A method for removing a serum albumin from a mixture of other compounds by contacting said mixture with a ligand a) having affinity for and enabling selective binding of the serum albumin and b) being attached to a base matrix insoluble in the aqueous media used or being possible to attach to such a matrix after having become bound to the serum albumin, characterized in that said ligand is derived from an albumin binding bacterial cell surface receptor and that the ligand lacks the IgG-binding and/or α2-macroglobulin-binding ability found in native forms of these type of bacterial receptors. An albumin-binding ligand derived from a cell surface bacterial receptor and attached covalently to a carrier matrix, characterized in that the ligand is monovalent with respect to ability to bind a serum albumin. A method for removal of serum albumin from a sample that is to be assayed for non-serum albumin components. The characteristic feature is to subject the sample to affinity adsorption by an albumin-binding ligand derived from an albumin-binding bacterial receptor.
**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<table>
<thead>
<tr>
<th>AL</th>
<th>Albania</th>
<th>ES</th>
<th>Spain</th>
<th>LS</th>
<th>Lesotho</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>Armenia</td>
<td>FI</td>
<td>Finland</td>
<td>LT</td>
<td>Lithuania</td>
</tr>
<tr>
<td>AT</td>
<td>Austria</td>
<td>FR</td>
<td>France</td>
<td>LU</td>
<td>Luxembourg</td>
</tr>
<tr>
<td>AU</td>
<td>Australia</td>
<td>GA</td>
<td>Gabon</td>
<td>LV</td>
<td>Latvia</td>
</tr>
<tr>
<td>AZ</td>
<td>Azerbaijan</td>
<td>GB</td>
<td>United Kingdom</td>
<td>MC</td>
<td>Monaco</td>
</tr>
<tr>
<td>BA</td>
<td>Bosnia and Herzegovina</td>
<td>GE</td>
<td>Georgia</td>
<td>MD</td>
<td>Republic of Moldova</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
<td>GH</td>
<td>Ghana</td>
<td>MG</td>
<td>Madagascar</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
<td>GN</td>
<td>Guinea</td>
<td>MK</td>
<td>The former Yugoslav</td>
</tr>
<tr>
<td>BF</td>
<td>Burkina Faso</td>
<td>GR</td>
<td>Greece</td>
<td>ML</td>
<td>Mali</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
<td>HU</td>
<td>Hungary</td>
<td>MN</td>
<td>Mongolia</td>
</tr>
<tr>
<td>BJ</td>
<td>Benin</td>
<td>IE</td>
<td>Ireland</td>
<td>MR</td>
<td>Mauritania</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
<td>IL</td>
<td>Israel</td>
<td>MW</td>
<td>Malawi</td>
</tr>
<tr>
<td>BV</td>
<td>Belarus</td>
<td>IS</td>
<td>Iceland</td>
<td>MX</td>
<td>Mexico</td>
</tr>
<tr>
<td>CA</td>
<td>Canada</td>
<td>IT</td>
<td>Italy</td>
<td>NE</td>
<td>Niger</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
<td>JP</td>
<td>Japan</td>
<td>NL</td>
<td>Netherlands</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
<td>KE</td>
<td>Kenya</td>
<td>NO</td>
<td>Norway</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
<td>KG</td>
<td>Kyrgyzstan</td>
<td>NZ</td>
<td>New Zealand</td>
</tr>
<tr>
<td>CI</td>
<td>Côte d'Ivoire</td>
<td>KP</td>
<td>Democratic People's Republic of Korea</td>
<td>PL</td>
<td>Poland</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
<td>KR</td>
<td>Republic of Korea</td>
<td>PT</td>
<td>Portugal</td>
</tr>
<tr>
<td>CN</td>
<td>China</td>
<td>KZ</td>
<td>Kazakhstan</td>
<td>RO</td>
<td>Romania</td>
</tr>
<tr>
<td>CU</td>
<td>Cuba</td>
<td>LC</td>
<td>Saint Lucia</td>
<td>RU</td>
<td>Russian Federation</td>
</tr>
<tr>
<td>CZ</td>
<td>Czech Republic</td>
<td>LI</td>
<td>Liechtenstein</td>
<td>SD</td>
<td>Sudan</td>
</tr>
<tr>
<td>DE</td>
<td>Germany</td>
<td>LK</td>
<td>Sri Lanka</td>
<td>SE</td>
<td>Sweden</td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
<td>LR</td>
<td>Liberia</td>
<td>SG</td>
<td>Singapore</td>
</tr>
<tr>
<td>EE</td>
<td>Estonia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| SI  | Slovenia           | SK  | Slovakia    | SN  | Senegal        |
| SZ  | Swaziland          |     |             |     |                |
| TD  | Chad               | TG  | Togo        | TJ  | Tajikistan     |
| TM  | Turkmenistan       | TR  | Turkey      | TT  | Trinidad and Tobago |
| UA  | Ukraine            | UG  | Uganda      | US  | United States of America |
| UZ  | Uzbekistan         | VN  | Viet Nam    | YU  | Yugoslavia     |
| ZW  | Zimbabwe           |     |             |     |                |
A method for the removal/purification of serum albumins and means for use in the method

Technical field

The present invention concerns a method for the separation/removal of a mammalian serum albumin from a solution containing a mixture of proteins in order to obtain a solution/preparation that is substantially devoid of the serum albumin. The invention also concerns novel immobilised forms of albumin-binding ligands deriving from native forms of bacterial receptors that are able to bind to one or more serum albumins.

Technical background

For a long time there has been a large demand for mammalian serum albumins, for instance serum albumin of human or bovine origin (HSA and BSA, respectively). For HSA this has mainly depended on its therapeutic use as a plasma volume expander. Originally serum albumins were obtained from sera/plasma of the appropriate species origin. For some years the focus has been to produce serum albumins recombinantly, in particular HSA. For bacterially produced recombinant forms, it has become urgent to remove host cell contaminants because they may be hazardous in vivo to mammals. For some time it has become apparent that producing HSA in transgenic animals should be beneficial, for instance in transgenic cows. This latter alternative, however, has the drawback that HSA will be present in mixture with the HSA analogue of the host animal (for instance with BSA if HSA produced in cows). This has created novel purification problems, for instance the specific removal of BSA from HSA.

Serum albumin preparations intended for use in vivo shall according to accepted practice contain < 0.01 %, such as < 0.001 % proteins that are heterologous to the species to which the preparation is to be administered. HSA preparations, for instance, that are to be used in humans
shall contain < 0.01 %, such as < 0.001 % BSA. All percentages are w/w.

Removal of serum albumins has also been a concern when assaying samples containing serum albumin for other components. Blood derived samples such as whole blood, serum and plasma are typical examples. In this kind of samples serum albumin is one of most abundant substances and may disturb assays for other substances that are present in lower amounts. In analyzing protein components after, for instance, two-dimensional gel electrophoresis or mass spectrometric fragmentation, serum albumin will easily disturb.

Various forms of affinity chromatography and/or ion exchange chromatography have earlier been applied to the purification of serum albumins. For affinity chromatography the general goal has been to find a chromatographic media (ligand attached to a chromatographic base matrix) that provide the sufficient specificity in order to remove either predetermined contaminants or the serum albumin desired as the end product from complex mixtures.

Illustrative examples of ligands previously used and having selectivity for serum albumins are given by Theodore Peters in All about Albumin - Biochemistry, Genetics and Medical Applications, (Ed. Theodore Peters, Jr., Academic Press (1996) pages 77-126. There are also known other compounds that bind serum albumins, even with species selectivity, that for various reasons have not found use in the selective/specific removal and/or purification of serum albumins. Examples are albumin binding receptors (proteins) present on the cell surface of certain bacteria, typically streptococci. See for instance Nygren et al., Eur. J. Biochem. 193 (1990) 143-148 (Protein G), Guss et al., WO 9507300 (Protein MAG), Jonsson et al., Infect. Immun. 63 (1995) 2968-2975 (Protein ZAG). These bacterial receptors frequently also bind to other proteins, for instance Protein G to IgG and Protein MAG and ZAG to IgG and $\alpha_2$-
macroglobulin. The various extra binding abilities of these proteins make them less suitable as ligands for the selective/specific removal of serum albumin from complex protein mixtures. The main use of their albumin-binding fragments has been as fusion partners, for instance in order to have an affinity handle attached to a protein to be purified. To the extent that immobilised forms have been produced it has been in order to make binding studies in relation to serum albumins from various mammalian species. See further Nygren et al (Eur. J. Biochem. 193 (1990) 143-148). Guss et al (WO 9507300) has outlined in a patent example to use intact protein MAG in an attempt to roughly purify albumin from mammalian blood. However it is apparent from Guss et al's results that also IgG and α2-macroglobulin were bound together with albumin.

Additional examples of known bacterial receptors binding to serum albumin are Protein H and M proteins, both from streptococci.

Native forms of this kind of receptors typically have more than one subsequence that is responsible for binding to albumin or IgG. The receptors may contain 1, 2 or 3 albumin-binding and/or IgG-binding regions. Functionally similar regions may differ in sequence.

Objectives of the invention

The first objective of the invention is to provide improved affinity methods for the removal of a serum albumin from a mixture of proteins in order to produce the serum albumin in pure form or a preparation essentially free of the removed serum albumin.

A second objective is to provide an affinity method as defined above which has an improved selectivity for a certain serum albumin that exists in mixture with one or more other serum albumins.
A third objective is to provide new affinity matrices carrying albumin-binding ligands having improved selectivities for serum albumins.

A fourth objective is an improved method for removal of serum albumin in samples that are to be assayed for one or more non-serum albumin components.

The methods for removal of serum albumin are based on matrices that carry an albumin binding ligand.

The invention

It has now surprisingly been found that the albumin binding capacity of the type of bacterial cell surface receptors mentioned above may advantageously be utilized for the selective removal and/or purification of serum albumins.

The inventive method encompasses that a mixture which contains (a) a serum albumin that is derived for a certain mammalian species and (b) other compounds, in particular proteins, is contacted with a ligand under conditions permitting binding (adsorption) of the serum albumin to the ligand. The ligand is preselected to have affinity for the serum albumin and is attached to a base matrix or is possible to attach to a base matrix after having become bound to the serum albumin. The invention has the characterizing feature that the ligand derives from an albumin-binding form of a bacterial receptor having affinity for a serum albumin, said form not encompassing the IgG- or α₂-macroglobulin-binding ability of native forms of these kind of receptors. The form is typically a fragment not encompassing the amino acid subsequences responsible for ability to bind to IgG- and/or α₂-macroglobulin.

The ligand

It is previously known that albumin-binding fragments can be obtained from the above-mentioned bacterial cell surface receptors, e.g. proteins G, MAG and ZAG and protein H and M-proteins. With present knowledge the most preferred
fragments to be used as ligands are monovalent with respect to albumin binding, e.g. forms of the native receptor in which all except one of the albumin binding regions have been neutralized. This does not exclude that also divalent, trivalent and other polyvalent fragments can be used as long as other disturbing binding abilities are not present. By recombinant techniques it is in principle possible to link any number of albumin binding regions to each other in series.

In streptococcal protein G (SPG) the various regions are arranged as described for strain G148. See for instance figure 1 in Sjölander et al., J. Immunol. Meth. 201 (1997) 115-123 which is based on Nygren et al., J. Mol. Recognit. 1 (1988) 69-:

<table>
<thead>
<tr>
<th>alb1</th>
<th>alb2</th>
<th>alb3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ss</td>
<td>E</td>
<td>A1</td>
</tr>
</tbody>
</table>

| serum albumin binding | IgG binding |

Alb1, alb2 and alb3 correspond to the three albumin binding regions of SPG from strain G148. The number of albumin-binding regions may differ between strains.

In addition to pure fragments various recombinant constructs derived from the native forms and their albumin binding fragments can also be used. Illustrative examples are mutated forms that retain their albumin binding capacity but lacks the ability to bind to IgG and/or $\alpha_2$-macroglobulin, and forms in which there have been added or inserted one or more amino acids, for instance cysteine, that facilitate covalent attachment to a carrier matrix. Addition/insertion of amino acid residues preferably takes place outside an albumin-binding site, for instance at the carboxy and/or amino terminal end. In case the change is within an albumin-binding site and the binding ability of
the site is to be retained the replacing amino acid residue should be conserved in relation to the replaced residue.

The sample containing the serum albumin to be
removed/purified

The serum albumin to be removed and/or purified typically exists in mixture with other proteins and/or biomolecules. The sample may be (a) blood preparations (such as plasma and serum), (b) fermentation liquids obtained from cultured host cells that have been transformed to express a serum albumin, (c) biological fluids obtained from transgenic mammals transformed to produce a serum albumin of another species, (d) and working up preparations derived from anyone of these types of liquids, etc. In case of liquids derived from transgenic animals the liquids will often contain also the normal serum albumin of the species concerned.

The matrix and the attachment of the ligand thereto.

In the preferred modes of the invention the ligand is attached to a base matrix that is insoluble in the aqueous media used. Such matrices often are based on polymers that expose a hydrophilic surface to the aqueous media used, i.e. expose hydroxy (−OH), carboxy (−COOH), carboxamido (−CONH₂, possibly in N- substituted forms), amino (−NH₂, possibly in substituted form), oligo- or polyethylenoxy groups on their external and, if present, also on internal surfaces. Typically the matrices are of the same kind as those normally used as chromatographic matrices. The polymers may, for instance, be based on polysaccharides, such as dextran, starch, cellulose, pullulan, agarose etc, which if necessary have been crosslinked, for instance with bisepoxides, epihalohydrins, 1,2,3-trihalo substituted lower hydrocarbons, to provide a suitable porosity and rigidity. The matrices may also be based on synthetic polymers, such as polyvinyl alcohol, poly hydroxyalkyl acrylates, poly hydroxyalkyl methacrylates, poly acrylamides,
polymethacrylamides etc. In case of hydrophobic polymers, such as those based on divinyl and monovinyl substituted benzenes, the surfaces of the matrices are often hydrophilized to expose hydrophilic groups as defined above to a surrounding aqueous liquid.

The matrices may also be of inorganic nature, e.g. silica, zirconium oxide etc.

Physically the insoluble matrices may be in the form of porous monoliths or in beaded/particle form that can be porous or non-porous. Matrices in beaded/particle form can be used as a packed bed or in a suspended form. Suspended forms include so called classified expanded beds and pure suspensions in which the particles/beads are moving round completely. In case of monoliths, packed bed and classified expanded beds, the separation procedure may be classified as a normal chromatography with a concentration gradient of adsorbed molecules being established along the flow direction. In case of pure suspension the separation procedure will be in the batch wise mode.

For suspensions, the beads/particles may contain a densifying filler material that will permit increased flow rates in case of classified expanded beds and facilitate sedimentation of the beads/particles after affinity binding (adsorption). See for instance WO-A-9717132 (Amersham Pharmacia Biotech AB) and WO-A-9200799 (Upfront Chromatography).

The ligand may be attached to the matrices via conventional coupling techniques utilising, e.g. amino and/or carboxy groups present in the ligand. In case a cysteine residue is present it may be utilized as well (thioether or disulfide attachments). Bisepoxides, epichlorohydrin, CNBr, N-hydroxysuccinimide (NHS) etc are typical coupling reagents.

Between the base matrix and the ligand there is often introduced a spacer that will improve the availability of the ligand and facilitate the chemical coupling of the
ligand to the matrix. Generally the spacer provides a hydrocarbon chain that has a length between 1-50 atoms. The hydrocarbon chain may be straight, branched or cyclic and optionally interrupted by one or more ether oxygen or amino nitrogen atoms and/or optionally substituted with one or more hydroxy, lower alkoxy, or amino group (-NH₂/NH₃⁺, where each hydrogen may be replaced with a lower alkyl or a lower acyl group). By lower alkyl or acyl group is primarily intended Cl-C10 alkyls/acyls. The spacer group may also, depending to coupling methodology, comprise ester, amido, thioether, etc groups that have the sufficient hydrolytic stability.

The spacer may also be polymeric providing attachments of several ligands per spacer. This type of spacers is often hydrophilic and called extenders, tentacles etc. See for instance International Patent Application PCT/SE98/00189 (Amersham Pharmacia Biotech AB).

It can be envisaged that the ligand may also be attached to the matrix by non-covalent bonding, such as physical adsorption or biospecific adsorption. For the latter type of binding the biotin-streapavidin system may be utilized.

As a potential alternative the ligand may be in soluble form that subsequent to binding to a serum albumin is insolubilized. This may be accomplished, for instance, by having the ligand conjugated to biotin and insolubilizing by contacting the formed complex between the serum albumin and the soluble ligand-biotin conjugate with a strepavidin-matrix.

**Procedural steps**

During the adsorption step the conditions are selected so as to promote binding between the ligand and the serum albumin intended. pH is typically selected between 4-8, the ionic strength in the interval corresponding to 0-3 M NaCl, and the temperature in the interval 0-40°C, with preference for 4-37°C. The exact values will depend on the species
origin of the serum albumin to be removed/purified and of the ligand attached to the matrix.

After adsorption the serum albumin bound to the ligand may be further worked up, for instance by first desorbing the bound serum albumin and subsequently subjecting it to further adsorption steps, for instance on an ion exchanger. Suitably desorption conditions may include change of pH, ionic strength, temperature and or addition of compounds interfering with binding. The main rules should then of course be not to change the conditions so that the serum albumin becomes irreversibly denatured.

Binding of the serum albumin to the ligand may be preceded by other adsorption steps. Such steps may for instance be based on ion exchange or on immune ligands.

In case the starting mixtures contains two serum albumins, one of which is adsorbed to the ligand and the other retained in the liquid, working up can continue on the liquid in order purify the serum albumin retained in the liquid.

In case the serum albumin has been removed in order to prepare samples low in serum albumin for assaying purposes, the sample is afterwards assayed, optionally after one or more additional conditioning steps. See above.

One separate aspect of the invention is an albumin-binding ligand derived from a cell surface bacterial receptor and attached covalently to a carrier matrix. This aspect is characterized in that the ligand is monovalent with respect to ability to bind a serum albumin. In the most preferred mode at the priority date the ligand essentially corresponds to region A3 of SPG. See above.

One further inventive aspect is to use anyone of the above-mentioned bacterial receptors and/or their albumin-binding modifications or fragments for the removal of serum albumin from a biological sample to be assayed for at least one other component that is not serum albumin. By including
also the IgG-binding and/or $\alpha_2$-macroglobulin subsequences of the receptors also IgG and/or $\alpha_2$-macroglobulin will be removed. The selection of matrix, coupling technology, conditions for the binding step etc are essentially the same as described above. After removal of serum albumin, possibly in combination with removal of IgG and/or $\alpha_2$-macroglobulin and, if so desired, one or more additional conditioning steps, the sample is subjected to the assay contemplated. Typically assays involve 2-dimensional gel electrophoresis and/or mass spectrometric fragmentation and final analyses of the individual proteins and/or their fragmentation patterns. In principle any protein can be assayed after the pretreatment described above. Exceptions are serum albumin, and depending upon ligand also IgG and/or $\alpha_2$-macroglobulin.

The invention is further defined in the appended claims and will now be further described by experimental support regarding the most preferred fragment.

EXPERIMENTAL PART

Immunisation: The protein used in this study was a 7.1 kDa fragment of Streptococcal protein G containing the complete albumin binding domain, A3 (fragment SPG-ABD3, aa 254-299 in Kraulis et al., FEBS letter 378 (1996) 190-194).

Approximately 2 mg of the fragment was immobilised on a 1 ml NHS-activated column, HiTrap, according to the manufacturers instruction (Amersham Pharmacia Biotech AB, Uppsala, Sweden) (NHS = N-hydroxy-succinimide).

Chromatography conditions: All samples were adjusted to pH 7.0 before loading onto the column. A buffer consisting of 20 mM sodium phosphate and 150 mM sodium chloride, pH 7.0, was used for equilibration and washing after sample loading on the column (Buffer A).
Elution was performed by decreasing pH to 2.7 by applying a 20 mM citrate, 150 mM sodium chloride buffer, pH 2.7 (Buffer B).

**Samples:** Human serum albumin HSA (Sigma, St Louis, Mo, U.S.A.) 2 mg/ml in Buffer A, bovine serum albumin BSA (Sigma, St Louis, Mo, U.S.A.) 2 mg/ml in Buffer A and acid treated bovine whey, plain or spiked with BSA or HSA. All samples was adjusted to pH 7.0 and filtered by a 45 µl depth filter prior to application.

**Chromatographic procedure:** The column was equilibrated with approximately 5 column volumes (Cv) of Buffer A. Subsequently 2 ml of sample was applied and the column was further washed with another 6 Cv of buffer A before elution was performed by applying 5 Cv of Buffer B. The procedure was followed by on-line UV detection at 280 nm. UV-adsorbing material passing through the column unhindered or eluted with buffer B was collected and further analysed by SDS-PAGE and silver staining for detection of proteins.

**Results:** When HSA was present in the sample, HSA was quantitatively bound to the column and subsequently eluted by Buffer B. When BSA was present in the sample, BSA was detected in the breakthrough and no BSA could be detected in the eluate. In the case of plain bovine whey all proteins detected in the sample by SDS-PAGE/silver staining could also be detected in the breakthrough while no proteins at all could be detected in the eluate. The results show that a fragment of an albumin binding bacterial cell surface receptor may have an extremely high selectivity for adsorbing HSA from mixtures containing both HSA and BSA.
1. A method for removing a serum albumin from a mixture of other compounds by contacting said mixture with a ligand a) having affinity for and enabling selective binding of the serum albumin and b) being attached to a base matrix insoluble in the aqueous media used or being possible to attach to such a matrix after having become bound to the serum albumin, characterized in that said ligand is derived from an albumin binding bacterial cell surface receptor, preferably a streptococci surface receptor, and that the ligand lacks the IgG-binding and/or $\alpha_2$-macroglobulin-binding ability found in native forms of these type of bacterial receptors.

2. The method of claim 1, characterized in that said mixture derives from a transgeneic mammal, preferably a cow, that has been transformed to express a serum albumin of a mammal that is of another species, preferably human, than the transgeneic mammal.

3. The method of anyone of claims 1-2, characterized in that said ligand contains one or more individual subunits, each of which are capable of binding serum albumin.

4. The method of claim 3, characterized in that said ligand contains only one serum albumin binding subunit.

5. The method of anyone of claims 1-4, characterized in that said albumin binding bacterial receptor is protein G.
6. The method of anyone of claims 1-5, characterized in that said mixture derives from a host in which said serum albumin is expressed as a heterologous protein, said serum albumin preferably being human serum albumin.

7. The method of anyone of claims 1-6, characterized in that said ligand is attached covalently to said matrix.

8. The method of claim 7, characterized in that said ligand contains an amino acid sequence and that said covalent attachment utilizes an amino group and/or a carboxy group and/or mercapto group, said groups being present in an amino acid in the amino acid sequence of the ligand.

9. The method of anyone of claims 1-8, characterized in that after the adsorption step said serum albumin is eluted from said affinity adsorbent and optionally further processed.

10. An albumin-binding ligand derived from a cell surface bacterial receptor and attached covalently to a carrier matrix, characterized in that the ligand is monovalent with respect to ability to bind a serum albumin.

11. The albumin-binding ligand of claim 10, characterized in that the ligand essentially corresponds to region A3 of SFG.

12. A method for removal of serum albumin from a sample that is to be assayed for non-serum albumin components, characterized in that the sample is subjected to affinity adsorption by an albumin-binding ligand derived from an albumin-binding bacterial receptor.
INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/00879

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/765
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)

IPC6: C07K

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

SE, DK, FI, NO classes as above

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>WO 9742835 A1 (GENZYME TRANSGENICS CORPORATION), 20 November 1997 (20.11.97), see claims 1, 5, 15 and 17</td>
<td>1-11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>WO 9602573 A1 (GENE PHARMING EUROPE BV), 1 February 1996 (01.02.96)</td>
<td>1-11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>The Journal of Immunology, Volume 140, No 5, March 1988, Ulf Sjöbring et al, &quot;Isolation and characterization of A 14-KDa Albumin-binding fragment of streptococcal protein G1&quot; page 1595 - page 1599</td>
<td>1-11</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search

10 November 1999

Date of mailing of the international search report

01 December 1999 (01.12.99)

Name and mailing address of the ISA/Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Carolina Palmcrantz/EÖ
Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)
1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the result of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.: 1-11

2. This communication is not the international search report which will be established according to Article 18 and Rule 43.

3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.

4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on the other parts of the international application for which such fees will have been paid.

### DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>WO 9742835 A1 (GENZYME TRANSGENICS CORPORATION), 20 November 1997 (20.11.97), see claims 1, 5, 15 and 17</td>
<td>1-11</td>
</tr>
<tr>
<td>A</td>
<td>WO 9602573 A1 (GENE PHARMING EUROPE BV), 1 February 1996 (01.02.96)</td>
<td>1-11</td>
</tr>
<tr>
<td>A</td>
<td>The Journal of Immunology, Volume 140, No 5, March 1988, Ulf Sjöbring et al, &quot;Isolation and characterization of A 14-KDa Albumin-binding fragment of streptococcal protein G\1&quot; page 1595 - page 1599</td>
<td>1-11</td>
</tr>
</tbody>
</table>

☐ Further documents are listed in a continuation Box. ☑ See patent family annex

---

* Special categories of cited documents:
  - **A** document defining the general state of the art which is not considered to be of particular relevance
  - **E** earlier document but published on or after the international filing date
  - **L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  - **O** document referring to an oral disclosure, use, exhibition or other means
  - **P** document published prior to the international filing date but later than the priority date claimed
  - **T** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  - **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
  - **&** document member of the same patent family

Form PCT/ISA/206 (Annex, first sheet) (July 1992)
INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/00879

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.: 1–11
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:

The wording "albumin-binding bacterial cell surface receptor" of the claims is considered to be a very broad expression covering many different receptors. The search has therefore been focused mainly on albumin-binding ligands derived from the bacterial receptor concerned in the application i.e. Protein G.

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 next 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.: 1–11

Remark on Protest
☐ The additional search fees were accompanied by the applicant’s protest.
☐ No protest accompanied the payment of additional search fees.
As is stated in Annex B to Administrative Instructions under the PCT, in force July 1, 1992 (PCT GAZETTE 1992, June 25, pages 7062-9, see page 7063 and example 5) unity of invention exists only when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding "special technical features"—i.e. features that define a contribution which each of the inventions makes over the prior art (c.f PCT Rule 13.2).

A search for this "special technical feature" mentioned in PCT Rule 13.2 among the independent claims did not reveal such a unifying, novel technical feature. Accordingly, the following inventions were found:

Invention 1, claims 1-11, concerns an albumin-binding ligand and a method for removing a serum albumin from a mixture of other compounds by contacting the mixture with the albumin-binding ligand. The ligand is derived from an albumin-binding bacterial cell surface receptor and lacks the IgG-binding and/or alpha2-macroglobulin-binding ability found in native forms of these type of bacterial receptors.

Invention 2, claim 12, concerns a method for removal of serum albumin from a sample that is to be assayed for non-serum albumin components. The method involves affinity adsorption using a ligand derived from an albumin-binding bacterial receptor.

The "special technical feature" of invention 1 is considered to be that the ligand is monovalent with respect to its ability to bind a serum albumin, that is, that it lacks binding abilities like IgG-binding and/or alpha2-macroglobulin-binding. However, invention 2 is not characterized by a monovalent albumin-binding ligand. The "special technical feature" of invention 2 is considered to be the use of an albumin-binding ligand derived from an albumin-binding bacterial receptor in an affinity adsorption method for removal of disturbing serum albumin from a sample that is to be assayed for non-serum albumin components.

Therefore, the international search has been restricted to invention 1.
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>WO 9742835 A1</td>
<td>20/11/97</td>
<td>AU 2940297 A</td>
<td>05/12/97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0923308 A</td>
<td>23/06/99</td>
</tr>
<tr>
<td>WO 9602573 A1</td>
<td>01/02/96</td>
<td>AU 2989495 A</td>
<td>16/02/96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2195202 A</td>
<td>01/02/96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0773961 A</td>
<td>21/05/97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GB 9414651 D</td>
<td>00/00/00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 10504289 T</td>
<td>28/04/98</td>
</tr>
</tbody>
</table>