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16 November 1995 (16.11.95)

(54) Title: ENZYMATIC PREPARATION OF POLYSACCHARIDES

(57) Abstract

Provided are *in vitro* processes for the production of polysaccharides such as hyaluronic acid, cellulose, polymannuronan, chitin, etc. These processes are carried out under reaction conditions that permit the recycling of nucleotide-phosphates required for the formation of sugar nucleotide precursors employed in the synthesis of these polysaccharides. Also provided are polysaccharides produced via these processes, and pharmaceutical and cosmetic compositions containing these polysaccharides.

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 95/00935

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12P19/26 C12P19/18 C12N11/04 C12N11/12 C12P19/04
A61L31/00 A61L27/00 A61L29/00 A61K31/715 A61K31/735
C08L1/02 C08L5/00 C08L5/08 C08L5/04 //C12N9/10,

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 C12P C12N C08L A61K A61L

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	BIOCHEM. J., vol.211, pages 181 - 189 PREHM P. 'Synthesis of hyaluronate in differentiated teratocarcinoma cells : characterization of the synthase' cited in the application see the whole document, especially figure 5. --- -/--	1,3-5, 11,12

[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
5 July 1995	23. 10. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	GAC, G

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 95/00935

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/54

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

B. FIELDS SEARCHED

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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.
Y	J. ORG. CHEM., vol.47, pages 5416 - 5418 WONG ET AL. 'Enzyme-catalyzed synthesis of N-acetyllactosamine with in-situ regeneration of uridine-5'-diphosphate-glucose and uridine-5'-diphosphate galactose' see page 5417, left column ---	1,3
Y	J. BIOL. CHEM., vol.254, no.14, 25 July 1979 pages 6252 - 6261 SUGAHARA ET AL. 'Biosynthesis of hyaluronic acid by Streptococcus' cited in the application see the whole document --- -/--	1,3

[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
5 July 1995	
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Authorized officer GAC, G

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INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/EP 95/00935

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,93 08205 (THE SCRIPPS REASEARCH INSTITUTE) 29 April 1993 see pages 7, 18, 23, 42, 43, 48-50, 57, 58.	1,3
A	ANALYTICAL BIOCHEMISTRY, vol.202, pages 215 - 238 ICHIKAWA ET AL. 'Enzyme-catalysed oligosaccharide synthesis' cited in the application see the whole document	1-22
A	J. BIOL. CHEM., vol.244, no.2, 25 January 1969 pages 236 - 246 STOOLMILLER ET AL. 'The biosynthesis of hyaluronic acid by Streptococcus' see the whole document	1,3
A	WO,A,92 16640 (THE SCRIPPS RESEARCH INSTITUTE) 1 October 1992 see the whole document	1-11
X	EP,A,0 144 019 (MILES LABORATORIES INC) 12 June 1985 see	13-19
X	WO,A,94 01468 (M.U.R.S.T ITALIAN MINISTRY FOR UNIV. AND SCIENTIFIC AND TECHNOL. RES.) 20 January 1994 see the whole document	20-22

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INTERNATIONAL SEARCH REPORT

Ir. ational application No.
PCT/EP 95/00935

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:

- claims 1-22
- claim 23
- claim 24
- claim 25
- claims 26-27

See additional sheet ISA/210

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 searches 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.:

1-22

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/EP95/00935

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

LACK OF UNITY OF INVENTION

-Enzymatic systems for the production of polysaccharides from their nucleotide-sugar precursors are known (see Sugahara et al., J. Biol. Chem. 1979, 254(14)6252-6261).
-The problem of the present invention is to provide further methods for the preparation of polysaccharides.
-The solution provided by the applicant is an enzymatic method with the regeneration of sugar nucleotides in-situ, more specifically:

1)An enzymatic method for the production of hyaluronic acid with regeneration of UDP-sugar precursors.
2)An enzymatic method for the production of chitin with regeneration of UDP-sugar precursors.
3)An enzymatic method for the production of cellulose with regeneration of UDP-sugar precursors.
4)An enzymatic method for the production of polymannuronic acid with regeneration of GDP-sugar precursors.
5)More generally, an enzymatic method for the production of a polysaccharide with regeneration of sugar-nucleotide precursors.

-However, systems for the regeneration in-situ of sugar-nucleotide precursors with recycling of nucleotide phosphates have already been disclosed (see WONG et al., J. Org. Chem.1982, 47, p.5416-18 and Ichikawa et al., Analyt. Biochem.1992, 202, p.215-238).
-In view of the fact that sugar nucleotides regeneration systems are known, due to the essential difference of the polysaccharide molecule synthetized as well as the enzymatic reaction used (different synthetising enzymes) and due to the fact that no other technical features can be distinguished which, in the light of the prior art, could be regarded as "special technical features", the ISA is of the opinion that there is no single inventive concept (Rule 13.1 PCT) underlying the present application.
-Therefore there is lack of unity between the following separate subjects: (Art.17(3) (a) PCT).

1. Claims:1-22: Process for producing hyaluronic acid in-vitro by incubating a protein (or a protein mixture) more specifically containing hyaluronic synthase, with a reaction mixture UTP, glucose-1-phosphate and N-acetylglucosamine -1-phosphate and allow the polymerization reaction to take place. Hyaluronic acid, pharmaceutical, cosmetic compositions, biomaterials containing it, use thereof.

2. Claim 23: Process for producing cellulose in-vitro: incubating cellulose synthase in a reaction mixture containing UTP and glucose-1-phosphate to form UDP-glucose; recovering cellulose.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

3. Claim 24: Process for producing polymannuronic acid in-vitro: incubating GDP-mannuronic acid polymerase in a reaction mixture containing GTP and mannose-1-phosphate to form GDP-mannuronic acid; recovering polymannuronic acid.

4. Claim 25: Process for producing chitin in-vitro: incubating chitin synthase in a reaction mixture containing N-acetylglucosamine-1-phosphate and UTP to form UDP-N-acetylglucosamine; recovering chitin.

5. Claims 26-27: Process for producing a polysaccharide in-vitro: incubating sugar-nucleotide synthase or polymerizing enzyme for said polysaccharide in a reaction mixture containing sugar precursors and nucleotide-triphosphate carriers to form sugar-nucleotide precursors; recovery of polysaccharide; and polysaccharide itself.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/EP 95/00935

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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