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(54) 发明名称

一种接触冷感性及吸水速干性赋予剂、以及
赋予物品接触冷感性及吸水速干性的方法

(57) 摘要

本发明的目的在于提供一种新型接触冷感性及吸水速干性赋予剂。本发明所涉及的接触冷感性及吸水速干性赋予剂含有改造丝心蛋白作为有效成分。

1. 一种接触冷感性及吸水速干性赋予剂,其中,其包含改造丝心蛋白作为有效成分。
2. 如权利要求1所述的接触冷感性及吸水速干性赋予剂,其中,所述改造丝心蛋白包括疏水性指标平均值(平均HI)超过0的改造丝心蛋白。
3. 如权利要求1或2所述的接触冷感性及吸水速干性赋予剂,其中,所述改造丝心蛋白包括改造蜘蛛丝丝心蛋白。
4. 如权利要求1至3中任一项所述的接触冷感性及吸水速干性赋予剂,其中,其为纤维形态。
5. 一种方法,其赋予物品接触冷感性及吸水速干性,其中,其包括使所述物品含有改造丝心蛋白的工序。

一种接触冷感性及吸水速干性赋予剂、以及赋予物品接触冷感性及吸水速干性的方法

技术领域

[0001] 本发明涉及一种接触冷感性及吸水速干性赋予剂、以及赋予物品接触冷感性及吸水速干性的方法。

背景技术

[0002] 近年来,夏季床上用品和衣物等直接接触皮肤的物品中,逐渐开始使用兼具接触冷感性及吸水速干性的高功能材料。例如,专利文献1公开了一种单丝纤度为0.5~3.0dtex的三醋酸短纤维A与纤维素短纤维B的复合纺织纱线,其特征在于,短纤维A与B的质量比率(A/B)在20/80~80/20范围内,进而3mm以上的毛羽指数为50个/m以下,且平均强力在100CN以上范围内。

[0003] 现有技术文献

[0004] 专利文献

[0005] 专利文献1:日本特开2015-98662号公报

发明内容

[0006] 发明所要解决的问题

[0007] 专利文献1中公开的复合纺织纱线利用了三醋酸短纤维的优点。但是,三醋酸短纤维等合成纤维的生物降解性差,存在产生环境负荷的问题。另一方面,关于天然纤维,丝绸虽然具有较高的接触冷感性,但是不能充分满足夏季床上用品和衣物等所要求的水平。而且,丝绸吸水速干性不充分,从防闷热等角度来看,也不具有足够的功能。综上所述,有关兼具接触冷感性及吸水速干性的高功能材料,尚未提供充分的选择。

[0008] 本发明的目的在于提供一种新型接触冷感性及吸水速干性赋予剂。本发明的目的还在于提供一种赋予指定物品接触冷感性及吸水速干性的方法。

[0009] 用于解决问题的方法

[0010] 本发明人等发现改造丝心蛋白具有优异的接触冷感性及吸水速干性。本发明基于该新见解而完成。

[0011] 本发明涉及例如下述各发明。

[0012] [1]

[0013] 一种接触冷感性及吸水速干性赋予剂,其中,其包含改造丝心蛋白作为有效成分。

[0014] [2]

[0015] 如[1]所述的接触冷感性及吸水速干性赋予剂,其中,所述改造丝心蛋白包括疏水性指标平均值(平均HI)超过0的改造丝心蛋白。

[0016] [3]

[0017] 如[1]或[2]所述的接触冷感性及吸水速干性赋予剂,其中,所述改造丝心蛋白包括改造蜘蛛丝丝心蛋白。

[0018] [4]

[0019] 如[1]至[3]中任一项所述的接触冷感性及吸水速干性赋予剂,其中,其为纤维形态。

[0020] [5]

[0021] 一种方法,其赋予物品接触冷感性及吸水速干性,其中,

[0022] 其包括使所述物品含有改造丝心蛋白的工序。

[0023] 发明效果

[0024] 根据本发明,可以提供一种新型接触冷感性及吸水速干性赋予剂。根据本发明,还可以提供一种赋予指定物品接触冷感性及吸水速干性的方法。

附图说明

[0025] 图1是表示改造丝心蛋白的结构域序列的一例的示意图。

[0026] 图2是表示天然来源的丝心蛋白的z/w(%)的值的分布的图。

[0027] 图3是表示天然来源的丝心蛋白的x/y(%)的值的分布的图。

[0028] 图4是表示改造丝心蛋白的结构域序列的一例的示意图。

[0029] 图5是表示改造丝心蛋白的结构域序列的一例的示意图。

具体实施方式

[0030] 以下,对本发明具体实施方式详细进行说明。但是,本发明并不限于以下实施方式。

[0031] (接触冷感性及吸水速干性赋予剂)

[0032] 本实施方式所涉及的接触冷感性及吸水速干性赋予剂含有改造丝心蛋白作为有效成分。

[0033] 改造丝心蛋白兼具接触冷感性(例如接触时感到凉爽的特性)及吸水速干性(例如大量吸收水分(例如汗液)且快速干燥等特性)。本实施方式所涉及的接触冷感性及吸水速干性赋予剂能利用改造丝心蛋白的这些特性,同时赋予指定物品接触冷感性及吸水速干性。

[0034] (改造丝心蛋白)

[0035] 本实施方式所涉及的改造丝心蛋白为包含式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。改造丝心蛋白可以在结构域序列的N末端侧及C末端侧中的任意一个末端侧或两个末端侧进一步添加氨基酸序列(N末端序列及C末端序列)。N末端序列及C末端序列并不限于此,典型为丝心蛋白中不具有特征性氨基酸基序重复的区域,并且由100个残基左右的氨基酸构成。

[0036] 在本说明书中,“改造丝心蛋白”是指人工制造的丝心蛋白(人造丝心蛋白)。改造丝心蛋白可以是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,也可以是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。在本说明书中,“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。

[0037] “改造丝心蛋白”可以是直接利用天然来源的丝心蛋白的氨基酸序列的丝心蛋白,

也可以是依据天然来源的丝心蛋白的氨基酸序列来改造该氨基酸序列的丝心蛋白(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造来改造氨基酸序列而得到的丝心蛋白),此外也可以为不依赖天然来源的丝心蛋白而人工设计和合成的丝心蛋白(例如,通过对编码所设计的氨基酸序列的核酸进行化学合成而具有所需氨基酸序列的丝心蛋白)。

[0038] 在本说明书中,“结构域序列”为生成丝心蛋白特有的结晶区(典型为相当于氨基酸序列的(A)_n基序)和非晶区(典型为相当于氨基酸序列的REP)的氨基酸序列,是指式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个。(A)_n基序的氨基酸残基数可以是2~20、4~27、4~20、8~20、10~20、4~16、8~16或10~16的整数。此外,只要(A)_n基序中的丙氨酸残基数相对于氨基酸残基总数的比例为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%(意味着其仅由丙氨酸残基构成)。在结构域序列中存在多个的(A)_n基序中的至少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可以是由10~200个氨基酸残基构成的氨基酸序列。m表示2~300的整数,也可以为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。

[0039] 本实施方式所涉及的改造丝心蛋白例如可以通过对克隆出的天然来源的丝心蛋白的基因序列进行相当于例如置换、缺失、插入及/或添加一个或多个氨基酸残基的氨基酸序列改造而得到。氨基酸残基的置换、缺失、插入及/或添加可以通过定点突变方法等本领域技术人员熟知的方法来进行。具体而言,可以根据Nucleic Acid Res.10,6487(1982)、Methods in Enzymology,100,448(1983)等文献中记载的方法来进行。

[0040] 天然来源的丝心蛋白是包含式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,例如,可以列举出昆虫或蜘蛛类产生的丝心蛋白。

[0041] 作为昆虫产生的丝心蛋白,可以列举例如家蚕(*Bombyx mori*)、野桑蚕(*Bombyx mandarina*)、天蚕(*Antheraea yamamai*)、柞蚕(*Antheraea pernyi*)、枫蚕(*Eriogyna pyretorum*)、蓖麻蚕(*Pilosamia Cynthia ricini*)、樗蚕(*Samia cynthia*)、樟蚕(*Caligula japonica*)、印度柞蚕(*Antheraea mylitta*)、琥珀蚕(*Antheraea assama*)等蚕产生的蚕丝蛋白及雀蜂(*Vespa simillima xanthoptera*)幼虫吐出的蜂丝蛋白。

[0042] 作为昆虫产生的丝心蛋白的更具体示例,可以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))。

[0043] 作为蜘蛛类产生的丝心蛋白,例如,可以列举出属于蜘蛛目(Araneae)的蜘蛛产生的蛛丝蛋白。更具体而言,可以列举大腹园蛛、十字园蛛、肥胖园蛛、五纹园蛛和野岛园蛛等属于园蛛属(*Araneus*属)的蜘蛛、青新园蛛、嗜水新园蛛、灌木新园蛛和类青新园蛛等属于新园蛛属(*Neoscona*属)的蜘蛛、小岬蛛等属于岬蛛属(*Pronus*属)的蜘蛛、蟾蜍曲腹蛛和对称曲腹蛛等属于曲腹蛛属(*Cyrtarachne*属)的蜘蛛、库氏棘腹蛛和乳突棘腹蛛等属于棘腹蛛属(*Gasteracantha*属)的蜘蛛、何氏瘤腹蛛和六刺瘤腹蛛等属于瘤腹蛛属(*Ordgarius*属)的蜘蛛、悦目金蛛、小悦目金蛛和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、红云斑蛛、花云斑蛛和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 (*Poltyys* 属) 的蜘蛛、八瘤艾蛛、四突艾蛛、圆腹艾蛛和黑尾艾蛛等属于艾蛛属 (*Cyclosa* 属) 的蜘蛛和日本壮头蛛等属于壮头蛛属 (*Chorizopes* 属) 的蜘蛛产生的蛛丝蛋白、以及前齿肖蛸、锥腹肖蛸、直伸肖蛸和鳞纹肖蛸等属于肖蛸属 (*Tetragnatha* 属) 的蜘蛛、纵条银鳞蛛、肩斑银鳞蛛和小肩斑银鳞蛛等属于银鳞蛛属 (*Leucauge* 属) 的蜘蛛、棒络新妇和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、红斑寇蛛、哈氏寇蛛、几何寇蛛和间斑寇蛛等属于寇蛛属 (*Latrodectus* 属) 的蜘蛛、以及属于育儿网蛛属 (*Euprosthenops* 属) 的蜘蛛等属于肖蛸科 (*Tetragnathidae* 科) 的蜘蛛产生的蛛丝蛋白。作为蛛丝蛋白, 可以列举例如 MaSp (MaSp1 和 MaSp2)、ADF (ADF3 和 ADF4) 等牵引丝蛋白、MiSp (MiSp1 和 MiSp2)、AcSp、PySp、Flag 等。

[0044] 作为蜘蛛类产生的蛛丝蛋白的更具体示例, 例如, 可以列举出 fibroin-3 (adf-3) [来自于 *Araneus diadematus*] (GenBank 登录号 AAC47010 (氨基酸序列)、U47855 (碱基序列))、fibroin-4 (adf-4) [来自于 *Araneus diadematus*] (GenBank 登录号 AAC47011 (氨基酸序列)、U47856 (碱基序列))、dragline silk protein spidroin 1 [来自于 *Nephila clavipes*] (GenBank 登录号 AAC04504 (氨基酸序列)、U37520 (碱基序列))、major ampullate spidroin 1 [来自于 *Latrodectus hesperus*] (GenBank 登录号 ABR68856 (氨基酸序列)、EF595246 (碱基序列))、dragline silk protein spidroin 2 [来自于 *Nephila clavata*] (GenBank 登录号 AAL32472 (氨基酸序列)、AF441245 (碱基序列))、major ampullate spidroin 1 [来自于 *Euprosthenops australis*] (GenBank 登录号 CAJ00428 (氨基酸序列)、AJ973155 (碱基序列))、以及 major ampullate spidroin2 [*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。

[0045] 作为天然来源的丝心蛋白的更具体示例, 可以进一步列举在 NCBI GenBank 中登记了序列信息的丝心蛋白。例如, 可以通过从 NCBI GenBank 所登记序列信息中包含 INV 作为分类码 (DIVISION) 的序列中提取出在定义 (DEFINITION) 中记载了蛛丝蛋白、壶状腺、丝心蛋白、“丝和多肽”或“丝和蛋白质”作为关键词的序列, 从 CDS 中提取出指定产物的字符串, 从来源 (SOURCE) 中提取出在组织类型 (TISSUE TYPE) 中记载了指定字符串的序列来确认。

[0046] 本实施方式所涉及的改造丝心蛋白可以是改造蚕丝 (丝绸) 丝心蛋白 (对蚕产生的蚕丝蛋白的氨基酸序列进行了改造的丝心蛋白), 也可以是改造蜘蛛丝丝心蛋白 (对蜘蛛类产生的蛛丝蛋白的氨基酸序列进行了改造的丝心蛋白)。

[0047] 作为改造丝心蛋白的具体示例, 可以列举出来自于蜘蛛大壶状腺产生的大吐丝管牵引丝蛋白的改造丝心蛋白 (第1改造丝心蛋白)、具有甘氨酸残基含量降低的结构域序列的改造丝心蛋白 (第2改造丝心蛋白)、具有 (A)_n 基序含量降低的结构域序列的改造丝心蛋白 (第3改造丝心蛋白)、甘氨酸残基含量及 (A)_n 基序含量降低的改造丝心蛋白 (第4改造丝

心蛋白)、具有包含局部疏水性指标大的区域的结构域序列的改造丝心蛋白(第5改造丝心蛋白)、及具有谷氨酰胺残基含量降低的结构域序列的改造丝心蛋白(第6改造丝心蛋白)。

[0048] 作为第1改造丝心蛋白,可以例举包含式1: $[(A)_n\text{基序-REP}]_m$ 所示的结构域序列的蛋白质。在第1改造丝心蛋白中, $(A)_n$ 基序的氨基酸残基数优选为3~20的整数,更优选为4~20的整数,进一步优选为8~20的整数,更进一步优选为10~20的整数,进一步还优选为4~16的整数,特别优选为8~16的整数,最优选为10~16的整数。在第1改造丝心蛋白中,式1中构成REP的氨基酸残基数优选为10~200个残基,更优选为10~150个残基,进一步优选为20~100个残基,更进一步优选为20~75个残基。在第1改造丝心蛋白中,式1: $[(A)_n\text{基序-REP}]_m$ 所示的氨基酸序列所含的甘氨酸残基、丝氨酸残基及丙氨酸残基的合计残基数相对于氨基酸残基总数优选为40%以上,更优选为60%以上,进一步优选为70%以上。

[0049] 第1改造丝心蛋白可以为以下多肽:包含式1: $[(A)_n\text{基序-REP}]_m$ 所示的氨基酸序列的单元,并且C末端序列为序列号1~3中任意一个所示的氨基酸序列或与序列号1~3中任意一个所示的氨基酸序列具有90%以上同源性的氨基酸序列。

[0050] 序列号1所示的氨基酸序列与由ADF3 (GI:1263287、NCBI) 氨基酸序列的C末端的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末端除去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末端除去29个残基后的氨基酸序列相同。

[0051] 作为第1改造丝心蛋白的更具体示例,可以列举出包含与(1-i)序列号4 (recombinant spider silk protein ADF3KaiLargeNRSH1) 所示的氨基酸序列或(1-ii)序列号4所示的氨基酸序列具有90%以上序列一致性的氨基酸序列的改造丝心蛋白。序列一致性优选为95%以上。

[0052] 在N末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氨基酸序列中,以使第1~13位的重复区域增加到约2倍且在第1154位氨基酸残基处终止翻译的方式突变,从而得到序列号4所示的氨基酸序列。序列号4所示的氨基酸序列的C末端的氨基酸序列与序列号3所示的氨基酸序列相同。

[0053] (1-i)的改造丝心蛋白可以由序列号4所示的氨基酸序列构成。

[0054] 第2改造丝心蛋白具有其结构域序列与天然来源的丝心蛋白相比甘氨酸残基含量降低的氨基酸序列。第2改造丝心蛋白与天然来源的丝心蛋白相比,可以具有相当于至少REP中的一个或多个甘氨酸残基被置换为其他氨基酸残基的氨基酸序列。

[0055] 第2改造丝心蛋白的结构域序列与天然来源的丝心蛋白相比,在REP中的选自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)中的至少一种基序序列中,可以具有相当于至少一个或多个该基序序列中的一个甘氨酸残基被置换为其他氨基酸残基的氨基酸序列。

[0056] 第2改造丝心蛋白中,上述甘氨酸残基被置换为其他氨基酸残基的基序序列的比例相对于全部基序序列可以为10%以上。

[0057] 第2改造丝心蛋白包含式1: $[(A)_n\text{基序-REP}]_m$ 所示的结构域序列,并且可以具有下述氨基酸序列:将从上述结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至上述结构域序列的C末端为止的序列后得到的序列中的全部REP所包含的由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氨基酸残基总数设为 z ,将从上述结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至上述结构域序列的C末端为止的序列后得到的序列中的氨基酸残基总数设为 w 时, z/w 为30%以上、40%以上、50%以上或50.9%以上。 $(A)_n$ 基序中的丙氨酸残基数相对于氨基酸残基总数可以为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%(意味着仅由丙氨酸残基构成)。

[0058] 第2改造丝心蛋白优选通过将GGX基序的一个甘氨酸残基替换为其他氨基酸残基来提高由XGX构成的氨基酸序列的含有比例。在第2改造丝心蛋白中,结构域序列中由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,进一步还优选为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可以通过与下述由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。

[0059] 对 z/w 的计算方法进一步进行详细说明。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(改造丝心蛋白或天然来源的丝心蛋白)中,从结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列,从而得到的序列所含的全部REP中提取由XGX构成的氨基酸序列。构成XGX的氨基酸残基总数为 z 。例如,提取了50个由XGX构成的氨基酸序列时(没有重复), z 为 $50 \times 3 = 150$ 。此外,例如,由XGXGX构成的氨基酸序列等存在包含在2个XGX中的X(中间的X)时,通过扣除重复部分来进行计算(XGXGX时为5个氨基酸残基)。 w 为从结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列后得到的序列所含的氨基酸残基总数。例如,为图1所示的结构域序列时, w 为 $4+50+4+100+4+10+4+20+4+30=230$ (已除去位于最靠C末端侧的 $(A)_n$ 基序)。然后,通过用 z 除以 w ,可以计算出 z/w (%)。

[0060] 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,通过例示在NCBI GenBank中登记有氨基酸序列信息的丝心蛋白的方法进行确认,提取出663种丝心蛋白(其中,来自于蜘蛛类的丝心蛋白为415种)。根据所提取的所有丝心蛋白中包含式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,通过上述计算方法计算出 z/w 。其结果如图2所示。图2的横轴表示 z/w (%),纵轴表示频率。由图2可知,天然来源的丝心蛋白中的 z/w 均小于50.9%(最大为50.86%)。

[0061] 在第2改造丝心蛋白中, 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,进一步还优选为80%以上。 z/w 的上限并没有特别限制,例如可以为95%以下。

[0062] 例如可以通过如下方式进行改造来得到第2改造丝心蛋白:从克隆出的天然来源的丝心蛋白的基因序列中对编码甘氨酸残基的碱基序列的至少一部分进行置换,使其编码其他氨基酸残基。此时,作为改造的甘氨酸残基,可以选择GGX基序及GPGXX基序中的一个甘氨酸残基,还可以置换成 z/w 为50.9%以上。此外,例如,也可以根据天然来源的丝心蛋白的氨基酸序列设计出满足上述方式的氨基酸序列,并且通过化学合成编码所设计的氨基酸序列的核酸而得到。在任何情况下,除了相当于从天然来源的丝心蛋白的氨基酸序列中将REP中的甘氨酸残基替换为其他氨基酸残基的改造外,还可以进一步进行相当于置换、缺失、插入及/或添加一个或多个氨基酸残基的氨基酸序列改造。

[0063] 作为上述的其他氨基酸残基,只要是除甘氨酸残基以外的氨基酸残基即可,并没有特别限制,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)残基及谷氨酸(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。

[0064] 作为第2改造丝心蛋白的更具体示例,可以列举出包含与(2-i)序列号6(Met-PRT380)、序列号7(Met-PRT410)、序列号8(Met-PRT525)或序列号9(Met-PRT799)所示的氨基酸序列、或者(2-ii)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%以上序列一致性的氨基酸序列的改造丝心蛋白。

[0065] 对(2-i)的改造丝心蛋白进行说明。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GGX全部置换为GQX,从而得到序列号6所示的氨基酸序列。从序列号6所示的氨基酸序列中从N末端侧向C末端侧使(A)_n基序每隔2个发生一个缺失,并进一步在C末端序列的近端插入一个[(A)_n基序-REP],从而得到序列号7所示的氨基酸序列。在序列号7所示的氨基酸序列的各(A)_n基序的C末端侧插入2个丙氨酸残基,进一步将部分谷氨酰胺(Q)残基置换为丝氨酸(S)残基,并且使C末端侧的部分氨基酸缺失以使得其与序列号7的分子量几乎相同,从而得到序列号8所示的氨基酸序列。在将序列号7所示的氨基酸序列中存在的20个结构域序列的区域(其中,该区域的C末端侧的数个氨基酸残基已被置换)重复4次后得到的序列的C末端添加指定的铰链序列和His标签序列,从而得到序列号9所示的氨基酸序列。

[0066] 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值分别为58.7%、70.1%、66.1%及70.0%。此外,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(在下文中说明)为1:1.8~11.3时的x/y值分别为15.0%、15.0%、93.4%、92.7%及89.8%。

[0067] (2-i)的改造丝心蛋白可以由序列号6、序列号7、序列号8或序列号9所示的氨基酸序列构成。

[0068] (2-ii)的改造丝心蛋白包含与序列号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%以上序列一致性的氨基酸序列。(2-ii)的改造丝心蛋白也为包含式1: [(A)_n基序-REP]_m所示的结构域序列的蛋白质。上述序列一致性优选为95%以上。

[0069] (2-ii)的改造丝心蛋白与序列号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%以上序列一致性,并且将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氨基酸残基总数设为z,将上述结构域序列中的REP的氨基酸残基总数设为w时,z/w优选为50.9%以上。

[0070] 第2改造丝心蛋白可以在N末端及C末端中的任意一端或两端包含标签序列。由此,可以实现改造丝心蛋白的分离、固定、检测及可视化等。

[0071] 作为标签序列,例如,可以列举出利用了与其他分子的特异性亲和性(结合性、亲和性)的亲和性标签。作为亲和性标签的具体示例,可以列举出组氨酸标签(His标签)。His标签是由4至10个左右的组氨酸残基排列而成的短肽,具有与镍等金属离子特异性结合的

性质,因此可以用于利用金属螯合层析(chelating metal chromatography)对改造丝心蛋白进行分离。作为标签序列的具体示例,例如,可以列举出序列号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)。

[0072] 此外,也可以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(MBP)等标签序列。

[0073] 还可以进一步使用利用了抗原抗体反应的“表位标签”。通过添加用于显示抗原性的肽(表位)作为标签序列,可以结合针对该表位的抗体。作为表位标签,可以列举出HA(流感病毒血凝素的肽序列)标签、myc标签、FLAG标签等。通过利用表位标签,可以很容易地以高特异性对改造丝心蛋白进行纯化。

[0074] 还可以进一步使用利用特定蛋白酶将标签序列切除而得到的标签。也可以通过对经由该标签序列吸附的蛋白质进行蛋白酶处理,来回收切除标签序列后的改造丝心蛋白。

[0075] 作为含有标签序列的改造丝心蛋白的更具体示例,可以列举出包含与(2-iii)序列号12(PRT380)、序列号13(PRT410)、序列号14(PRT525)或序列号15(PRT799)所示的氨基酸序列、或者(2-iv)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%以上序列一致性的氨基酸序列的改造丝心蛋白。

[0076] 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末端添加序列号11所示的氨基酸序列(包含His标签序列及铰链序列),从而得到序列号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。

[0077] (2-iii)的改造丝心蛋白可以由序列号12、序列号13、序列号14或序列号15所示的氨基酸序列构成。

[0078] (2-iv)的改造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%以上序列一致性的氨基酸序列。(2-iv)的改造丝心蛋白也为包含式1: $[(A)_n\text{基序-REP}]_m$ 所示的结构域序列的蛋白质。上述序列一致性优选为95%以上。

[0079] (2-iv)的改造丝心蛋白与序列号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%以上序列一致性,并且将REP中所含的由XGX(其中,X为甘氨酸以外的氨基酸残基)构成的氨基酸序列的氨基酸残基总数设为 z ,将上述结构域序列中的REP的氨基酸残基总数设为 w 时, z/w 优选为50.9%以上。

[0080] 第2改造丝心蛋白可以含有用于将重组蛋白生产系统所生产的蛋白质释放到宿主外部的分泌信号。分泌信号的序列可以根据宿主的种类来进行适当设定。

[0081] 第3改造丝心蛋白具有其结构域序列与天然来源的丝心蛋白相比 $(A)_n$ 基序的含量降低的氨基酸序列。第3改造丝心蛋白的结构域序列与天然来源的丝心蛋白相比,可以具有相当于缺失至少一个或多个 $(A)_n$ 基序的氨基酸序列。

[0082] 第3改造丝心蛋白可以具有相当于从天然来源的丝心蛋白中缺失10~40% $(A)_n$ 基序的氨基酸序列。

[0083] 第3改造丝心蛋白的结构域序列与天然来源的丝心蛋白相比,可以具有相当于至少从N末端侧向C末端侧每1~3个 $(A)_n$ 基序缺失一个 $(A)_n$ 基序的氨基酸序列。

[0084] 第3改造丝心蛋白的结构域序列与天然来源的丝心蛋白相比,可以具有相当于至少从N末端侧向C末端侧依次重复发生两个连续 $(A)_n$ 基序缺失及一个 $(A)_n$ 基序缺失的氨基酸序列。

[0085] 第3改造丝心蛋白的结构域序列可以具有相当于至少从N末端侧向C末端侧每隔2个发生一个(A)_n基序缺失的氨基酸序列。

[0086] 第3改造丝心蛋白包含式1: [(A)_n基序-REP]_m所示的结构域序列,并且可以具有下述氨基酸序列:从N末端侧向C末端侧依次比较相邻的2个[(A)_n基序-REP]单元的REP的氨基酸残基数,将氨基酸残基数少的REP的氨基酸残基数设为1时,将另一个REP的氨基酸残基数的比值为1.8~11.3的相邻的2个[(A)_n基序-REP]单元的氨基酸残基数相加而得到的合计值的最大值设为x,将结构域序列的氨基酸残基总数设为y时,x/y为20%以上、30%以上、40%以上或50%以上。(A)_n基序中的丙氨酸残基数相对于氨基酸残基总数可以为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%(意味着仅由丙氨酸残基构成)。

[0087] 参照图1进一步详细说明x/y的计算方法。图1示出了从改造丝心蛋白中除去N末端序列及C末端序列后的结构域序列。该结构域序列从N末端侧(左侧)起具有(A)_n基序-第1REP(50个氨基酸残基)-(A)_n基序-第2REP(100个氨基酸残基)-(A)_n基序-第3REP(10个氨基酸残基)-(A)_n基序-第4REP(20个氨基酸残基)-(A)_n基序-第5REP(30个氨基酸残基)-(A)_n基序的序列。

[0088] 以不重复的方式从N末端侧向C末端侧依次选择相邻的2个[(A)_n基序-REP]单元。此时,也可以存在未被选择的[(A)_n基序-REP]单元。图1示出了模式1(第1REP与第2REP的比较、以及第3REP与第4REP的比较)、模式2(第1REP与第2REP的比较、以及第4REP与第5REP的比较)、模式3(第2REP与第3REP的比较、以及第4REP与第5REP的比较)、模式4(第1REP与第2REP的比较)。另外,除此之外还存在选择方法。

[0089] 接下来,对各模式的所选择的相邻的2个[(A)_n基序-REP]单元中的各REP的氨基酸残基数进行比较。比较方法是將氨基酸残基数较少一方设为1,然后求出另一个氨基酸残基数的比值。例如,比较第1REP(50个氨基酸残基)与第2REP(100个氨基酸残基)时,将氨基酸残基数较少的第1REP设为1,则第2REP的氨基酸残基数的比值为100/50=2。同样地,比较第4REP(20个氨基酸残基)与第5REP(30个氨基酸残基)时,将氨基酸残基数较少的第4REP设为1,则第5REP的氨基酸残基数的比值为30/20=1.5。

[0090] 图1中,用实线表示将氨基酸残基数较少一方设为1时,另一方氨基酸残基数的比值为1.8~11.3的[(A)_n基序-REP]单元的组。在本说明书中,将该比值称为锯齿比率。用虚线表示将氨基酸残基数较少一方设为1时,另一方氨基酸残基数的比值小于1.8或大于11.3的[(A)_n基序-REP]单元的组。

[0091] 在各模式中,将实线所示的相邻的2个[(A)_n基序-REP]单元的所有氨基酸残基数相加(不只是REP,也是(A)_n基序的氨基酸残基数)。而且,对相加后的合计值进行比较,并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所示的示例中,模式1的合计值最大。

[0092] 接下来,可以通过x除以结构域序列的氨基酸残基总数y来计算出x/y(%)。

[0093] 在第3改造丝心蛋白中,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,进一步还优选为75%以上,特别优选为80%以上。x/y的上限并没有特别限制,例如可以为100%以下。锯齿比率为1:1.9~11.3时,x/y优选为89.6%以上,锯齿比率为1:1.8~3.4时,x/y优选为77.1%以上,锯齿比率为1:1.9~8.4时,

x/y优选为75.9%以上,锯齿比率为1:1.9~4.1时,x/y优选为64.2%以上。

[0094] 第3改造丝心蛋白为结构域序列中存在多个的(A)_n基序中的至少7个仅由丙氨酸残基构成的改造丝心蛋白时,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,进一步还优选为70%以上,特别优选为80%以上。x/y的上限并没有特别限制,为100%以下即可。

[0095] 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,通过例示在NCBI GenBank中登记有氨基酸序列信息的丝心蛋白的方法进行确认,提取出663种丝心蛋白(其中,来自于蜘蛛类的丝心蛋白为415种)。根据所提取的所有丝心蛋白中由式1:[(A)_n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,通过上述计算方法计算出x/y。图3表示锯齿比率为1:1.9~4.1时的结果。

[0096] 图3的横轴表示x/y(%),纵轴表示频率。由图3可知,天然来源的丝心蛋白中的x/y均小于64.2%(最大为64.14%)。

[0097] 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缺失一个或多个编码(A)_n基序的序列而使x/y为64.2%以上,从而得到第3改造丝心蛋白。此外,例如也可以设计出相当于从天然来源的丝心蛋白的氨基酸序列中缺失一个或多个(A)_n基序而使x/y为64.2%以上的氨基酸序列,并且通过化学合成编码所设计的氨基酸序列的核酸而得到。在任何情况下,除了相当于从天然来源的丝心蛋白的氨基酸序列中缺失(A)_n基序的改造外,还可以进一步进行相当于置换、缺失、插入及/或添加一个或多个氨基酸残基的氨基酸序列改造。

[0098] 作为第3改造丝心蛋白的更具体示例,可以列举出包含与(3-i)序列号17(Met-PRT399)、序列号7(Met-PRT410)、序列号8(Met-PRT525)或序列号9(Met-PRT799)所示的氨基酸序列、或者(3-ii)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%以上序列一致性的氨基酸序列的改造丝心蛋白。

[0099] 对(3-i)的改造丝心蛋白进行说明。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从N末端侧向C末端侧使(A)_n基序每隔2个发生一个缺失,并进一步在C末端序列的近端插入一个[(A)_n基序-REP],从而得到序列号17所示的氨基酸序列。序列号7、序列号8或序列号9所示的氨基酸序列如第2改造丝心蛋白中说明所示。

[0100] 序列号10所示的氨基酸序列(相当于天然来源的丝心蛋白)的锯齿比率为1:1.8~11.3中的x/y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值均为93.4%。序列号8所示的氨基酸序列中的x/y值为92.7%。序列号9所示的氨基酸序列中的x/y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值分别为46.8%、56.2%、70.1%、66.1%及70.0%。

[0101] (3-i)的改造丝心蛋白可以由序列号17、序列号7、序列号8或序列号9所示的氨基酸序列构成。

[0102] (3-ii)的改造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%以上序列一致性的氨基酸序列。(3-ii)的改造丝心蛋白也为包含式1:[(A)_n基序-REP]_m所示的结构域序列的蛋白质。上述序列一致性优选为95%以上。

[0103] (3-ii)的改造丝心蛋白与序列号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%以上序列一致性,并且从N末端侧向C末端侧依次比较相邻的2个[(A)_n基序-

REP]单元的REP的氨基酸残基数,将氨基酸残基数少的REP的氨基酸残基数设为1时,将另一个REP的氨基酸残基数的比值为1.8~11.3(锯齿比率为1:1.8~11.3)的相邻的2个[(A)_n基序-REP]单元的氨基酸残基数相加而得到的合计值的最大值设为x,将结构域序列的氨基酸残基总数设为y时,x/y优选为64.2%以上。

[0104] 第3改造丝心蛋白可以在N末端及C末端中的任意一端或两端包含上述标签序列。

[0105] 作为含有标签序列的改造丝心蛋白的更具体示例,可以列举出包含与(3-iii)序列号18(PRT399)、序列号13(PRT410)、序列号14(PRT525)或序列号15(PRT799)所示的氨基酸序列、或者(3-iv)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%以上序列一致性的氨基酸序列的改造丝心蛋白。

[0106] 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末端添加序列号11所示的氨基酸序列(包含His标签序列及铰链序列),从而得到序列号18、序列号13、序列号14及序列号15所示的氨基酸序列。

[0107] (3-iii)的改造丝心蛋白可以由序列号18、序列号13、序列号14或序列号15所示的氨基酸序列构成。

[0108] (3-iv)的改造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%以上序列一致性的氨基酸序列。(3-iv)的改造丝心蛋白也为包含式1: [(A)_n基序-REP]_m所示的结构域序列的蛋白质。上述序列一致性优选为95%以上。

[0109] (3-iv)的改造丝心蛋白与序列号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%以上序列一致性,并且从N末端侧向C末端侧依次比较相邻的2个[(A)_n基序-REP]单元的REP的氨基酸残基数,将氨基酸残基数少的REP的氨基酸残基数设为1时,将另一个REP的氨基酸残基数的比值为1.8~11.3的相邻的2个[(A)_n基序-REP]单元的氨基酸残基数相加而得到的合计值的最大值设为x,将结构域序列的氨基酸残基总数设为y时,x/y优选为64.2%以上。

[0110] 第3改造丝心蛋白可以含有用于将重组蛋白生产系统所生产的蛋白质释放到宿主外部的分泌信号。分泌信号的序列可以根据宿主的种类来进行适当设定。

[0111] 第4改造丝心蛋白的结构域序列与天然来源的丝心蛋白相比,具有(A)_n基序含量降低且甘氨酸残基含量降低的氨基酸序列。第4改造丝心蛋白的结构域序列与天然来源的丝心蛋白相比,可以具有相当于缺失至少一个或多个(A)_n基序且至少REP中的一个或多个甘氨酸残基被其他氨基酸残基置换的氨基酸序列。即,第4改造丝心蛋白是兼具上述第2改造丝心蛋白和第3改造丝心蛋白的特征的改造丝心蛋白。具体实施方式等如第2改造丝心蛋白及第3改造丝心蛋白中说明所示。

[0112] 作为第4改造丝心蛋白的更具体示例,可以列举出包含与(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)或序列号15(PRT799)所示的氨基酸序列、或者(4-ii)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所示的氨基酸序列具有90%以上序列一致性的氨基酸序列的改造丝心蛋白。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造丝心蛋白的具体实施方式如上所述。

[0113] 第5改造丝心蛋白的结构域序列与天然来源的丝心蛋白相比,可以具有相当于REP中的一个或多个氨基酸残基被置换为疏水性指标大的氨基酸残基及/或在REP中插入一个

或多个疏水性指标大的氨基酸残基的包含局部疏水性指标大的区域的氨基酸序列。

[0114] 局部疏水性指标大的区域优选由连续的2~4个氨基酸残基构成。

[0115] 上述疏水性指标大的氨基酸残基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)中的氨基酸残基。

[0116] 第5改造丝心蛋白与天然来源的丝心蛋白相比,除了相当于REP中的一个或多个氨基酸残基被置换为疏水性指标大的氨基酸残基及/或在REP中插入一个或多个疏水性指标大的氨基酸残基的改造外,进一步与天然来源的丝心蛋白相比,还可以有相当于置换、缺失、插入及/或添加一个或多个氨基酸残基的氨基酸序列改造。

[0117] 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将REP中的一个或多个亲水性氨基酸残基(例如疏水性指标为负的氨基酸残基)置换为疏水性氨基酸残基(例如疏水性指标为正的氨基酸残基)、及/或在REP中插入一个或多个疏水性氨基酸残基,从而得到第5改造丝心蛋白。此外,例如也可以设计出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置换为疏水性氨基酸残基及/或在REP中插入一个或多个疏水性氨基酸残基的氨基酸序列,并且通过化学合成编码所设计的氨基酸序列的核酸而得到。在任何情况下,除了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置换为疏水性氨基酸残基及/或在REP中插入一个或多个疏水性氨基酸残基的改造外,还可以进一步进行置换、缺失、插入及/或添加一个或多个氨基酸残基的氨基酸序列改造。

[0118] 第5改造丝心蛋白包含式1: $[(A)_n \text{ 基序-REP}]_m$ 所示的结构域序列,并且可以具有下述氨基酸序列:从上述结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至上述结构域序列的C末端为止的序列后得到的序列所含的所有REP中,将连续的4个氨基酸残基的疏水性指标的平均值为2.6以上的区域所含的氨基酸残基总数设为p,将从上述结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至上述结构域序列的C末端为止的序列后得到的序列所含的氨基酸残基总数设为q时,p/q为6.2%以上。

[0119] 关于氨基酸残基的疏水性指标,使用公知的指标(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标(亲水指数,以下也记为“HI”)如下述表1所示。

[0120] [表1]

[0121]

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	天门冬氨(Asn)	-3.5
蛋氨酸(Met)	1.9	天门冬氨酸(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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[0122] 对 p/q 的计算方法进一步进行详细说明。在计算中,使用从式1: $[(A)_n \text{基序-REP}]_m$ 所示的结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列后得到的序列(以下称为“序列A”)。首先,在序列A所包含的所有REP中,计算连续的4个氨基酸残基的疏水性指标的平均值。疏水性指标的平均值通过连续的4个氨基酸残基中所含有的各氨基酸残基的HI总和除以4(氨基酸残基数)而求出。疏水性指标的平均值针对所有连续的4个氨基酸残基而求出(各氨基酸残基用于1~4次平均值的计算)。接下来,确定连续的4个氨基酸残基的疏水性指标的平均值为2.6以上的区域。即使某个氨基酸残基属于多处“疏水性指标的平均值为2.6以上的连续的4个氨基酸残基”,也会作为一个氨基酸残基包含在区域中。而且,该区域中所含的氨基酸残基总数为 p 。此外,序列A中所含的氨基酸残基总数为 q 。

[0123] 例如,提取到20处“疏水性指标的平均值为2.6以上的连续的4个氨基酸残基”时(没有重复),在连续的4个氨基酸残基的疏水性指标的平均值为2.6以上的区域中含有20处连续的4个氨基酸残基(没有重复), p 为 $20 \times 4 = 80$ 。此外,例如,2处“疏水性指标的平均值为2.6以上的连续的4个氨基酸残基”仅有一个氨基酸残基重复存在时,在连续的4个氨基酸残基的疏水性指标的平均值为2.6以上的区域中,含有7个氨基酸残基($p = 2 \times 4 - 1 = 7$,-1”为扣除重复部分)。例如,为图4所示的结构域序列时,“疏水性指标的平均值为2.6以上的连续的4个氨基酸残基”不重复且存在7个,因此 p 为 $7 \times 4 = 28$ 。此外,例如为图4所示的结构域序列时, q 为 $4 + 50 + 4 + 40 + 4 + 10 + 4 + 20 + 4 + 30 = 170$ (不包含位于C末端侧最后的 $(A)_n$ 基序)。然后,通过 p 除以 q ,可以计算出 p/q (%)。图4时为 $28/170 = 16.47\%$ 。

[0124] 在第5改造丝心蛋白中, 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,进一步还优选为30%以上。 p/q 的上限并没有特别限制,例如可以为45%以下。

[0125] 例如,可以通过将克隆出的天然来源的丝心蛋白的氨基酸序列的REP中的一个或多个亲水性氨基酸残基(例如疏水性指标为负的氨基酸残基)置换为疏水性氨基酸残基(例如疏水性指标为正的氨基酸残基)、及/或在REP中插入一个或多个疏水性氨基酸残基,使其满足上述 p/q 的条件,来将其改造成包含局部疏水性指标大的区域的氨基酸序列,从而得到第5改造丝心蛋白。此外,例如,也可以根据天然来源的丝心蛋白的氨基酸序列设计出满足上述 p/q 条件的氨基酸序列,并且通过化学合成编码所设计的氨基酸序列的核酸而得到。在任何情况下,与天然来源的丝心蛋白相比,除了相当于REP中的一个或多个氨基酸残基被置换为疏水性指标大的氨基酸残基及/或在REP中插入一个或多个疏水性指标大的氨基酸残基的改造外,还可以进一步进行相当于置换、缺失、插入及/或添加一个或多个氨基酸残基的改造。

[0126] 作为疏水性指标大的氨基酸残基,并没有特别限制,优选为异亮氨酸(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),更优选为缬氨酸(V)、亮氨酸(L)及异亮氨酸(I)。

[0127] 作为第5改造丝心蛋白的更具体示例,可以列举出包含与(5-i)序列号19(Met-PRT720)、序列号20(Met-PRT665)或序列号21(Met-PRT666)所示的氨基酸序列、或者(5-ii)序列号19、序列号20或序列号21所示的氨基酸序列具有90%以上序列一致性的氨基酸序列

的改造丝心蛋白。

[0128] 对(5-i)的改造丝心蛋白进行说明。相对于序列号7(Met-PRT410)所示的氨基酸序列,除C末端侧的终端的结构域序列外,每隔一个REP插入2处分别由3个氨基酸残基构成的氨基酸序列(VLI),进而将一部分的谷氨酰胺(Q)残基替换为丝氨酸(S)残基,并且使C末端侧的一部分氨基酸缺失,从而得到序列号19所示的氨基酸序列。相对于序列号8(Met-PRT525)所示的氨基酸序列,每隔一个REP插入1处分别由3个氨基酸残基构成的氨基酸序列(VLI),从而得到序列号20所示的氨基酸序列。相对于序列号8所示的氨基酸序列,每隔一个REP插入2处分别由3个氨基酸残基构成的氨基酸序列(VLI),从而得到序列号21所示的氨基酸序列。

[0129] (5-i)的改造丝心蛋白可以由序列号19、序列号20或序列号21所示的氨基酸序列构成。

[0130] (5-ii)的改造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%以上序列一致性的氨基酸序列。(5-ii)的改造丝心蛋白也为包含式1: $[(A)_n \text{基序} - \text{REP}]_m$ 所示的结构域序列的蛋白质。上述序列一致性优选为95%以上。

[0131] (5-ii)的改造丝心蛋白与序列号19、序列号20或序列号21所示的氨基酸序列具有90%以上序列一致性,并且从结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列后得到的序列所含的所有REP中,将连续的4个氨基酸残基的疏水性指标的平均值为2.6以上的区域所含的氨基酸残基总数设为p,将从结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列后得到的序列所含的氨基酸残基总数设为q时, p/q 优选为6.2%以上。

[0132] 第5改造丝心蛋白可以在N末端及C末端中的任意一端或两端包含标签序列。

[0133] 作为含有标签序列的改造丝心蛋白的更具体示例,可以列举出包含与(5-iii)序列号22(PRT720)、序列号23(PRT665)或序列号24(PRT666)所示的氨基酸序列、或者(5-iv)序列号22、序列号23或序列号24所示的氨基酸序列具有90%以上序列一致性的氨基酸序列的改造丝心蛋白。

[0134] 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末端添加序列号11所示的氨基酸序列(包含His标签序列及铰链序列),从而得到序列号22、序列号23及序列号24所示的氨基酸序列。

[0135] (5-iii)的改造丝心蛋白可以由序列号22、序列号23或序列号24所示的氨基酸序列构成。

[0136] (5-iv)的改造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%以上序列一致性的氨基酸序列。(5-iv)的改造丝心蛋白也为包含式1: $[(A)_n \text{基序} - \text{REP}]_m$ 所示的结构域序列的蛋白质。上述序列一致性优选为95%以上。

[0137] (5-iv)的改造丝心蛋白与序列号22、序列号23或序列号24所示的氨基酸序列具有90%以上序列一致性,并且从结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列后得到的序列所含的所有REP中,将连续的4个氨基酸残基的疏水性指标的平均值为2.6以上的区域所含的氨基酸残基总数设为p,将从结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列后得到的序列所含的氨基酸残基总数设为q时, p/q 优选为6.2%以上。

[0138] 第5改造丝心蛋白可以含有用于将重组蛋白生产系统所生产的蛋白质释放到宿主外部的分泌信号。分泌信号的序列可以根据宿主的种类来进行适当设定。

[0139] 第6改造丝心蛋白与天然来源的丝心蛋白相比,具有谷氨酰胺残基含量降低的氨基酸序列。

[0140] 第6改造丝心蛋白优选在REP的氨基酸序列中包含选自GGX基序及GPGXX基序中的至少1种基序。

[0141] 第6改造丝心蛋白在REP中包含GPGXX基序时,GPGXX基序含有率通常为1%以上,也可以为5%以上,优选为10%以上。GPGXX基序含有率的上限并没有特别限制,可以为50%以下,也可以为30%以下。

[0142] 在本说明书中,“GPGXX基序含有率”是通过以下方法计算出的值。

[0143] 在包含式1: $[(A)_n \text{基序-REP}]_m$ 或式2: $[(A)_n \text{基序-REP}]_m - (A)_n$ 基序所示的结构域序列的丝心蛋白(改造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自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列后得到的序列所含的所有REP中,将该区域所含的GPGXX基序的总个数乘以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自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列且进一步除去 $(A)_n$ 基序后得到的所有REP的氨基酸残基总数设为t时,以s/t来计算GPGXX基序含有率。

[0144] 在GPGXX基序含有率的计算中,将“从结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列后得到的序列”作为对象是为了排除下述影响:“位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列”(相当于REP的序列)中有时包含在丝心蛋白中与特征性序列相关性低的序列,m较小时(即,结构域序列短时)会影响GPGXX基序含有率的计算结果。另外,“GPGXX基序”位于REP的C末端时,即使“XX”例如为“AA”,也作为“GPGXX基序”进行处理。

[0145] 图5是表示改造丝心蛋白的结构域序列的示意图。参照图5来具体说明GPGXX基序含有率的计算方法。首先,在图5所示的改造丝心蛋白的结构域序列(“ $[(A)_n \text{基序-REP}]_m - (A)_n$ 基序”类型)中,所有REP都包含在“从结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列后得到的序列”(图5中“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 $7 \times 3 = 21$ 。同样地,由于所有REP都包含在“从结构域序列中除去自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列后得到的序列”(图5中“区域A”所示的序列)中,因此从该序列中进一步除去 $(A)_n$ 基序后得到的所有REP的氨基酸残基总数t为 $50 + 40 + 10 + 20 + 30 = 150$ 。接下来,通过s除以t,可以计算出s/t(%),为图5的改造丝心蛋白时,为 $21/150 = 14.0\%$ 。

[0146] 在第6改造丝心蛋白中,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%。

[0147] 在本说明书中,“谷氨酰胺残基含有率”是通过以下方法计算出的值。在包含式1: $[(A)_n \text{基序-REP}]_m$ 或式2: $[(A)_n \text{基序-REP}]_m - (A)_n$ 基序所示的结构域序列的丝心蛋白(改造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自位于最靠C末端侧的 $(A)_n$ 基序至结构域序列的C末端为止的序列后得到的序列(相当于图5的“区域A”的序列)所含的所有REP中,将该区域所含的谷氨酰胺残基总数设为u,将从结构域序列中除去自位于最靠C末端

侧的(A)_n基序至结构域序列的C末端为止的序列且进一步除去(A)_n基序后得到的所有REP的氨基酸残基总数设为t时,以u/t来计算谷氨酰胺残基含有率。在谷氨酰胺残基含有率的计算中,以“从结构域序列中除去自位于最靠C末端侧的(A)_n基序至结构域序列的C末端为止的序列后得到的序列”为对象的理由与上述理由相同。

[0148] 第6改造丝心蛋白的结构域序列与天然来源的丝心蛋白相比,可以具有相当于缺失REP中的一个或多个谷氨酰胺残基或将其替换为其他氨基酸残基的氨基酸序列。

[0149] “其他氨基酸残基”只要是谷氨酰胺残基以外的氨基酸残基即可,优选为疏水性指标大于谷氨酰胺残基的氨基酸残基。氨基酸残基的疏水性指标如表1所示。

[0150] 如表1所示,作为疏水性指标大于谷氨酰胺残基的氨基酸残基,可以列举出选自异亮氨酸(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)中的氨基酸残基。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)中的氨基酸残基,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)中的氨基酸残基。

[0151] 在第6改造丝心蛋白中,REP的疏水性程度优选为-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的疏水性程度的上限并没有特别限制,可以为1.0以下,也可以为0.7以下。

[0152] 在本说明书中,“REP的疏水性程度”为通过以下方法计算出的值。

[0153] 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(改造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自位于最靠C末端侧的(A)_n基序至结构域序列的C末端为止的序列后得到的序列(相当于图5的“区域A”的序列)所含的所有REP中,将该区域的各氨基酸残基的疏水性指标总和设为v,将从结构域序列中除去自位于最靠C末端侧的(A)_n基序至结构域序列的C末端为止的序列且进一步除去(A)_n基序后得到的所有REP的氨基酸残基总数设为t时,以v/t来计算REP的疏水性程度。在REP的疏水性程度的计算中,以“从结构域序列中除去自位于最靠C末端侧的(A)_n基序至结构域序列的C末端为止的序列后得到的序列”为对象的理由与上述理由相同。

[0154] 第6改造丝心蛋白的结构域序列与天然来源的丝心蛋白相比,除了相当于缺失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替换为其他氨基酸残基的改造外,还可以进一步进行相当于置换、缺失、插入及/或添加一个或多个氨基酸残基的氨基酸序列改造。

[0155] 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替换为其他氨基酸残基,从而得到第6改造丝心蛋白。此外,例如也可以设计出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替换为其他氨基酸残基的氨基酸序列,并且通过化学合成编码所设计的氨基酸序列的核酸而得到。

[0156] 作为第6改造丝心蛋白的更具体示例,可以列举出包含(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) 或序列号42 (Met-PRT1028) 所示的氨基酸序列的改造丝心蛋白,或者包含与(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或序列号42所示的氨基酸序列具有90%以上序列一致性的氨基酸序列的改造丝心蛋白。

[0157] 对(6-i)的改造丝心蛋白进行说明。将序列号7所示的氨基酸序列 (Met-PRT410) 中的QQ全部置换为VL,从而得到序列号25所示的氨基酸序列。将序列号7所示的氨基酸序列中的QQ全部置换为TS,并且将剩余的Q置换为A,从而得到序列号26所示的氨基酸序列。将序列号7所示的氨基酸序列中的QQ全部置换为VL,并且将剩余的Q置换为I,从而得到序列号27所示的氨基酸序列。将序列号7所示的氨基酸序列中的QQ全部置换为VI,并且将剩余的Q置换为L,从而得到序列号28所示的氨基酸序列。将序列号7所示的氨基酸序列中的QQ全部置换为VF,并且将剩余的Q置换为I,从而得到序列号29所示的氨基酸序列。

[0158] 将序列号8所示的氨基酸序列 (Met-PRT525) 中的QQ全部置换为VL,从而得到序列号30所示的氨基酸序列。将序列号8所示的氨基酸序列中的QQ全部置换为VL,并且将剩余的Q置换为I,从而得到序列号31所示的氨基酸序列。

[0159] 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2次后得到的序列中的QQ全部置换为VF,并且将剩余的Q置换为I,从而得到序列号32所示的氨基酸序列。

[0160] 将序列号7所示的氨基酸序列中的QQ全部置换为LI,并且将剩余的Q置换为V,从而得到序列号41所示的氨基酸序列 (Met-PRT917)。将序列号7所示的氨基酸序列中的QQ全部置换为IF,并且将剩余的Q置换为T,从而得到序列号42所示的氨基酸序列 (Met-PRT1028)。

[0161] 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)。

[0162] [表2]

[0163]	改造丝心蛋白	谷氨酰胺残基含有率	GPGXX 基序含有率	REP 疏水性程度
	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

[0164] (6-i)的改造丝心蛋白可以由序列号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。

[0165] (6-ii)的改造丝心蛋白包含与序列号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%以上序列一致性的氨基酸序列。(6-ii)的改造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。上述序列一致性优选为95%以上。

[0166] (6-ii)的改造丝心蛋白的谷氨酰胺残基含有率优选为9%以下。此外,(6-ii)的改造丝心蛋白的GPGXX基序含有率优选为10%以上。

[0167] 第6改造丝心蛋白可以在N末端及C末端中的任意一端或两端包含标签序列。由此,可以实现改造丝心蛋白的分离、固定、检测及可视化等。

[0168] 作为含有标签序列的改造丝心蛋白的更具体示例,可以列举出包含(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)或序列号44(PRT1028)所示的氨基酸序列的改造丝心蛋白,或者包含与(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或序列号44所示的氨基酸序列具有90%以上序列一致性的氨基酸序列的改造丝心蛋白。

[0169] 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末端添加序列号11所示的氨基酸序列(包含His标签序列及铰链序列),从而得到序列号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。由于仅在N末端添加了标签序列,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)。

[0170] [表3]

[0171]	改造丝心蛋白	谷氨酰胺残基含有率	GPGXX 基序含有率	REP 疏水性程度
	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

[0172] (6-iii)的改造丝心蛋白可以由序列号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。

[0173] (6-iv)的改造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%以上序列一致性的氨基酸序列。(6-iv)的改造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。上述序列一致性优选为95%以上。

[0174] (6-iv)的改造丝心蛋白的谷氨酰胺残基含有率优选为9%以下。此外,(6-iv)的改造丝心蛋白的GPGXX基序含有率优选为10%以上。

[0175] 第6改造丝心蛋白可以含有用于将重组蛋白生产系统所生产的蛋白质释放到宿主外部的分泌信号。分泌信号的序列可以根据宿主的种类来进行适当设定。

[0176] 改造丝心蛋白可以为兼具第1改造丝心蛋白、第2改造丝心蛋白、第3改造丝心蛋白、第4改造丝心蛋白、第5改造丝心蛋白及第6改造丝心蛋白所具有特征中的至少2种以上特征的改造丝心蛋白。

[0177] 作为改造丝心蛋白,可以是亲水性改造丝心蛋白,也可以是疏水性改造丝心蛋白。在本说明书中,“亲水性改造丝心蛋白”是指疏水性指标的平均值(平均HI)为0以下的改造丝心蛋白。在本说明书中,“疏水性改造丝心蛋白”是指平均HI超过0的改造丝心蛋白。平均HI是求出构成改造丝心蛋白的所有氨基酸残基的疏水性指标(HI)总和,然后用该总和除以氨基酸残基总数后得到的值。疏水性指标如表1所示。亲水性改造丝心蛋白具有充分的接触冷感性,且吸水速干性优异。疏水性改造丝心蛋白不仅接触冷感性优异,且具有充分的吸水速干性。

[0178] 作为亲水性改造丝心蛋白,例如,可以列举出包含序列号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造丝心蛋白。

[0179] 作为疏水性改造丝心蛋白,例如,可以列举出包含序列号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,以及序列号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造丝心蛋白。

[0180] 本实施方式所涉及的改造丝心蛋白可以使用编码该改造丝心蛋白的核酸,利用常规方法进行制造。编码该改造丝心蛋白的核酸可以根据碱基序列信息进行化学合成,也可以利用PCR法等进行合成。此外,所制造的改造丝心蛋白的分离和纯化也可以利用常用方法来进行。

[0181] (接触冷感性及吸水速干性赋予剂)

[0182] 本实施方式所涉及的接触冷感性及吸水速干性赋予剂可以仅包含1种改造丝心蛋白,也可以组合包含2种以上改造丝心蛋白。

[0183] 本实施方式所涉及的接触冷感性及吸水速干性赋予剂通过接触冷暖感测试仪进行评估的热流量峰值($q_{\max}$, W/cm²)可以是0.055W/cm²以上,可以是0.060W/cm²以上,可以是0.065W/cm²以上,还可以是0.070W/cm²以上。热流量峰值的上限并没有特别限制,通常为0.2W/cm²以下。热流量峰值例如可以通过后述实施例中记载的方法来进行评估。

[0184] 本实施方式所涉及的接触冷感性及吸水速干性赋予剂按照JIS L 1907进行评估的吸水性可以为60秒以下,可以为30秒以下,可以为20秒以下,可以为10秒以下,还可以为5秒以下。吸水性例如可以通过后述实施例中记载的方法来进行评估。

[0185] 本实施方式所涉及的接触冷感性及吸水速干性赋予剂通过测量扩散性残留水分率进行评估的速干性可以为100分钟以下,可以为90分钟以下,可以为80分钟以下,还可以

为70分钟以下。扩散性残留水分率(%)是通过下式计算出的值。扩散性残留水分率(%) = 各时间的水的重量(g) / 测量开始时的水的重量(g) × 100。速干性是指扩散性残留水分率降至10%以下所需要的时间。速干性例如可以通过后述实施例中记载的方法来进行评估。

[0186] 本实施方式所涉及的接触冷感性及吸水速干性赋予剂根据其形态、用途等还可以包含其他添加剂(有效成分以外的成分)。作为添加剂,例如,可以列举出增塑剂、流平剂、交联剂、结晶成核剂、抗氧化剂、紫外线吸收剂、着色剂、填料及合成树脂。添加剂的含量可以在不损害本发明效果的范围内进行设定。

[0187] 本实施方式所涉及的接触冷感性及吸水速干性赋予剂例如可以是粉末状、糊状、液状(例如悬浊液、溶液)中的任意一种形态。本实施方式所涉及的接触冷感性及吸水速干性赋予剂还可以是例如纤维、薄膜、凝胶、多孔质体、颗粒等形态。本实施方式所涉及的接触冷感性及吸水速干性赋予剂的形态可以根据赋予接触冷感性及吸水速干性的对象物品的种类及用途来适当设定。

[0188] 本实施方式所涉及的接触冷感性及吸水速干性赋予剂由于包含改造丝心蛋白作为主成分,因此可以成型为上述任意形态。该成型体可以是将改造丝心蛋白本身成型的成型体,也可以是将改造丝心蛋白与其他材料组合成型的成型体。

[0189] 以粉末状形态制备本实施方式所涉及的接触冷感性及吸水速干性赋予剂时,例如也可以对通过上述改造丝心蛋白的制造方法得到的蛋白质进行干燥来制成粉末状。在蛋白质粉末中,根据需要还可以包含其他添加剂。

[0190] 以液状(例如溶液)形态制备本实施方式所涉及的接触冷感性及吸水速干性赋予剂时,例如也可以利用可溶解改造丝心蛋白的溶剂将通过上述改造丝心蛋白的制造方法得到的蛋白质溶解来制成液状(改造丝心蛋白溶液)。改造丝心蛋白溶液中根据需要还可以包含其他添加剂。作为可溶解改造丝心蛋白的溶剂,例如,可以列举出二甲基亚砜(DMSO)、N,N-二甲基甲酰胺(DMF)、甲酸和六氟异丙醇(HFIP)等。该溶剂中可以添加无机盐作为溶解促进剂。

[0191] 以纤维形态制备本实施方式所涉及的接触冷感性及吸水速干性赋予剂时,例如可以将上述改造丝心蛋白溶液作为掺杂液,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进行纺丝来制成纤维(改造丝心蛋白纤维)。作为纤维形态,可以是单纱线,也可以是混纺纱、混纤纱、混纺针织纱、交捻纱、并捻纱以及包芯纱等复合纱,还可以是无纺布等。

[0192] 改造丝心蛋白纤维可以是短纤维,也可以是长纤维。此外,改造丝心蛋白纤维可以单独使用改造丝心蛋白纤维,也可以与其他纤维组合使用。即,仅由改造丝心蛋白纤维构成的单纱、将改造丝心蛋白纤维与其他纤维组合而成的复合纱可以分别单独使用,或者也可以组合使用。上述单纱及上述复合纱可以是将短纤维加捻而成的纺纱,也可以是将长纤维加捻或未加捻而成的长纱。此外,改造丝心蛋白纤维可以是短纤维,也可以是长纤维,还能够以不加工成纱的纤维状态单独使用或与其他纤维组合使用。作为其他纤维,例如,可以列举出尼龙、聚酯等合成纤维,铜氨纤维、人造丝等再生纤维,棉、亚麻、兽毛纤维、丝绸等天然纤维。与其他纤维组合使用时,以纤维总量为基准,改造丝心蛋白纤维含量优选为20质量%以上,更优选为30质量%以上,进一步优选为40质量%以上,进一步更优选为50质量%以上。

[0193] 以薄膜、凝胶、多孔质体、颗粒等形态制备本实施方式所涉及的接触冷感性及吸水速干性赋予剂时,例如可以按照日本特开2009-505668号公报、日本特开2009-505668号公报、日本特许第5678283号公报、日本特许第4638735号公报等中记载的方法来制造。

[0194] (赋予物品接触冷感性及吸水速干性的方法)

[0195] 本实施方式所涉及的赋予物品接触冷感性及吸水速干性的方法包括使物品含有改造丝心蛋白的工序。本发明所涉及的改造丝心蛋白由于兼具接触冷感性及吸水速干性,因此通过使物品含有该改造丝心蛋白,可以赋予物品接触冷感性及吸水速干性。

[0196] 物品只要是需赋予接触冷感性及吸水速干性的物品即可,并没有特别限制。具体而言,可以列举出纤维、纺织物、编织物、无纺布、棉、海绵、薄膜、树脂、复合材料(与本实施方式所涉及的接触冷感性及吸水速干性赋予剂的形态无关)、以及利用它们制作的各种物品等。

[0197] 使物品含有改造丝心蛋白的工序也可以是通过使物品含有上述本发明所涉及的接触冷感性及吸水速干性赋予剂来使物品含有改造丝心蛋白的工序。作为使物品含有改造丝心蛋白的方法,并没有特别限制,可以是在材料(原料)中混合改造丝心蛋白的方法,也可以是将以上述成型体形态制备的接触冷感性及吸水速干性赋予剂与其他材料(成型体等)组合来形成物品的方法。此外,还可以是通过将改造丝心蛋白(根据需要也可以包含其他添加剂)本身成型来形成物品(成型体)的方法。

[0198] 以物品总量为基准,物品中的改造丝心蛋白的含量优选为20质量%以上,更优选为30质量%以上,进一步优选为40质量%以上,进一步更优选为50质量%以上。以物品总量为基准,改造丝心蛋白的含量的上限可以为100质量%,也可以为90质量%以下。通过调节物品中改造丝心蛋白的含量,可以控制物品的接触冷感性及吸水速干性。即,由于本发明所涉及的改造丝心蛋白兼具接触冷感性及吸水速干性,因此随着提高物品中改造丝心蛋白的含量,可以进一步提高物品的接触冷感性及吸水速干性。

[0199] 上述本发明还可视为用于赋予物品接触冷感性及吸水速干性的改造丝心蛋白的用途,即视为包括使物品含有改造丝心蛋白的工序的用途。

[0200] 实施例

[0201] 下面,将基于实施例等更具体地说明本发明。但是,本发明并不限于以下实施例。

[0202] (试验例1:改造丝心蛋白纤维的制造)

[0203] (1) 表达载体的制备

[0204] 设计出具有序列号40所示的氨基酸序列的改造丝心蛋白(PRT966)及具有序列号15所示的氨基酸序列的改造丝心蛋白(PRT799)。合成编码所设计的改造丝心蛋白的核酸。该核酸中,在5'末端附加有NdeI位点、在终止密码子下游附加有EcoRI位点。将该核酸克隆至克隆载体(pUC118)中。然后,利用NdeI和EcoRI对该核酸进行限制酶处理并将其切出后,重组到蛋白质表达载体pET-22b(+)中,得到表达载体。

[0205] (2) 蛋白质的表达

[0206] 用得到的表达载体转化大肠杆菌BLR(DE3)。将该转化的大肠杆菌在含有氨苄西林的2mL LB培养基中培养15小时。将该培养液添加到含有氨苄西林的100mL的种子培养用培养基(表4)中,以使 OD_{600} 达到0.005。将培养液温度保持在30℃,进行烧瓶培养直至 OD_{600} 达到5为止(约15小时),得到种子培养液。

[0207] [表4]

[0208] 种子培养用培养基

[0209]	试剂	浓度(g/L)
	葡萄糖	5.0
	KH ₂ PO ₄	4.0
	K ₂ HPO ₄	9.3
	酵母提取物	6.0
	氨苄西林	0.1

[0210] 将该种子培养液添加到加有500ml生产培养基(表5)的发酵罐中,以使OD₆₀₀达到0.05。将培养液温度保持在37℃,将pH值恒定控制在6.9下进行培养。此外,将培养液中的溶解氧浓度维持在溶解氧饱和浓度的20%。

[0211] [表5]

[0212] 生产培养基

[0213]	试剂	浓度(g/L)
	葡萄糖	12.0
	KH ₂ PO ₄	9.0
	MgSO ₄ ·7H ₂ O	2.4
	酵母提取物	15
	FeSO ₄ ·7H ₂ O	0.04
	MnSO ₄ ·5H ₂ O	0.04
	CaCl ₂ ·2H ₂ O	0.04
	ADEKANOL (ADEKA、LG-295S)	0.1 (mL/L)

[0214] 在生产培养基中的葡萄糖完全消耗后,立即以1mL/分钟的速度添加进料液(葡萄糖455g/1L、酵母提取物120g/1L)。将培养液温度保持在37℃,将pH值恒定控制在6.9下进行培养。此外,将培养液中的溶解氧浓度维持在溶解氧饱和浓度的20%,培养20小时。然后,向培养液中添加1M的异丙基-β-硫代半乳糖吡喃糖苷(IPTG)以使其最终浓度为1mM,并表达诱导改造丝心蛋白。在添加IPTG后经过20小时的时间点,离心分离培养液,回收菌体。使用由添加IPTG前和添加IPTG后的培养液制备的菌体进行SDS-PAGE,根据依赖于IPTG添加的目标改造丝心蛋白尺寸的条带的出现,确认到目标改造丝心蛋白的表达。

[0215] (3) 蛋白质的纯化

[0216] 将添加IPTG后经过2小时回收的菌体用20mM Tris-HCl buffer (pH7.4) 进行清洗。将清洗后的菌体悬浮在含有约1mM的PMSF的20mM Tris-HCl缓冲液(pH7.4)中,用高压均质器(GEA Niro Soavi公司制)来破碎细胞。对破碎后的细胞进行离心分离,得到沉淀物。利用20mM Tris-HCl缓冲液(pH7.4)清洗所得到的沉淀物,直至达到高纯度为止。将清洗后的沉淀物以达到100mg/mL浓度的方式悬浊在8M胍缓冲液(8M胍盐酸盐、10mM磷酸二氢钠、20mM NaCl、1mM Tris-HCl、pH7.0)中,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的纤维素管36/32)用水进行透析。通过离心分离来回收透析后得到的白色凝聚蛋白质,用冷冻干燥机除去水分,通过回收冻干粉,而获得改造丝心蛋白

(PRT966及PRT799)。

[0217] PRT966是平均HI超过0的疏水性改造丝心蛋白。PRT799是平均HI为0以下的亲水性改造丝心蛋白。

[0218] (4) 改造丝心蛋白纤维的制造

[0219] 准备以4.0质量%溶解有LiCl的二甲基亚砜(DMSO)作为溶剂,向其添加改造丝心蛋白的冻干粉使其浓度达到24质量%,并使用振荡器溶解3小时。然后,除去不溶物和气泡,得到改造丝心蛋白溶液(纺丝原液)。

[0220] 将制备的纺丝原液以60℃用网孔为5 μ m的金属过滤器过滤,然后将其放入30mL的不锈钢注射器内静置并使其脱泡后,从针头直径为0.2mm的实心喷嘴喷到100质量%的甲醇凝固浴槽中。喷出温度为60℃。凝固后,卷取所得到的原丝并使其自然干燥,得到改造丝心蛋白纤维(原料纤维)。

[0221] (试验例2:吸水速干性的评估)

[0222] (1) 针织物的制造

[0223] 使用试验例1准备的各种原料纤维,使用横编织机进行横编织来制造针织物。为了比较,准备市售的丝绸纤维、棉纤维及聚酯纤维作为原料纤维。

[0224] 使用PRT966纤维作为原料纤维的针织物,其纤度:1/30Nm(毛线支数单丝),针距:18。使用PRT799纤维作为原料纤维的针织物,其纤度:1/30Nm(毛线支数单丝),针距:16。调整使用了其他原料纤维的针织物的纤度及针距,使其具有与使用PRT966纤维及PRT799纤维的针织物大致相同的覆盖因子。具体而言,如下所述。

[0225] 丝绸纤度:2/60Nm(双股纱),针距:14

[0226] 棉纤度:2/34Nm(双股纱),针距:14

[0227] 聚酯纤度:1/60Nm(单丝),针距:14

[0228] (2) 吸水性评估

[0229] 吸水性通过基于JIS L 1907(纤维制品的吸水性试验方法滴注法)的试验进行评估。具体而言,在标准环境(温度20 $\pm$ 2℃/湿度65 $\pm$ 4%RH)下,从滴管向上述准备的针织物表面滴下1滴水,测量滴下的水滴被针织物吸收(镜面反射消失)为止的时间(最长测量时间为60秒)。结果如表6所示。

[0230] (3) 速干性评估

[0231] 速干性通过扩散性残留水分率的测量进行评估。具体而言,在标准环境(温度20 $\pm$ 2℃/湿度65 $\pm$ 4%RH)下,将0.6mL的自来水向针织物的里侧滴下,每经过一定时间(每5分钟)测量针织物重量,由此求出水的重量,通过下式计算扩散性残留水分率。

[0232] 扩散性残留水分率(%) = 各时间的水的重量(g) / 测量开始时的水的重量(g) $\times$ 100进行测量直到扩散性残留水分率为10%以下(即,水的重量为0.6mL的10% = 60 μ L(60mg)),并求出直到扩散性残留水分率为10%所需要的时间。结果如表6所示。

[0233] [表6]

[0234]

	吸水性(秒)	速干性(分)
改造丝心蛋白(PRT966)	>60	54.1
改造丝心蛋白(PRT799)	3	65.9
丝绸	>60	134.3

棉	1	125.7
聚酯	>60	300.8

[0235] 可以理解为,改造丝心蛋白(PRT799及PRT966)具有优异的吸水速干性。可以理解为,特别是亲水性改造丝心蛋白(PRT799)的吸水性和速干性均优异。

[0236] (试验例3:接触冷感性的评估)

[0237] (1) 针织物的制造

[0238] 使用试验例1准备的各种原料纤维,使用横编织机进行横编织来制造针织物。为了比较,准备市售的羊毛纤维、羊绒纤维、丝绸纤维、棉纤维、人造丝纤维及聚酯纤维作为原料纤维。

[0239] 使用PRT966纤维作为原料纤维的针织物,其纤度:1/30Nm(毛线支数单丝),针距:18。使用PRT799纤维作为原料纤维的针织物,其纤度:1/30Nm(毛线支数单丝),针距:16。调整使用了其他原料纤维的针织物的纤度及针距,使其具有与使用PRT966纤维及PRT799纤维的针织物大致相同的覆盖因子。具体而言,如下所述。

[0240] 羊毛纤度:2/30Nm(双股纱),针距:14

[0241] 羊绒纤度:1/32Nm(单丝),针距:14

[0242] 丝绸纤度:2/60Nm(双股纱),针距:14

[0243] 棉纤度:2/34Nm(双股纱),针距:14

[0244] 人造丝纤度:1/38Nm(单丝),针距:14

[0245] 聚酯纤度:1/60Nm(单丝),针距:14

[0246] (2) 接触冷感性评估

[0247] 裁成10cm×10cm的试验片(试样),置于试验环境(温度20±2℃,相对湿度65±4%)中4小时以上。然后,使用设定为30℃的接触冷感测试仪(KES-F7ThermoLabII型,由日本Katotech株式会社制造),在其热板上放置传感器使传感器温度保持恒定后,使传感器接触试验片的背面,在与试验片的温度差(ΔT)10℃的条件下,测量了各试验片的热流量峰值($q_{\max}$,W/cm²)。热流量峰值越大,意味着从接触物(皮肤等)到试验片上的热移动量越大,可以评估为接触冷感性高。结果如表7所示。

[0248] [表7]

原料纤维	热流量峰值($q_{\max}$,W/cm ²)
PRT966	0.140
PRT799	0.073
羊毛	0.049
羊绒	0.053
丝绸	0.101
棉	0.054
人造丝	0.103
聚酯	0.082

[0250] 可以理解为,改造丝心蛋白(PRT966及PRT799,尤其是PRT966)具有优异的接触冷感性。

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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 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 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 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

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 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 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 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
Pro Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala Ala		
	165	170
Ala Ala Ala Ala Ala Gly Ser Gly Gln Gln Gly Pro Gly Gln Tyr Gly		
	180	185
Pro Tyr Ala Ser Ala Ala Ala Ala Ala Ala Gly Ser Tyr Gly Ser		
	195	200
Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Gln Ser Gly Ser Gly		
	210	215
Gln Gln Gly Pro Gly Gln Gln Gly Pro Tyr Ala Ser Ala Ala Ala Ala		
225	230	235
Ala Ala Ala Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ser Ser		
	245	250
Ala Ala Ala Ala Ala Ala Ala Gly Ser Tyr Gly Tyr Gly Pro Gly Gln		
	260	265
Gln Gly Pro Tyr Gly Pro Gly Ala Ser Gly Gln Asn Gly Pro Gly Ser		
	275	280
Gly Gln Tyr Gly Pro Gly Gln Gln Gly Pro Gly Pro Ser Ala Ala Ala		
	290	295
Ala Ala Ala Ala Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Ala		
305	310	315
Ser Ala Ala Ala Ala Ala Ala Ala Gly Ser Tyr Gly Pro Gly Gln Gln		
	325	330
Gly Pro Gly Gln Tyr Gly Pro Gly Ser Ser Gly Pro Gly Gln Gln Gly		
	340	345
Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala Ala Ala Gly Ser		
	355	360
Tyr Gly Pro Gly Gln Gln Gly Pro Tyr Gly Pro Gly Pro Ser Ala Ala		
	370	375
Ala Ala Ala Ala Ala Gly Ser Tyr Gln Gln Gly Pro Gly Gln Gln Gly		
385	390	395
Pro Tyr Gly Pro Gly Ala Ser Gly Pro Gly Gln Gln Gly Pro Tyr Gly		
	405	410
Pro Gly Ala Ser Ala Ala Ala Ala Ala Ala Gly Pro Gly Gln Tyr		
	420	425
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

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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 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 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 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 Ala Gly Pro		
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 Ala Gly Pro Gly Gln Gln		
865	870	875
Gly Pro Tyr Gly Pro Gly Ala Ser Ala 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 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 Ala Gly Pro Gly Gln Tyr Gly		
980	985	990
Pro Gly Gln Gln Gly Pro Ser Ala Ser Ala 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 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 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 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 Gly Pro	Gly Gln Tyr
1565	1570	1575
Gly Pro Gly Gln Gln Gly	Pro Ser Ala Ser 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 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	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 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	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	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 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 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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20	25	30	
Gly Ser Ala 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 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	80
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	
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		
595		

<210> 11

<211> 12

<212> PRT

<213> 人工序列

<220>

<223> His标签

<400> 11

Met His His His His His His Ser Ser Gly Ser Ser

1 5 10

<210> 12

<211> 608

<212> PRT

<213> 人工序列

<220>

<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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Gln Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Gly Gln
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
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Gln Gly Pro Tyr Gly Pro Gly Gln Ser Ala Ala Ala Ala Gly Ser
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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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Gln Gly Pro Gly Gln Ser Ala Ala Ala Ala Gly Pro Gly Gln Gln		
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Tyr Gly Gln Gly Pro Tyr Gly Pro Gly Ala Ser Gly Pro Gly Gln Tyr		
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Ser Gly Gln Gln Gly Pro Gly Gln Tyr Gly Pro Tyr Ala Ser Ala Ala		
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Pro Tyr Ala Ser Ala Ala Ala Ala Ala Gly Pro Gly Gln Gln Gly Pro		
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Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala Gly Gln Tyr Gly Tyr		
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Tyr Gly Pro Gly Gln Gln	Gly Pro Ser Ala Ser	Ala Ala Ala Ala
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Gln Gly Pro Tyr Gly Pro	Gly Gln Ser Gly Ser	Gly Gln Gln Gly
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Pro Gly Gln Gln Gly Pro	Tyr Ala Ser Ala Ala	Ala Ala Ala Gly
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Pro Gly Gln Gln Gly Pro	Tyr Gly Pro Gly Ser	Ser Ala Ala Ala
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Ala Ala	Gly Gln Tyr Gly Tyr	Gly Pro Gly Gln Gln	Gly Pro Tyr
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Tyr Ala Ser Ala Ala Ala Ala Gly Val Tyr Gly Ser Gly Pro Gly	
195 200 205	
Leu Ile Gly Pro Tyr Gly Pro Gly Val Ser Gly Ser Gly Leu Ile Gly	
210 215 220	
Pro Gly Leu Ile Gly Pro Tyr Ala Ser Ala Ala Ala Ala Gly Pro	
225 230 235 240	
Gly Leu Ile Gly Pro Tyr Gly Pro Gly Ser Ser Ala Ala Ala Ala	

				245						250						255
Gly	Val	Tyr	Gly	Tyr	Gly	Pro	Gly	Leu	Ile	Gly	Pro	Tyr	Gly	Pro	Gly	
				260						265						270
Ala	Ser	Gly	Val	Asn	Gly	Pro	Gly	Ser	Gly	Val	Tyr	Gly	Pro	Gly	Leu	
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Ile	Gly	Pro	Gly	Val	Ser	Ala	Ala	Ala	Ala	Ala	Gly	Pro	Gly	Leu	Ile	
				290						295						300
Gly	Pro	Tyr	Gly	Pro	Gly	Ala	Ser	Ala	Ala	Ala	Ala	Ala	Gly	Val	Tyr	
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Gly	Pro	Gly	Leu	Ile	Gly	Pro	Gly	Val	Tyr	Gly	Pro	Gly	Ser	Ser	Gly	
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Pro	Gly	Leu	Ile	Gly	Pro	Tyr	Gly	Pro	Gly	Ser	Ser	Ala	Ala	Ala	Ala	
				340						345						350
Ala	Gly	Val	Tyr	Gly	Pro	Gly	Leu	Ile	Gly	Pro	Tyr	Gly	Pro	Gly	Val	
				355						360						365
Ser	Ala	Ala	Ala	Ala	Ala	Gly	Val	Tyr	Leu	Ile	Gly	Pro	Gly	Leu	Ile	
				370						375						380
Gly	Pro	Tyr	Gly	Pro	Gly	Ala	Ser	Gly	Pro	Gly	Leu	Ile	Gly	Pro	Tyr	
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Gly	Pro	Gly	Ala	Ser	Ala	Ala	Ala	Ala	Ala	Gly	Pro	Gly	Val	Tyr	Gly	
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Pro	Gly	Leu	Ile	Gly	Pro	Ser	Ala	Ser	Ala	Ala	Ala	Ala	Ala	Gly	Val	
				420						425						430
Tyr	Gly	Ser	Gly	Pro	Gly	Val	Tyr	Gly	Pro	Tyr	Gly	Pro	Gly	Val	Ser	
				435						440						445
Gly	Pro	Gly	Ser	Gly	Leu	Ile	Gly	Val	Gly	Pro	Tyr	Gly	Pro	Gly	Ala	
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Ser	Ala	Ala	Ala	Ala	Ala	Gly	Val	Tyr	Gly	Pro	Gly	Leu	Ile	Gly	Pro	
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Tyr	Gly	Pro	Gly	Val	Ser	Ala	Ala	Ala	Ala	Ala	Gly	Pro	Gly	Ser	Gly	
				485						490						495
Val	Tyr	Gly	Pro	Gly	Ala	Ser	Gly	Val	Asn	Gly	Pro	Gly	Ser	Gly	Val	
				500						505						510
Tyr	Gly	Pro	Gly	Leu	Ile	Gly	Pro	Gly	Val	Ser	Ala	Ala	Ala	Ala	Ala	
				515						520						525
Gly	Val	Tyr	Leu	Ile	Gly	Pro	Gly	Leu	Ile	Gly	Pro	Tyr	Gly	Pro	Gly	
				530						535						540
Ala	Ser	Ala	Ala	Ala	Ala	Ala	Gly	Val	Tyr	Gly	Ser	Gly	Pro	Gly	Leu	
545																560
				550						555						560

Ile Gly Pro Tyr Gly Pro Gly Val Ser Gly Ser Gly Leu Ile Gly Pro		
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Gly Leu Ile Gly Pro Tyr Ala Ser Ala Ala Ala Ala Ala Gly Pro Gly		
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Ser Gly Leu Ile Gly Pro Gly Ala Ser		
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Met His His His His His His Ser Ser Gly Ser Ser Gly Pro Gly Ile		
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Phe Gly Pro Tyr Gly Pro Gly Ala Ser Ala Ala Ala Ala Ala Gly Thr		
	20	25 30
Gly Pro Gly Ser Gly Ile Phe Gly Pro Gly Thr Ser Gly Thr Tyr Gly		
	35	40 45
Pro Gly Ile Phe Gly Pro Gly Ile Phe Gly Pro Gly Ser Ser Ala Ala		
	50	55 60
Ala Ala Ala Gly Pro Gly Thr Tyr Gly Pro Gly Ile Phe Gly Pro Ser		
65	70	75 80
Ala Ser Ala Ala Ala Ala Ala Gly Pro Gly Ser Gly Ile Phe Gly Pro		
	85	90 95
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		
	115	120 125
Pro Gly Ile Phe Gly Pro Tyr Gly Ser Ala Ala Ala Ala Ala Gly Pro		
	130	135 140
Gly Ser Gly Thr Tyr Gly Thr Gly Pro Tyr Gly Pro Gly Ala Ser Gly		
145	150	155 160
Pro Gly Thr Tyr Gly Pro Gly Ile Phe Gly Pro Ser Ala Ser Ala Ala		
	165	170 175
Ala Ala Ala Gly Ser Gly Ile Phe Gly Pro Gly Thr Tyr Gly Pro Tyr		
	180	185 190
Ala Ser Ala Ala Ala Ala Ala Gly Thr Tyr Gly Ser Gly Pro Gly Ile		
	195	200 205

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

515	520	525
Ile Phe Gly Pro Gly	Ile Phe Gly Pro Tyr Gly	Pro Gly Ala Ser Ala
530	535	540
Ala Ala Ala Ala Gly	Thr Tyr Gly Ser Gly	Pro Gly Ile Phe Gly Pro
545	550	555
Tyr Gly Pro Gly Thr	Ser Gly Ser Gly Ile Phe Gly	Pro Gly Ile Phe
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Gly Pro Tyr Ala Ser	Ala Ala Ala Ala Gly	Pro Gly Ser Gly Ile
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Phe Gly Pro Gly Ala	Ser	
595		

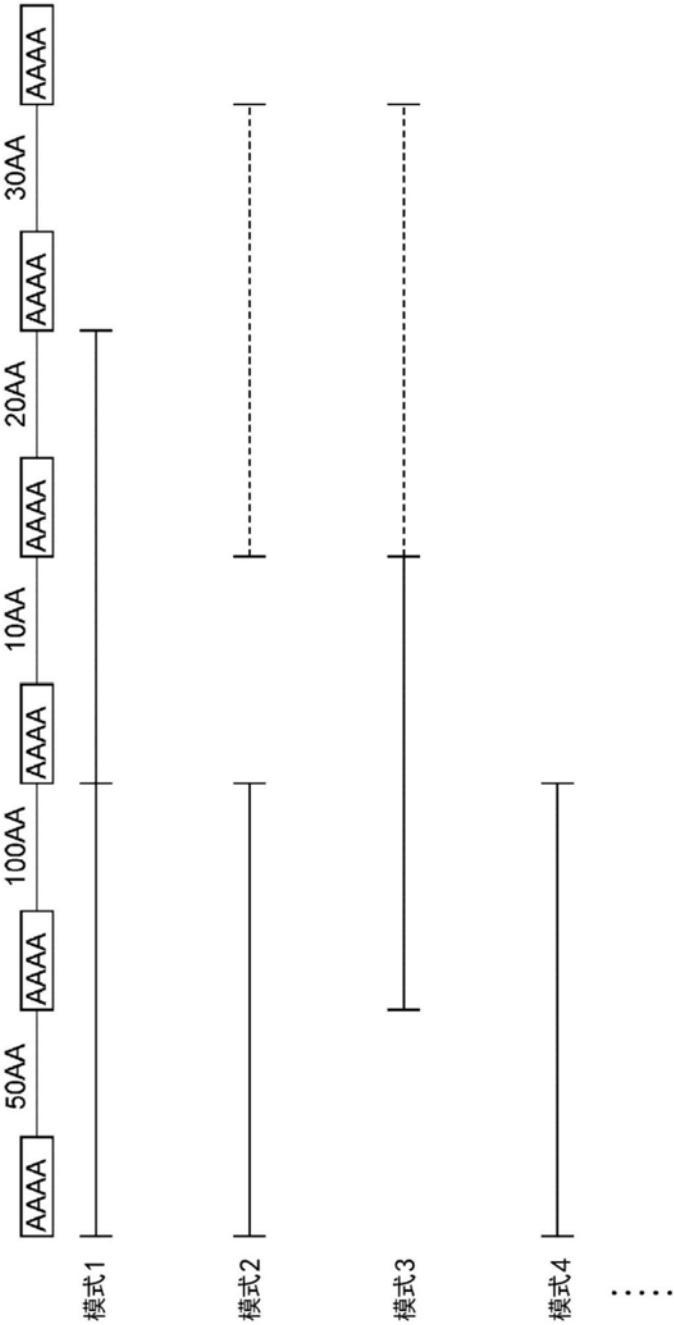


图1

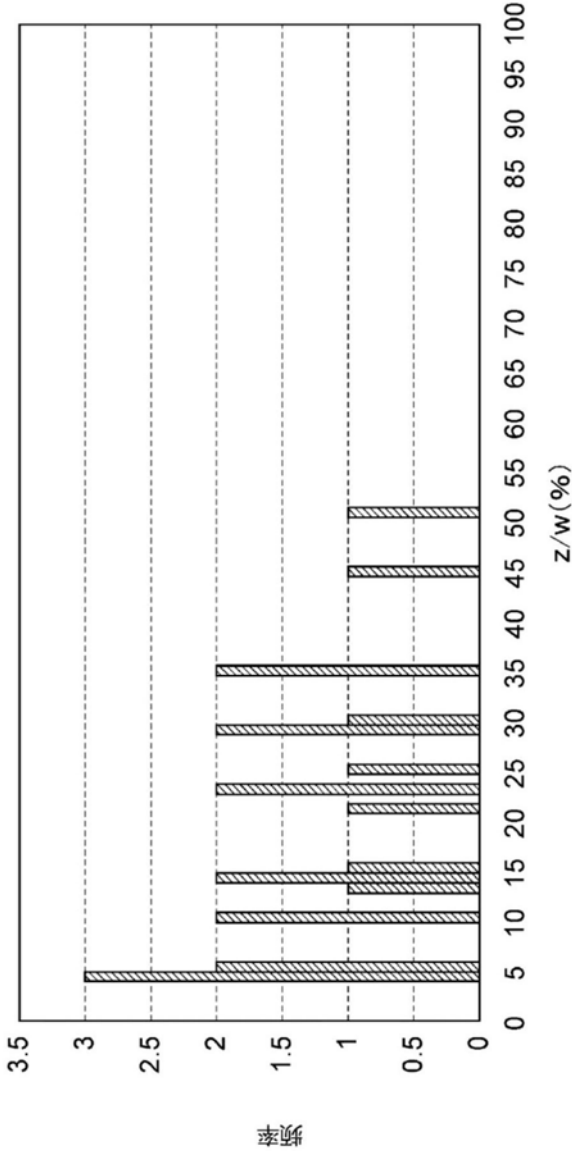


图2

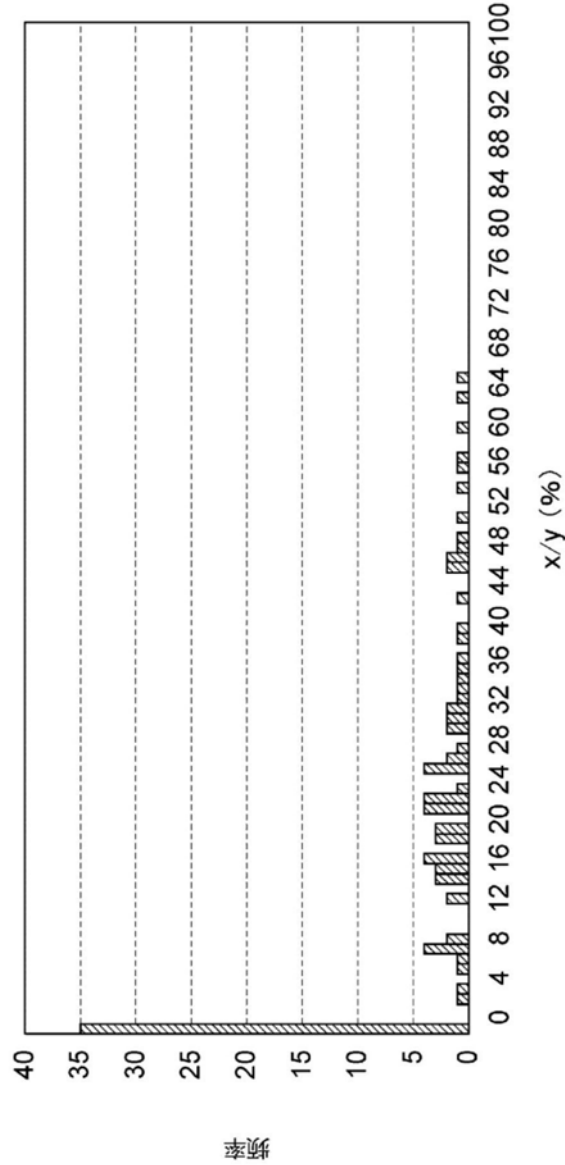


图3

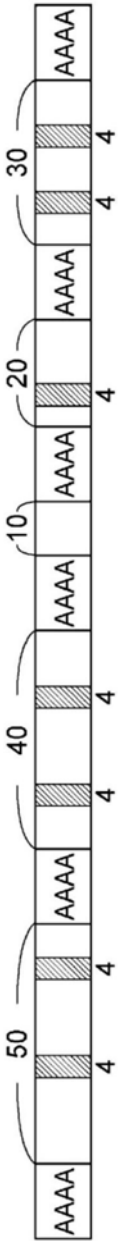


图4

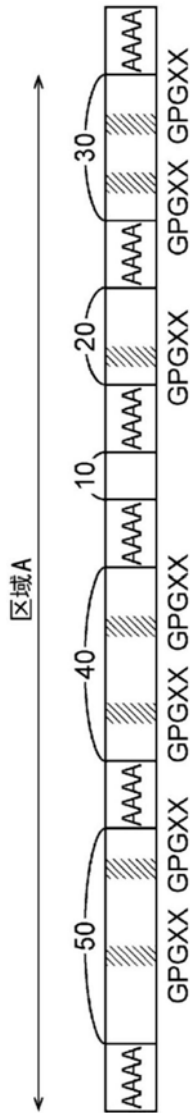


图5