770
Ala Ser Gly Gln Gln Gly	Pro Tyr Gly Pro Gly	Ala Ser Ala Ala
1775	1780	1785
Ala Ala Ala Gly Gln Asn	Gly Pro Gly Ser Gly	Gln Gln Gly Pro
1790	1795	1800
Gly Gln Ser Gly Gln Tyr	Gly Pro Gly Gln Gln	Gly Pro Gly Gln
1805	1810	1815
Gln Gly Pro Gly Ser Ser	Ala Ala Ala Ala Ala	Gly Pro Gly Gln
1820	1825	1830
Tyr Gly Pro Gly Gln Gln	Gly Pro Ser Ala Ser	Ala Ala Ala Ala
1835	1840	1845
Ala Gly Pro Gly Ser Gly	Gln Gln Gly Pro Gly	Ala Ser Gly Gln
1850	1855	1860
Tyr Gly Pro Gly Gln Gln	Gly Pro Gly Gln Gln	Gly Pro Gly Ser
1865	1870	1875
Ser Ala Ala Ala Ala Ala	Gly Gln Tyr Gly Ser	Gly Pro Gly Gln
1880	1885	1890
Gln Gly Pro Tyr Gly Ser	Ala Ala Ala Ala Ala	Gly Pro Gly Ser
1895	1900	1905
Gly Gln Tyr Gly Gln Gly	Pro Tyr Gly Pro Gly	Ala Ser Gly Pro
1910	1915	1920
Gly Gln Tyr Gly Pro Gly	Gln Gln Gly Pro Ser	Ala Ser Ala Ala
1925	1930	1935
Ala Ala Ala Gly Ser Gly	Gln Gln Gly Pro Gly	Gln Tyr Gly Pro
1940	1945	1950
Tyr Ala Ser Ala Ala Ala	Ala Ala Gly Gln Tyr	Gly Ser Gly Pro
1955	1960	1965
Gly Gln Gln Gly Pro Tyr	Gly Pro Gly Gln Ser	Gly Ser Gly Gln
1970	1975	1980
Gln Gly Pro Gly Gln Gln	Gly Pro Tyr Ala Ser	Ala Ala Ala Ala
1985	1990	1995
Ala Gly Pro Gly Gln Gln	Gly Pro Tyr Gly Pro	Gly Ser Ser Ala
2000	2005	2010

Ala Ala Ala Ala Gly Gln Tyr Gly Tyr Gly Pro Gly Gln Gln Gly		
2015	2020	2025
Pro Tyr Gly Pro Gly Ala Ser Gly Gln Asn Gly Pro Gly Ser Gly		
2030	2035	2040
Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Ser Ala Ala Ala		
2045	2050	2055
Ala Ala Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser		
2060	2065	2070
Ala Ala Ala Ala Ala Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro		
2075	2080	2085
Gly Gln Tyr Gly Pro Gly Ser Ser Gly Pro Gly Gln Gln Gly Pro		
2090	2095	2100
Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala Ala Gly Gln Tyr Gly		
2105	2110	2115
Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Ala Ala Ala		
2120	2125	2130
Ala Ala Gly Gln Tyr Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr		
2135	2140	2145
Gly Pro Gly Ala Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro		
2150	2155	2160
Gly Ala Ser Ala Ala Ala Ala Ala Gly Pro Gly Gln Tyr Gly Pro		
2165	2170	2175
Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala Ala Ala Ala Gly Gln		
2180	2185	2190
Tyr Gly Ser Gly Pro Gly Gln Tyr Gly Pro Tyr Gly Pro Gly Gln		
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Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala Ala Ala Gly Ser		
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Tyr Gly Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala Ser		
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Val Leu Ile Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala		
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Ala Ala Gly Ser Tyr Gly Pro Gly Gln Gln Gly Pro Gly Gln Tyr Gly		
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Val Leu Ile Gly Pro Tyr Gly Pro Gly Pro Ser Ala Ala Ala Ala Ala		
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Pro Gly Ala Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala		
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Ser Ala Ala Ala Ala Ala Ala Ala Gly Pro Gly Gln Tyr Val Leu Ile		
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 Pro Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Ser Tyr Gly Pro
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Tyr Gly Pro Gly Ala Ser Gly Pro Gly Gln Tyr Gly Pro Gly Gln Gln			
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Gln Val Leu Ile Gly Pro Gly Gln Tyr Val Leu Ile Gly Pro Tyr Ala			
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Gly Pro Gly Gln Gln Val Leu Ile Gly Pro Tyr Val Leu Ile Gly Pro			
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Gly Ser Ser Ala Ala Ala Ala Ala Ala Gly Ser Tyr Gly Tyr Gly			
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Pro Tyr Val Leu Ile Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala Ala			
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Val Leu Gly Pro Ser Ala Ser Ala Ala Ala Ala Gly Ser Gly Val			
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Pro Tyr Gly Pro Gly Ala Ser Gly Ala Asn Gly Pro Gly Ser Gly Ala		
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50						55				60					
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Pro	Gly	Val	Leu	Gly	Pro	Gly	Val	Leu	Gly	Pro	Gly	Ser	Ser	Ala	Ala
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Ile	Tyr	Gly	Pro	Gly	Ser	Ser	Gly	Pro	Gly	Val	Leu	Gly	Pro	Tyr	Gly

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Tyr Val Leu Gly Pro Gly Val Leu Gly Pro Tyr Gly Pro Gly Ala Ser			
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245          250          255
Pro Tyr Gly Pro Gly Ala Ser Gly Leu Asn Gly Pro Gly Ser Gly Leu
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Tyr Gly Pro Gly Val Ile Gly Pro Gly Leu Ser Ala Ala Ala Ala Ala
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Pro	Tyr	Gly	Pro	Gly	Ala	Ser	Gly	Ile	Asn	Gly	Pro	Gly	Ser	Gly	Ile	260	265	270	

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Pro Gly Ser Gly Leu Tyr Gly Leu Gly Pro Tyr Gly Pro Gly Ala Ser		
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Gly Pro Gly Leu Tyr Gly Pro Gly Val Ile Gly Pro Ser Ala Ser Ala		
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Ala Ala Ala Ala Gly Ser Gly Val Ile Gly Pro Gly Leu Tyr Gly Pro		
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Tyr Ala Ser Ala Ala Ala Ala Ala Gly Leu Tyr Gly Ser Gly Pro Gly		
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Val Ile Gly Pro Tyr Gly Pro Gly Leu Ser Gly Ser Gly Val Ile Gly		
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Pro Gly Val Ile Gly Pro Tyr Ala Ser Ala Ala Ala Ala Gly Pro		
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Gly Val Ile Gly Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala		
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Gly Leu Tyr Gly Tyr Gly Pro Gly Val Ile Gly Pro Tyr Gly Pro Gly		
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Ala Ser Gly Leu Asn Gly Pro Gly Ser Gly Leu Tyr Gly Pro Gly Val		
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Ile Gly Pro Gly Leu Ser Ala Ala Ala Ala Gly Pro Gly Val Ile		
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Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Gly Leu Tyr		
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Gly Pro Gly Val Ile Gly Pro Gly Leu Tyr Gly Pro Gly Ser Ser Gly		
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Pro Gly Val Ile Gly Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala		
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Ala Gly Leu Tyr Gly Pro Gly Val Ile Gly Pro Tyr Gly Pro Gly Leu		
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Ser Ala Ala Ala Ala Gly Leu Tyr Val Ile Gly Pro Gly Val Ile		
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Gly Pro Tyr Gly Pro Gly Ala Ser Gly Pro Gly Val Ile Gly Pro Tyr		
385	390	395
Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Pro Gly Leu Tyr Gly		
405	410	415

Pro Gly Val Ile Gly Pro Ser Ala Ser Ala Ala Ala Ala Gly Leu
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 Tyr Gly Ser Gly Pro Gly Leu Tyr Gly Pro Tyr Gly Pro Gly Leu Ser
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 Gly Pro Gly Ser Gly Val Ile Gly Leu Gly Pro Tyr Gly Pro Gly Ala
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 Ser Ala Ala Ala Ala Ala Gly Leu Tyr Gly Pro Gly Val Ile Gly Pro
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 Tyr Gly Pro Gly Leu Ser Ala Ala Ala Ala Gly Pro Gly Ser Gly
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Tyr Ala Ser Ala Ala Ala Ala Gly Ile Tyr Gly Ser Gly Pro Gly			
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Val Phe Gly Pro Tyr Gly Pro Gly Ile Ser Gly Ser Gly Val Phe Gly			
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Pro Gly Val Phe Gly Pro	Ser Ala Ser Ala Ala Ala	Ala Gly Ile
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Gly Ser Gly Val Leu Gly Pro	Gly Gln Tyr Gly Pro Tyr	Ala Ser Ala
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Gly Pro Tyr Gly Pro Gly Gln	Ser Gly Ser Gly Val Leu	Gly Pro Gly
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Val Leu Gly Pro Tyr Ala Ser	Ala Ala Ala Ala Ala Gly	Pro
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Pro Gly Ala Ser Gly Gln Asn	Gly Pro Gly Ser Gly Gln	Tyr Gly Pro
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Gly Val Leu Gly Pro Gly Pro	Ser Ala Ala Ala Ala Ala	Gly
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Pro Gly Val Leu Gly Pro Tyr	Gly Pro Gly Ala Ser Ala	Ala Ala Ala
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 Gly Ser Tyr Val Leu Gly Pro Gly Val Leu Gly Pro Tyr Gly Pro Gly
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 Gly Ser Gly Val Leu Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala
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Ile Tyr Gly Pro Gly Val Leu Gly Pro Gly Val Leu Gly Pro Gly Ser		
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Gly Ile Gly Pro Tyr Gly Pro Gly Ala Ser Gly Pro Gly Ile Tyr Gly		
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Pro Gly Val Leu Gly Pro Ser Ala Ser Ala Ala Ala Ala Ala Ala Ala		
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Pro Gly Ala Ser Gly Ile Asn Gly Pro Gly Ser Gly Ile Tyr Gly Pro		
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Gly Val Leu Gly Pro Gly Pro Ser Ala Ala Ala Ala Ala Ala Ala Gly		
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Pro Gly Val Leu Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala		

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Tyr Ala Ser Ala Ala Ala Ala Gly Ile Tyr Gly Ser Gly Pro Gly			
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Val Phe Gly Pro Tyr Gly Pro Gly Ile Ser Gly Ser Gly Val Phe Gly			
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Gly Ile Tyr Gly Tyr Gly Pro Gly Val Phe Gly Pro Tyr Gly Pro Gly			
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Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Ile Tyr			
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Ala Gly Ile Tyr Gly Pro Gly Val Phe Gly Pro Tyr Gly Pro Gly Ile		
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Ile Tyr Gly Pro Gly Ala Ser Gly Ile Asn Gly Pro Gly Ser Gly Ile		
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Phe Gly Pro Tyr Gly Pro Gly Ile Ser Gly Ser Gly Val Phe Gly Pro		
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595	600	605
Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Ile Asn Gly Pro		
610	615	620
Gly Ser Gly Val Phe Gly Pro Gly Ile Ser Gly Ile Tyr Gly Pro Gly		

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Ala Gly Pro Gly Ile Tyr Gly Pro Gly	Val Phe Gly Pro Ser Ala Ser		
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Ser Gly Ile Tyr Gly Pro Gly Val Phe Gly Pro Gly	Val Phe Gly Pro		
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Val Phe Gly Pro Tyr Gly Ser Ala Ala Ala Ala Gly Pro Gly Ser			
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Gly Ile Tyr Gly Ile Gly Pro Tyr Gly Pro Gly Ala Ser Gly Pro Gly			
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Ile Tyr Gly Pro Gly Val Phe Gly Pro Ser Ala Ser Ala Ala Ala Ala			
755	760	765	
Ala Gly Ser Gly Val Phe Gly Pro Gly Ile Tyr Gly Pro Tyr Ala Ser			
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Pro Tyr Gly Pro Gly Ile Ser Gly Ser Gly Val Phe Gly Pro Gly Val			
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Gly Tyr Gly Pro Gly Val Phe Gly Pro Tyr Gly Pro Gly Ala Ser Gly			
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Ile Asn Gly Pro Gly Ser Gly Ile Tyr Gly Pro Gly Val Phe Gly Pro			
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Val Phe Gly Pro Gly Ile Tyr Gly Pro Gly Ser Ser Gly Pro Gly Val			
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Phe Gly Pro Ser Ala Ser Ala Ala Ala Ala Gly Ile Tyr Gly			
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Pro Tyr Gly Pro Gly Ala Ser Gly Val Asn Gly Pro Gly Ser Gly Val
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Tyr Gly Pro Gly Leu Ile Gly Pro Gly Val Ser Ala Ala Ala Ala Ala
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Ile Phe Gly Pro Ser Ala Ser Ala Ala Ala Gly Pro Gly Ser
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Gly Ile Phe Gly Pro Gly Ala Ser Gly Thr Tyr Gly Pro Gly Ile Phe
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Gly Pro Gly Ile Phe Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly
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Thr Tyr Gly Ser Gly Pro Gly Ile Phe Gly Pro Tyr Gly Ser Ala Ala
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Ala Ala Ala Gly Pro Gly Ser Gly Thr Tyr Gly Thr Gly Pro Tyr Gly
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Pro Gly Ala Ser Gly Pro Gly Thr Tyr Gly Pro Gly Ile Phe Gly Pro
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Thr Tyr Gly Pro Tyr Ala Ser Ala Ala Ala Ala Gly Thr Tyr Gly
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Tyr Gly Pro Gly Ala Ser Gly Thr Gly Pro Gly Ser Gly Thr Tyr Gly
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Gly Thr Tyr Gly Pro Gly Ile Phe Gly Pro Gly Thr Ser Ala Ala Ala		
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Ala Ala Gly Thr Tyr Ile Phe Gly Pro Gly Ile Phe Gly Pro Tyr Gly		
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Pro Gly Ala Ser Ala Ala Ala Ala Gly Thr Tyr Gly Ser Gly Pro		
	530	535
Gly Ile Phe Gly Pro Tyr Gly Pro Gly Thr Ser Gly Ser Gly Ile Phe		
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Ala Ala Ala Ala Gly Pro Gly Val Tyr Gly Pro Gly Leu Ile Gly Pro	
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Ser Ala Ser Ala Ala Ala Ala Gly Pro Gly Ser Gly Leu Ile Gly	
85 90 95	
Pro Gly Ala Ser Gly Val Tyr Gly Pro Gly Leu Ile Gly Pro Gly Leu	
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Ile Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly Val Tyr Gly Ser	
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Gly Ala Ser Gly Thr Tyr Gly Pro Gly Ile Phe Gly Pro Gly Ile Phe		
	100	105 110
Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly Thr Tyr Gly Ser Gly		
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Pro Gly Ile Phe Gly Pro Tyr Gly Ser Ala Ala Ala Ala Gly Pro		
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Gly Ser Gly Thr Tyr Gly Thr Gly Pro Tyr Gly Pro Gly Ala Ser Gly		
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Pro Gly Thr Tyr Gly Pro Gly Ile Phe Gly Pro Ser Ala Ser Ala Ala		
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Pro Gly Thr Ser Ala Ala Ala Ala Ala Gly Pro Gly Ile Phe Gly Pro		
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Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Thr Tyr Gly Pro		
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Gly Ile Phe Gly Pro Gly Thr Tyr Gly Pro Gly Ser Ser Gly Pro Gly		
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Thr Tyr Gly Pro Gly Ile Phe Gly Pro Tyr Gly Pro Gly Thr Ser Ala		
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Gly Ser Gly Ile Phe Gly Thr Gly Pro Tyr Gly Pro Gly Ala Ser Ala		
	450	455 460
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Pro Gly Thr Ser Ala Ala Ala Ala Ala Gly Pro Gly Ser Gly Thr Tyr		
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Gly Pro Gly Ala Ser Gly Thr Gly Pro Gly Ser Gly Thr Tyr Gly Pro		
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Gly Ile Phe Gly Pro Gly Thr Ser Ala Ala Ala Ala Ala Gly Thr Tyr		

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Tyr Gly Pro Gly Thr	Ser Gly Ser Gly Ile Phe Gly	Pro Gly Ile Phe
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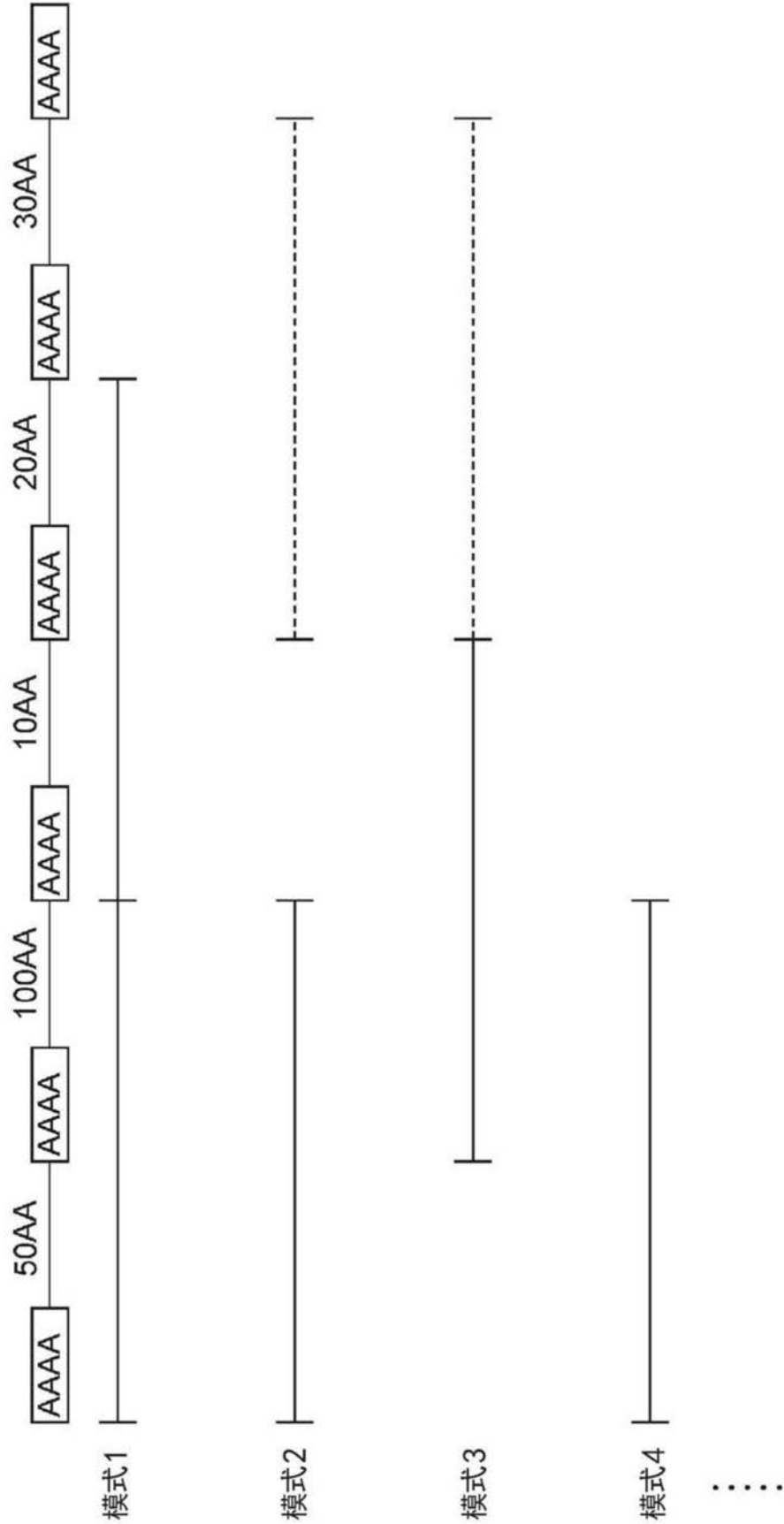


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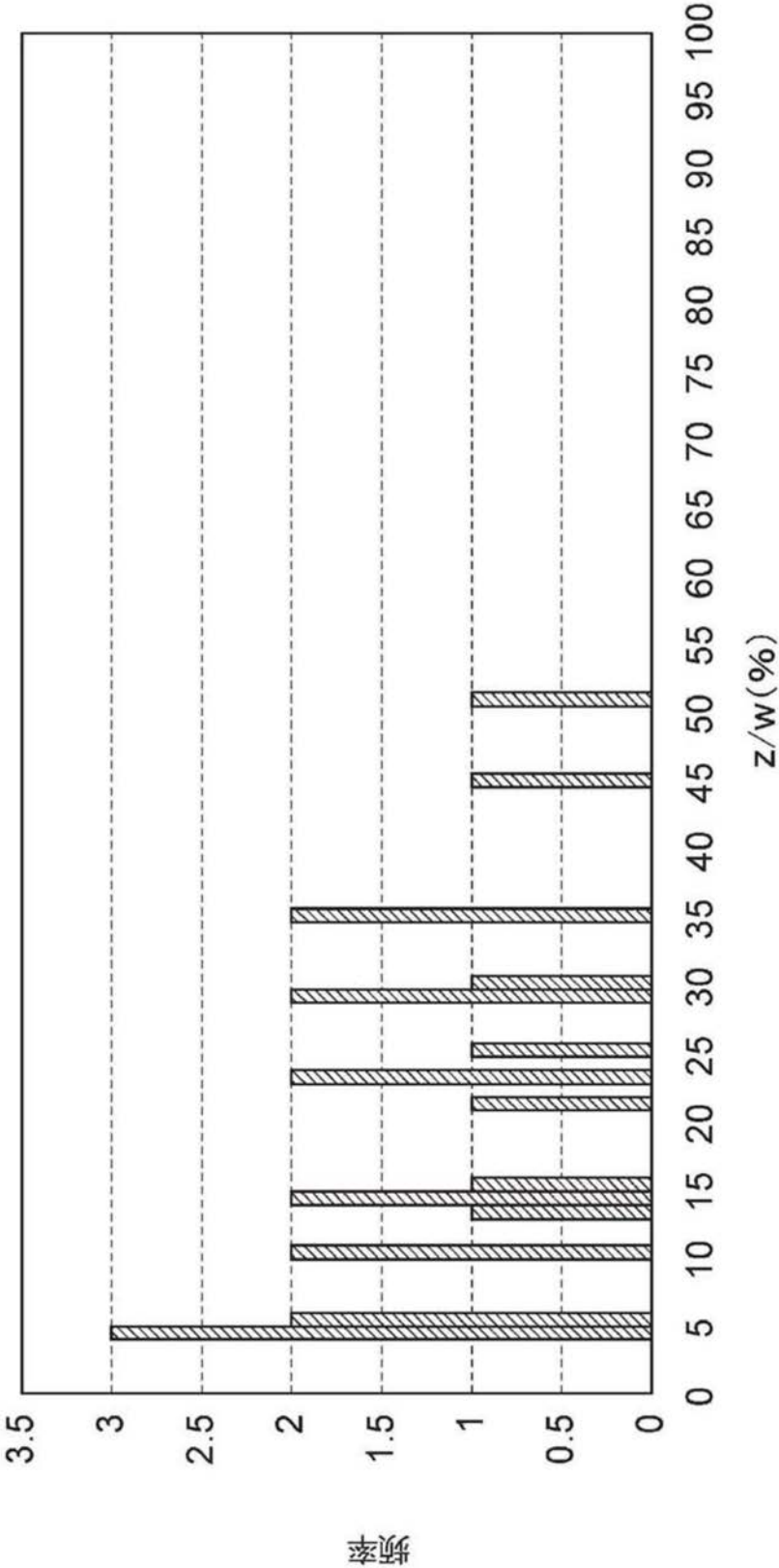


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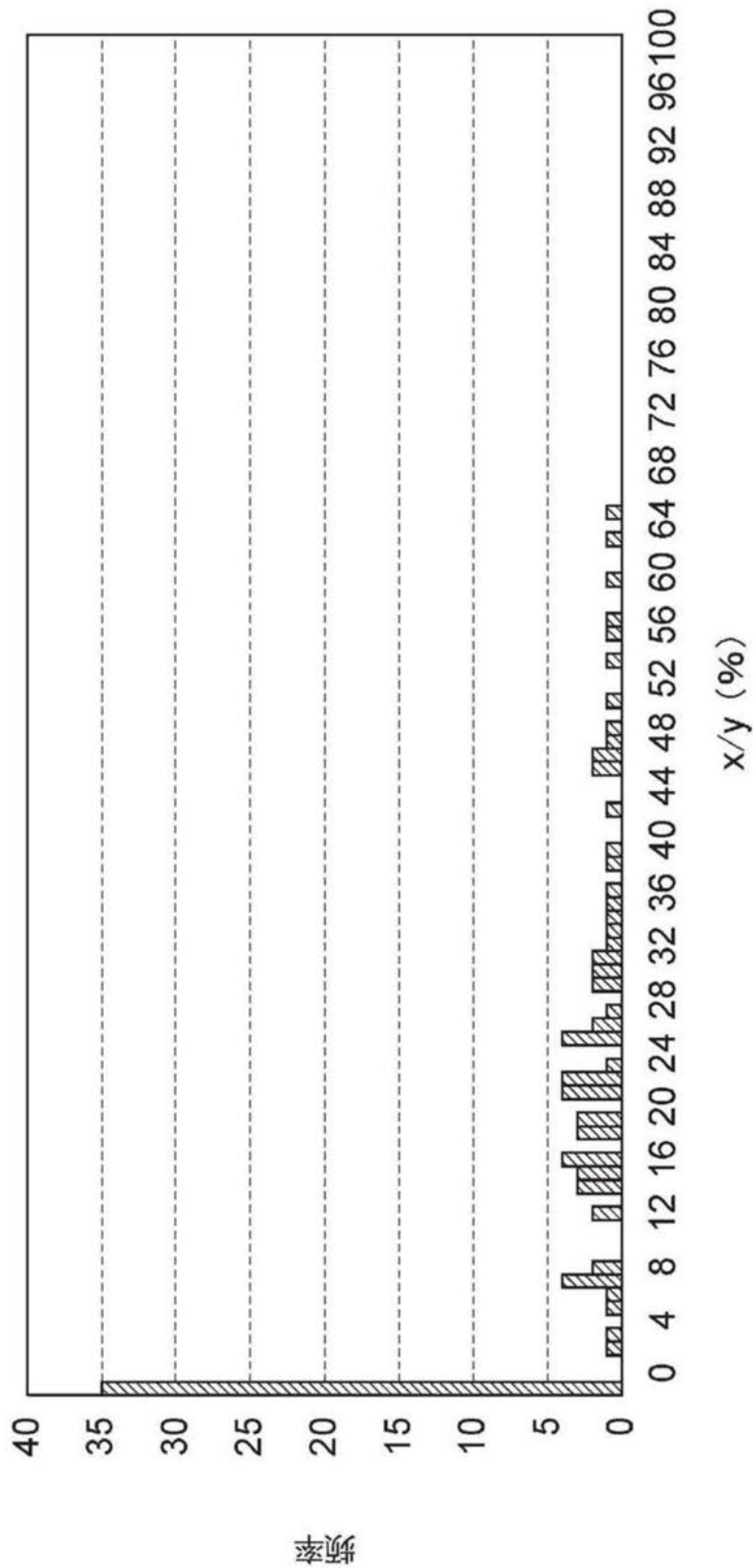


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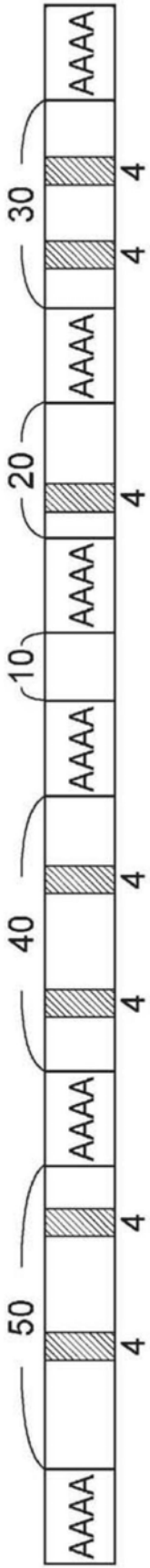


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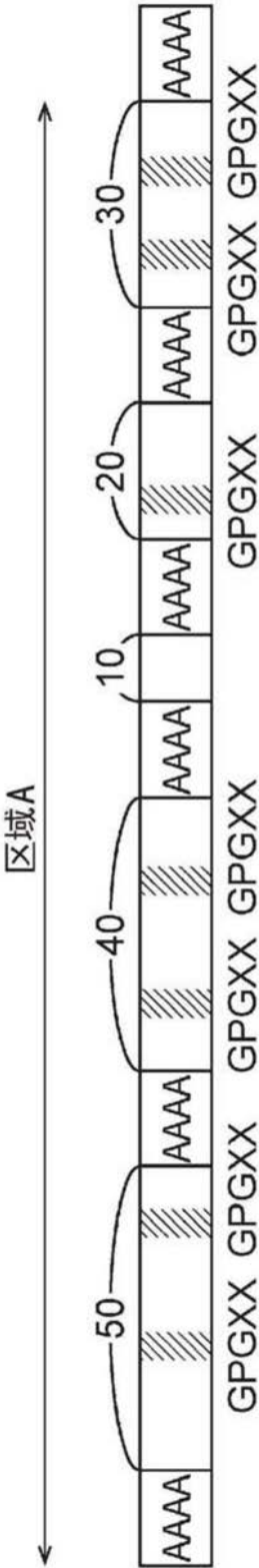


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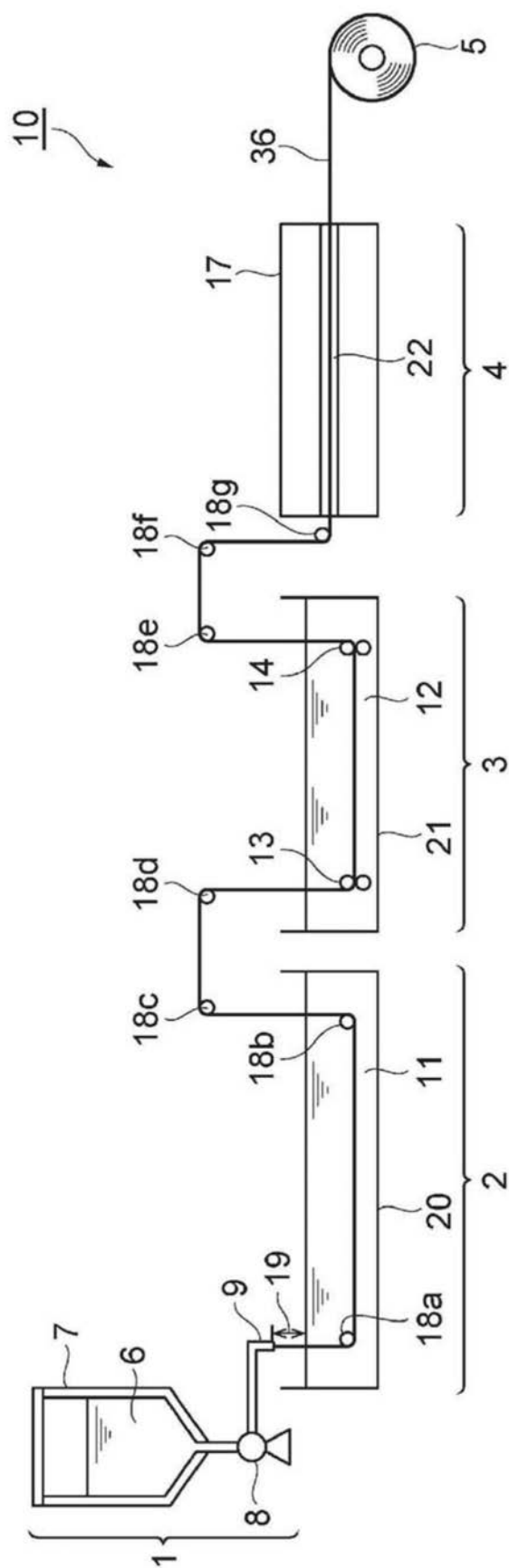


图6

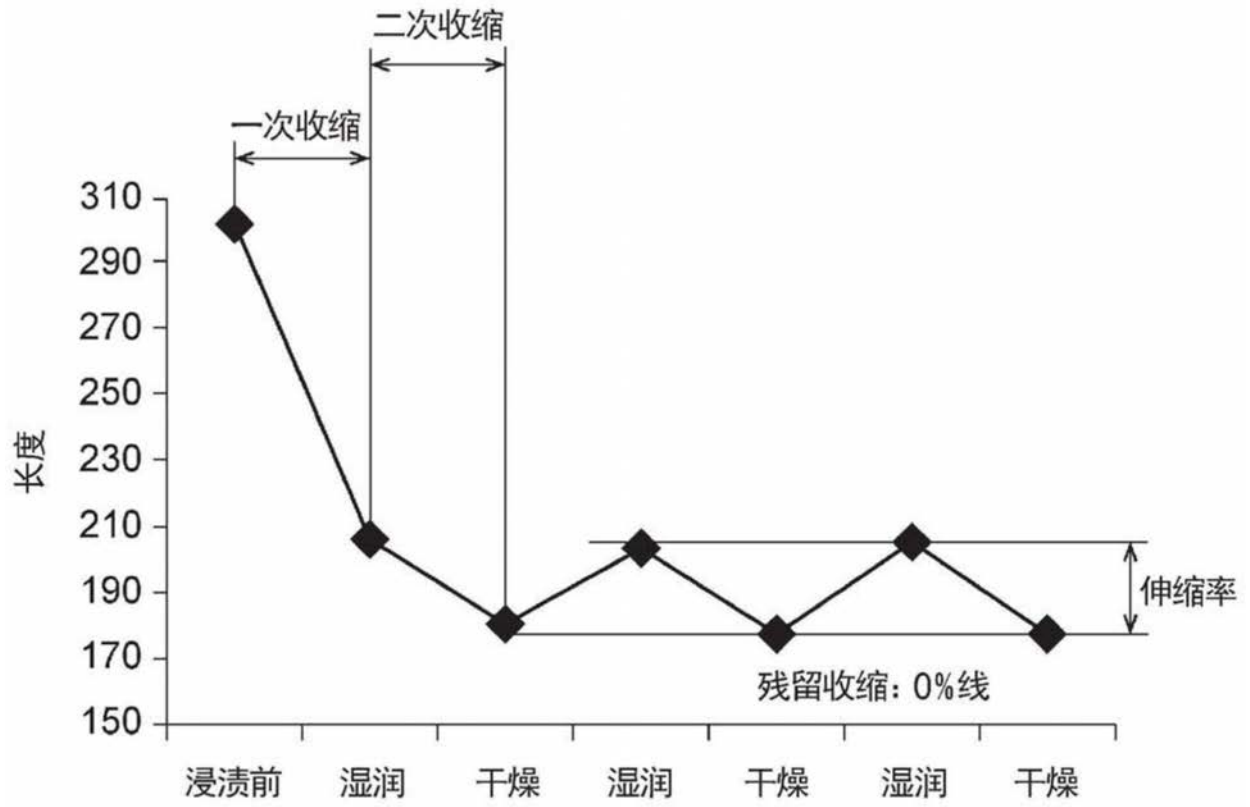


图7