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(21) International Application Number: PCT/US99/00048 (22) International Filing Date: 11 January 1999 (11.01.99) (30) Priority Data: 60/071,247 12 January 1998 (12.01.98) US (71) Applicant (for all designated States except US): G.D. SEARLE & CO. [US/US]; Corporate Patent Dept., P.O. Box 5110, Chicago, IL 60680-5110 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): VIOLAND, Bernard, N. [US/US]; 1710 Woods Bend Lane, Glencoe, MO 63038 (US). POLAZZI, Joseph, O. [US/US]; Chesterfield, MO (US). CASPERSON, Gerald, F. [US/US]; Ballwin, MO (US). (74) Agents: WILLIAMS, Roger, A. et al.; G.D. Searle & Co., Corporate Patent Dept., P.O. Box 5110, Chicago, IL 60680-5110 (US).	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 11 November 1999 (11.11.99)
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(54) Title: METHOD OF REFOLDING ANGIOSTATIN

The diagram illustrates the domain architecture of several protein constructs. pMON24624 is the full-length protein with domains SP, NTP, K1, K2, K3, K4, and K5. The following constructs show different domain combinations:

- pMON24642: K1, K2, K3, K4
- pMON24643: K1, K2, K3, K4
- pMON24644: K2, K3
- pMON24645: K2, K3, K4
- pMON24646: K1, K2, K3
- pMON24649: K1

(57) Abstract

An efficient method for expressing, solubilizing, refolding, and purifying, angiotensinogen from inclusion bodies expressed in bacteria is disclosed. Nucleic acids encoding full-length angiotensinogen and subfragments encoding kringle domains (K1-4, K2-3, K2-4, K1-3, and K1) are also disclosed.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/00048

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N9/68 C07K1/113

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YIHAI CAO ET AL.: "Kringle domains of human angiostatin" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 46, 15 November 1996 (1996-11-15), pages 29461-29467, XP002086445 MD US cited in the application	1-3, 19-22, 33,34, 37-40, 45-47
Y	page 29462, right-hand column, paragraph 3 --- -/-	4-18, 23-32, 35,36, 41-44,48

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
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Date of the actual completion of the international search

28 April 1999

Date of mailing of the international search report

24.09.99

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/00048

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCOTT CLEARY ET AL.: "Purification and characterization of tissue plasminogen activator kringle-2 domain expressed in Escherichia coli" BIOCHEMISTRY, vol. 28, no. 4, 21 February 1989 (1989-02-21), pages 1884-1891, XP002101498 EASTON, PA US	1-3,15, 16, 19-22, 33,34, 45-47
Y	page 1885, left-hand column, paragraph 5 -right-hand column, paragraph 1; figure 2 page 1886, right-hand column, paragraph 2 -page 1887, right-hand column, paragraph 1 ---	4-14,17, 18, 23-32, 35,36, 41-44,48
X	EP 0 523 296 A (ELI LILLY AND COMPANY) 20 January 1993 (1993-01-20)	1-9, 11-14, 19,20, 29-32
Y	page 3, line 18 - line 21; examples 34,36 ---	10, 15-18, 23-28, 35,36, 41-44,48
Y	BERNHARD FISCHER ET AL.: "Isolation, renaturation, and formation of disulfide bonds of eukaryotic proteins expressed in Escherichia coli as inclusion bodies" BIOTECHNOLOGY AND BIOENGINEERING INCLUDING: SYMPOSIUM BIOTECHNOLOGY IN ENERGY PRODUCTION AND CONSERVATION., vol. 41, no. 1, 5 January 1993 (1993-01-05), pages 3-13, XP000605146 NEW YORK US cited in the application page 4, left-hand column, paragraph 2 - last paragraph page 5, left-hand column, paragraph 2 -page 9, right-hand column, paragraph 1; table VI ---	4-9, 11-18, 23-32, 35,36, 41-44
Y	WO 97 41824 A (ABBOTT LABORATORIES) 13 November 1997 (1997-11-13) abstract; figure 3 -----	10,48

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/ 00048

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows.

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-48

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99/00048

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-48

Method for producing angiostatin including a step of refolding at an elevated pH

2. Claim : 49

Polypeptide encoded by a sequence selected from SEQ ID NO:10 through 29.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/00048

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 523296 A	20-01-1993	CA 2047063 A JP 5076360 A	16-01-1993 30-03-1993
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