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D E C I S I O N
of 10 October 2002

Case Number: T 0150/98 - 3.3.4

Application Number: 88309824.6

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Language of the proceedings: EN

Title of invention:
Method of purifying protein

Patentee:
SCHERING CORPORATION

Opponent:
Boehringer Ingelheim GmbH

Headword:
Protein purification/SCHERING CORPORATION

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step (yes)"

Decisions cited:
G 0009/91, G 0010/91, T 0176/84

Catchword:
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Case Number: T 0150/98 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 10 October 2002

Appellant: (Proprietor of the patent)
SCHERING CORPORATION
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Kenilworth,
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Representative:
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Respondent: (Opponent)
Boehringer Ingelheim
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Decision under appeal: Decision of the Opposition Division of the European Patent Office posted 21 November 1997 revoking European patent No. 0 313 343 pursuant to Article 102(1) EPC.

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: M. R. J. Wieser
S. U. Hoffmann

Summary of Facts and Submissions

I. The appeal was lodged by the patent proprietors (appellants) against the decision of the opposition division, whereby the European Patent No. 0 313 343, which had been opposed by one party under Article 100(a) on the grounds of lack of novelty (Article 54 EPC) and lack of inventive step (Article 56 EPC) of the claims of a main and an auxiliary request, both filed on 16 September 1997, was revoked pursuant to Article 102(1) EPC.

Claim 1 of the main request reads:

"A method for separating a crude protein mixture into a first protein fraction containing a desired protein and a second protein fraction containing an undesired protein which is structurally closely related to the desired protein, which method comprises contacting the crude protein mixture at a selected pH with an ion exchange resin, characterised in that the ion exchange resin is a strong ion exchange resin and the pH of the crude protein mixture is adjusted to a selected pH value at which only one of the desired and undesired proteins is bound by the resin and the other is not, which selected pH value is chosen by:

(a) determining the isoelectric points of a plurality of proteins in the crude protein mixture;

(b) selecting a pH value which lies between and is only fractional pH units away from said determined isoelectric points of the desired and undesired proteins of the fractions to be separated whereby at said selected pH value said desired and undesired proteins in the first and second protein fraction possess very small net electric charges, are oppositely

charged, and only one of the desired and undesired proteins binds to the ion exchanger resin,

and recovering the desired protein either from the unbound fraction, or if the desired protein is in the bound fraction, eluting the desired protein from the resin."

Claim 1 of the auxiliary request differed from this claim by stating in item (a), that the determination of the isoelectric points was carried out by "using computer simulation or isoelectric focusing gels."

The opposition division decided that, while the requirements of novelty were met, claim 1 of both requests did not involve an inventive step, in particular in the light of the following documents:

(6) Pharmacia Biotechnology Aktuell, no. 2, vol. 2,(1987), pages 1 to 27; and

(8) J.Chromatography, vol. 266, (1983), L.A.Haff, et al., pages 409 to 425.

II. The appeal fee was paid and a statement of the grounds of appeal was filed by the appellants.

III. In reply to the statements of grounds of appeal the opponents (respondents) made written submissions wherein they referred to the disclosure of the patent, which they considered to be insufficient.

IV. In reply thereto, the appellants filed written submissions, containing comments concerning sufficiency of disclosure.

V. The board issued a communication informing the parties

that Article 83 EPC, which has been discussed by both parties in their last submissions, was not a ground of opposition, and their attention was drawn to the decisions of the Enlarged Board of Appeal G 9/91 (OJ EPO 1993, 408) and G 10/91 (OJ EPO 1993, 420).

VI. In reply thereto the appellants informed the board that they do not agree to the introduction of a new ground of opposition based on Article 83 EPC, and filed a new auxiliary request, with only one claim, which reads:

"A method for separating GM-CSF from Δ4 GM-CSF which comprises contacting a protein mixture comprising GM-CSF and Δ4 GM-CSF with a strong ion exchange resin, characterised in that the protein mixture is adjusted to a selected pH value at which only one of the GM-CSF and Δ4 GM-CSF is bound by the resin and the other is not, wherein said selected pH value lies between and is only fractional pH units away from the determined isoelectric points of the GM-CSF and Δ4 GM-CSF, whereby at said selected pH value the GM-CSF and Δ4 GM-CSF possess very small electrical charges, are oppositely charged, and only one of the GM-CSF and Δ4 GM-CSF binds to the ion exchange resin, and recovering GM-CSF."

VII. Oral proceedings took place on 10 October 2002. At the oral proceedings, the appellants filed a new auxiliary request, in replacement of the former one, consisting of claim 1 as cited in section VI above, and dependent claims 2 and 3, and an amended description.

VIII. In addition to the documents already cited above, the present decision refers to document

(15) US-A-4 626 355.

IX. The submissions by the appellants may be summarized as

follows:

Document (8) was the closest state of the art. With regard to the main request, this document, contrary to claim 1, referred to a process where separation of proteins, which in a first step were bound to a strong ion exchange resin, was achieved subsequently by salt gradient elution. The isoelectric points of the enzyme isoforms to be separated were not indicated.

A skilled person would not have considered document (15), using a weak ion exchanger, all the more as this document referred to a technically far remote area. Besides the use of a strong ion exchanger, claim 1 differed from this teaching insofar as the proteins to be separated, at the chosen pH value, possessed very small net electrical charges and were oppositely charged. Knowledge of the isoelectric points of proteins did not allow to draw conclusions on their charge in a conclusive way, which, as can be seen from document (6), page 2, left paragraph, lines 11 to 14, depended from more than one factor.

With regard to the auxiliary request, the underlying problem was not addressed in the prior art. Ion chromatography was not known in the art as a tool to separate recombinant proteins from undesired impurities.

X. The submissions by the respondents may be summarised as follows:

Document (15) was considered as closest state of the art. With regard to the main request, the skilled person would not have disregarded its teaching as being technically far remote. The replacement of the weak ion exchanger there, with a strong ion exchanger in the

claim was considered to be obvious for a skilled person in the light of the disclosure in document (6). No other differences between document (15) and claim 1 were identified.

With regard to the auxiliary request, a skilled person would obviously have applied the teaching of the closest prior art to the specific problem posed. The reason why isoforms of specific proteins possess varying isoelectric points was of no importance for finding a method for their separation.

XI. The appellants requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request filed 16 September 1997, or on the basis of the auxiliary request and amended description pages 3 to 7, filed during the oral proceedings on 10 October 2002.

The respondents requested that the appeal be dismissed.

Reasons for the Decision

The main request

Articles 54, 84 and 123 EPC

1. The board is convinced by the appellants argument, that the wording of claim 1, i.e *"..at which only one of the desired and undesired proteins is bound.."*, is clear under Article 84 EPC and expresses the idea of the claimed invention, as the term "one of A and B" has the meaning of "either A or B".

No objections, either by the respondents or by the board, have been brought forward with regard to the

requirements of Articles 54, 123(2) and 123(3) EPC.

Article 56 EPC

2. Controversial is the question which prior art document should be used as starting point for the evaluation of inventive step. The appellants are of the opinion, that, in view of what is claimed, document (15) refers to technical field that is far remote, and would not have been taken into consideration by a skilled person. This document, referring to a medical assay for determining the alcohol consumption of an individual, is not regarded to concern a similar purpose as the claimed invention, referring to an industrial process for obtaining a protein in pure form. According to the principle that the closest state of the art has to be concerned with the same purpose or effect as the claimed invention, they consider document (8) as closest state of the art. It refers to the use of electrophoretic titration curves for predicting optimal chromatographic conditions for fast ion-exchange chromatography of proteins. Test mixtures containing isoforms of beef heart and muscle lactic dehydrogenase are subjected to electrophoretic titration and thereafter loaded onto a strong ion exchange resin at a pH where all isoforms are bound. The retention in terms of salt concentration required for elution, is found to be dependent upon the charge density of the proteins (see page 411, last full paragraph, Figures 3 and 4).

The respondents consider that both documents (8) and (15) are suitable springboards for an analysis of inventive step. With regard to document (8) they mention that the point in time when the actual separation takes place, either at the loading step or at a later gradient elution step, is not relevant, as long as the result of the process is identical. They

put forward, that document (15), although it refers to a diagnostic method, is essentially concerned with the solution of a problem in the field of protein separation.

3. In the boards judgement, document (15), referring to a diagnostic assay, and thus to the medical field, is not far remote from the present invention, which refers to the purification of proteins, which in a preferred embodiment are recombinantly produced proteins for medical use. A skilled person, working in the field of purification of pharmaceutically useful proteins, would consider documents referring to the development of diagnostic assays, based on protein separation, as coming from a technically neighbouring field. In line with the case law of the boards of appeal (T 176/84, OJ EPO 1986, 50), a skilled person would, as well as considering the state of the art in the specific technical field of the application, look for suggestions in neighbouring fields or a broader general technical field if the same or similar problems arose, and if he could be expected to be aware of such general fields.

Thus, document (15) is considered by the board as closest state of the art.

4. The assay described there is based on an ion-chromatographic separation process which makes use of an isoelectric point (Ip) allotropism of specific transferrin isoforms to be determined. By loading a weak polyion exchanger with a solution containing the different isoforms at a pH-value lying between their respective isoelectric points, those isoforms having a higher Ip will pass through the column, and those having a lower Ip will bind to the column (column 4, first full paragraph). The Ip's of the six relevant

isoforms are determined according to example 1, whereby a transferrin sample is applied to a column at a pH high enough so that all isoforms are negatively charged and therefore attach to the ion exchanger (see column 6, second full paragraph and table). By lowering the pH by applying a pH gradient, the charge of each isotransferrin changes from negative to less negative and finally to zero, where it is released from the ion exchanger at its Ip. Appellant's argument, that it cannot be derived from document (15), that any two isotransferrins mentioned, are oppositely charged and possess very small net electrical charges at a pH-value between their respective Ip´s, cannot be accepted. Taking asialotransferrin and monosialotransferrin as an example, both at the initial pH of 6,8 are negatively charged and bound by the anion exchanger. Upon lowering of the pH to 6,1, asialotransferrin is eluted, as a result of the fact that at this pH the charge of the protein is zero. The next protein to be eluted upon further lowering the pH, is monosialotransferrin at pH 5,9. The charge of asialotransferrin at a pH above its Ip of 6,1, where it is zero, has become positive. Thus, at a pH between 5,9 (Ip of monosialotransferrin) and 6,1 (Ip of asialotransferrin), monosialotransferrin has a very small negative electric charge and asialotransferrin has a very small positive electric charge. This is used in separating the two isoforms by loading a mixture on an anion exchanger at a pH of 6,00 +/- 0,02, whereby the (weakly)positive charged asialotransferrin passes through the column, while the (weakly) negative charged monosialotransferrin is bound by the column (see column 4, line 4 to 29; scenario (C)).

5. In the light of this teaching, the underlying, objective technical problem is the provision of an alternative method for the separation of structurally

closely related proteins.

6. This problem has been solved by the method of claim 1, which is distinguished from the disclosure in the closest prior art by the use of strong ion exchange resin. The resin used in document (15) is a weak exchanger having a high buffering capacity for a pH in a range between the Ip's of the proteins to be separated.

7. Document (15) does not contain a hint to replace the used weak ion exchanger with another resin. The skilled person, in order to solve the problem, would turn to publications dealing with general principles and embodiments of ion exchange chromatography, such as document (6), a folder of the employer of the inventors of document (15), published some years later. This document explains on page 2, right column, the technical principle of the present invention ("Um an einen Anionenaustauscher zu binden, benötigen Proteine einen pH-Wert, der über ihrem IEP liegt; bei Kationenaustauschern findet eine Bindung nur bei pH-Werten unterhalb des IEP statt"). On pages 3 to 4 the differences between strong and weak ion exchangers are specified, and on page 4, left column, a table is given, indicating three major advantages of strong resins over weak ones. The weak polyion exchanger used in document (15), PBE 94, is discussed on page 6 (Figure 9). Document (6) states at the bottom of page 4, left column, that the four most modern products of the company publishing the document are strong exchangers.

A skilled person being confronted with the problem to provide an alternative process to the method of document (15), would obviously consider to replace the resin used therein, with the most modern and

advantageous products disclosed in document (6).

8. For these reasons, claim 1 is found to lack an inventive step and thus the request of which it is part is not allowable under Article 56 EPC.

The auxiliary request

Articles 54, 83, 84 and 123 EPC

9. The board is convinced that the claims of the auxiliary request meet the requirements of Articles 123(2) and (3) EPC. Clarity, support by the description, and novelty of claims 1 to 3 have not been objected by the respondents, and do not form a hindrance to patentability in the eyes of the board as well.

Sufficiency of disclosure does not have to be considered, as the auxiliary request refers to a preferred embodiment of the main request, for which Article 83 EPC was not a ground of opposition (see Facts and Submissions, section VI above).

Article 56 EPC

10. Claim 1 differs from the main request in that it is restricted to a method for separating GM-CSF from Δ4 GM-CSF. This corresponds to the preferred embodiment of the invention (column 2, lines 31 to 35; column 4, line 52 to column 6, line 1; example 1 and claims 7, 8 and 11 of the granted patent). Dependent claims 2 and 3 correspond to claims 9 and 11 of the patent as granted.

11. Document (15), also for the auxiliary request, is considered to represent the closest state of the art. However, the objective technical problem is a different

one, namely the finding of a method to separate GM-CSF from Δ4 GM-CSF. This problem is not mentioned in the prior art documents, and no solution to it is provided, accordingly.

12. The Δ4 impurity is a degradation product of GM-CSF, missing 4 N-terminal amino acids, which is formed during recombinant production of GM-CSF (column 4, lines 54 to 58 of the granted patent).

The skilled person knows that the absence or presence of one or a few amino acids in the sequence of a protein can have essential bearings as well on the structural (molecular weight, size, primary-, secondary- and tertiary structure) as well as on the functional features of the protein. Especially when used for a medical purpose (GM-CSF is the Granulocyte Macrophage Colony Stimulating Factor), it is of utmost importance to separate the pure protein from non-functional or even harmful impurities.

As a result of the multiplicity of possible changes, that can be effected by the missing of 4 N-terminal amino acids from a complex protein, the skilled person could consider a manifold of processes to separate the desired from the undesired protein. Among the parameters the skilled person can select as basis for a separation process, such as size, weight, shape, immunoaffinity and charge, several have been found by the appellants as not effecting a satisfying result (column 4, line 58 to column 5, line 4 of the granted patent).

13. Document (15) discloses a ion-chromatographic method for separating 6 isoforms of transferrin. While all of them are present in the blood of addicted alcohol consumers, only 4 can be detected in the blood of

normal patients. The method is based on an Ip allotropism of the isoforms, resulting from the different number of sialic acid groups contained therein. No indication of a general applicability of the disclosed method is given, nor is there any suggestion to use the method for separating proteins produced by recombinant DNA technology from contaminations having a different amino acid sequence.

Document (8) discloses the separation of isoforms of beef heart and muscle lactic dehydrogenase (M_2H_2 type A and B, which have identical primary structure and represent the two possible arrangements of two subunits of two different types in a tetrahedral structure) by loading them onto a strong ion exchange resin and eluting them with a salt gradient.

Document (6) is concerned with the influence of the charge of proteins on their chromatographic behaviour. The central role of the Ip in this respect is acknowledged (Figures 2 and 3), and a detailed description of the principles of ion exchange chromatography is provided (page 3, right column, last paragraph to page 5; Figures 6 and 7).

Neither document (8) nor (6) disclose a separation process carried out at a pH-value where only one of a desired and an undesired, structurally closely related, protein contained in a mixture binds to an ion exchange resin.

14. A skilled person in order to solve the problem underlying the present invention, and being aware of the relevant state of the art, would consider various possible routes to separate GM-CSF from its Δ4 impurity and would, thus, have to make a choice not knowing in advance which route might lead to success. The

decision, to make use of the Ip allotropism of the two proteins and to separate them by ion exchange chromatography according to claim 1, therefore in the board's judgement, cannot be arrived in a straightforward and thus non-inventive manner.

The board therefore concludes that claims 1 to 3 of the auxiliary request fulfil the requirements of Article 56 EPC.

Order

For these reasons it is decided:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent on the basis of the auxiliary request, claims 1 to 3, and an amended description, pages 3 to 7, both filed during the oral proceedings on 10 October 2002.

The Registrar: The Chairwoman:

P. Cremona U. M. Kinkeldey