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D E C I S I O N
of 5 November 1998

Case Number: T 0746/94 - 3.3.4

Application Number: 85902779.9

Publication Number: 0183776

IPC: C12P 21/00

Language of the proceedings: EN

Title of invention:
A process for isolating a substantially insoluble polypeptide using non-ionic detergents

Patentee:
Berlex Laboratories, Inc.

Opponent:
Boehringer Mannheim GmbH Patentabteilung

Headword:
Polypeptide isolation/BERLEX

Relevant legal provisions:
EPC Art. 123(2), 54, 56

Keyword:
"Main request: added subject-matter - yes"
"Auxiliary request: novelty - yes, inventive step - yes"

Decisions cited:
T 0013/84, T 0433/86

Catchword:
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Case Number: T 0746/94 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 5 November 1998

Appellant: (Opponent)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 16 August 1994 concerning maintenance of European patent No. 0 183 776 in amended form.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
W. Moser

Summary of Facts and Submissions

I. European patent No. 0 183 776 with the title "A process for isolating a substantially insoluble polypeptide using non-ionic detergents" was granted with 6 claims on the basis of European patent application No. 85 902 779.9.

Claim 1 as granted read as follows:

"1. A process for isolating a substantially insoluble polypeptide being produced by a genetically engineered organism which does not naturally produce said gene product, characterised in that the process comprising the steps of:

a) contacting substantially insoluble polypeptide contained in said lysate with a solution containing a non-ionic detergent at a concentration sufficient to solubilize impurities while maintaining the insolubility of the polypeptide; and

b) separating the insoluble polypeptide from the soluble impurities;

with the proviso that the insoluble polypeptide cannot be selected from chymosin, precursors of chymosin and fusion products thereof capable of displaying milk clotting activity."

II. A notice of opposition was filed requesting the revocation of the patent under Article 100(a) to (c) EPC.

III. By an interlocutory decision within the meaning of Article 106(3) EPC, the Opposition Division maintained the patent in amended form according to Article 102(3) EPC on the basis of the second auxiliary request and of a correspondingly amended description filed during the oral proceedings.

Claim 1 of this request read as follows:

"1. A process for isolating a substantially insoluble polypeptide being produced by a genetically engineered microorganism which does not naturally produce said gene product, characterised in that the process comprising the steps of:

a) preparing a lysate from the microorganism;

b) reducing the viscosity of the lysate by physical methods;

c) contacting substantially insoluble polypeptide contained in said lysate with a solution containing a non-ionic detergent at a concentration sufficient to solubize impurities while maintaining the insolubility of the polypeptide; and

d) separating the insoluble polypeptide from the soluble impurities;

with the proviso that the insoluble polypeptide cannot be selected from chymosin, precursors of chymosin and fusion products thereof capable of displaying milk clotting activity."

Dependent claims 2 to 6 related to further embodiments of the process of claim 1.

IV. The Appellant (Opponent) filed an appeal, paid the appeal fee and submitted the grounds for the appeal.

V. The Respondent (Patentee) answered to the Appellant's submissions.

VI. A communication was sent according to Article 11(2) of the Rules of Procedure of the Boards of Appeal, setting out the Board's provisional, non-binding opinion.

VII. Oral proceedings took place on 5 November 1998. The Respondent defended the patent in suit on the basis of a main request containing the claims as maintained by the Opposition Division and filed an auxiliary request with three claims. Claims 2 and 3 of the auxiliary request were the same as claims 2 and 5 of the main request. Claim 1 of the auxiliary request read as follows:

"1. A process for isolating and activating a substantially insoluble polypeptide being produced by a genetically engineered micro-organism which does not naturally produce said gene product, characterised in that the process comprises the steps of:

(a) preparing a lysate from the microorganism;

(b) reducing the viscosity of the lysate by physical methods;

(c) contacting the substantially insoluble polypeptide

contained in the said treated lysate with a solution containing a non-ionic detergent at a concentration sufficient to solubilize impurities while maintaining the insolubility of the polypeptide;

(d) separating the insoluble polypeptide from the soluble impurities;

(e) the insoluble polypeptide after separation from soluble impurities is solubilized by contact with a chaotropic agent; and

(f) the solubilized polypeptide is activated by removal of the chaotropic agent

and further characterised in that said non-ionic detergent comprises Triton X-100 with the proviso that the insoluble polypeptide cannot be selected from chymosin, precursors of chymosin and fusion products thereof capable of displaying milk clotting activity."

VIII. The following documents were inter alia considered:

(1): El-Afifi, S.I., Egypt. J. Microbiol., 13, No. 1-2, pages 107 to 119, 1978,

(2): Shepherd, R. J. et al., Virology, 102, pages 389 to 400, 1980,

(3): Kleid, D.G. et al., Science, 214, pages 1125 to 1129, 1981,

(4): EP-A 0 123 928.

IX. The submissions in writing and during oral proceedings by the Appellant can be summarized as follows:

- There were two reasons why claim 1 of the main request (see point III above) failed to fulfil the requirements of Article 123(2) EPC.

  Firstly, the claimed process for the separation of an insoluble protein from cellular impurities was not disclosed as such in the application as filed. What this application disclosed was a process for restoring biological activity to a cloned gene product initially obtained in an insoluble (inactive) form which comprised the separation of the insoluble protein from the cellular impurities followed by its denaturation and renaturation into an active form.

  On page 1 to 6 of the application as filed, the invention was repeatedly identified as the restoration of biological activity to the insoluble protein: the separation of the insoluble protein was never described independently from its recovery in active form, the last steps of denaturation and renaturation were never considered facultative.

  Secondly, the application as filed did not contain any disclosure of non-ionic detergents in general. To the contrary, the specific non-ionic detergent Triton X-100 was described as essential since Tween-20, the other non-ionic detergent cited, was found not to be active.

- Document (4) was novelty destroying to claim 1 under Article 54(3) EPC as it disclosed the claimed process for use in the purification of recombinant chymosin and, thus, taught that this process could be applied to proteins in general.

- Document (2) disclosed a process for the purification of a foreign protein present in plant cells in the form of inclusion bodies. Said protein was separated from the other cellular components by treatment with Triton X-100, which solubilised these components, whereas the inclusion bodies remained insoluble. It was obvious to transfer this technology from plants to bacteria, even if the inclusion bodies in plants were considered to have a morphology different from that of the inclusion bodies in bacteria, because the term "inclusion bodies" always defined the same entity i.e. an insoluble, aggregated foreign protein. Furthermore, document (3) disclosed the use of a non-ionic detergent in a process for the isolation of inclusion bodies from bacteria where the bacterial components were solubilised while the inclusion bodies remained insoluble. The combination of document (1) or (2) with document (3) rendered the claimed process non inventive.

With regard to the auxiliary request, no objections were raised under Articles 123(2), (3) and 83 EPC. However, the same objections under Articles 54 and 56 EPC prevailed against this request as against the main request.

X. The Respondent answered essentially as follows:

- The process of separating the insoluble recombinant protein was only a part of a much more complex process which included cloning and expression of the foreign gene as well as the recovery of the protein in an active form. Although each of these subprocesses was essential if the recombinant active protein was to be recovered, there was no necessity to recite them all in the claim. The skilled person finding that the recombinant protein formed aggregates would be perfectly aware that two independent steps had to be achieved to retrieve it in active form, but would not necessarily choose to perform both these steps as the biologically inactive protein had uses on its own, for example the determination of the amino-acids composition or of the N terminal sequence of the molecule. The conclusion was, thus, that even if the two processes were concomitantly described, they would be considered distinct.

  There existed a formal basis in the application as filed for claiming the isolation process as such on page 3, line 10 where mention was made of "the novel processes for producing chymosin utilising Triton X-100 (a non-ionic detergent) as a reagent for the protein purification and urea and alkali as reagents for solubilization and renaturation." The plural form of the word "process" showed that the purification and the recovery in active form of the recombinant protein were to be considered independently.

The basis in the application as filed for the term "non-ionic detergents" was to be found in claim 6 which related to detergents in general, together with page 3, line 12 where it was specified that Triton X-100 was a non-ionic detergent. This attracted the reader's attention to non-ionic detergents in general.

- Document (4) could not be novelty-destroying for the subject-matter of claim 1 because it was solely directed to the purification of chymosin. The generic teaching on pages 1 to 3 of said document was not directed to protein purification in general which was only mentioned on the last line of page 3.

- Documents (1) and (2) were concerned with the purification of plant inclusion bodies which were morphologically different from inclusion bodies produced by bacteria. The use of the non-ionic detergent NP-40 in document (3) for the recovery of a recombinant protein was in no way connected to the purification of the protein itself but with the lysis of the recombinant cells. The combination of document (1) or (2) with document (3) thus could not destroy the inventive step of claim 1.

XI. The Appellant requested that the decision under appeal be set aside and the European patent No. 0 183 776 be revoked.

The Respondent requested that the appeal be dismissed or, as an auxiliary request, that the decision under

appeal be set aside and the patent be maintained on the basis of the claims 1 to 3 filed during oral proceedings.

Reasons for the Decision

1. The appeal is admissible.

Main request

Article 123(2) EPC

2. Claim 1 of the main request relates to a process for separating a substantially insoluble recombinant polypeptide from soluble impurities. It addresses the problem of the purification of said polypeptide quite irrespective of its activity.

3. The Respondent argued that a formal basis in the application as filed on which to acknowledge that the process for polypeptide purification is a separate process may be found on page 3, lines 10 to 14: "An aspect of the present invention discloses novel **processes** for producing active chymosin, utilizing Triton X-100 (a non-ionic detergent) as a reagent for protein purification and urea and alkali as reagents for solubilization and renaturation."(emphasis added).

4. The Board has to examine whether this statement read in the light of the common general knowledge at the priority date of the patent in suit can serve as a direct and unambiguous disclosure of the now claimed

process: that of purifying the protein with a non-ionic detergent.

5. The application as filed defines the invention on page 1 as "the restoration of biological activity to inactive protein". On page 5, line 26, it is stated: "the present invention requires several specific steps to achieve efficient recoveries of active chymosin". Figure 2 shows a quantitative study of the claimed process whereby the amount of chymosin recovered is measured by its activity. In the same manner, the relative effectiveness of various renaturation procedures is evaluated in Table I by the activity of chymosin, i.e after denaturation and renaturation have been carried out. Finally, the sentence following the statement quoted by the Respondent reads:" Thus the present invention is a novel and original procedure for protein renaturation", and originally filed claim 1 relates to a process for restoring the biological activity of an insoluble recombinant polypeptide which comprises a first step of separating the polypeptide from soluble impurities and a second step of restoring its activity.

6. In the Board's judgment, all this is evidence that in the application as filed, the separation of the insoluble recombinant protein per se was only mentioned as a preliminary step in solving **the** problem of restoring the protein's activity which is dealt with in technical detail. This means that in the claim in question, compared to the original disclosure, emphasis is put on one single step of a whole process for recovering an **active** protein.

7. In accordance with the case law of the Boards of Appeal, the reformulation of a problem is not precluded by Article 123(2) EPC if the problem could be deduced by a skilled person from the application as filed (cf. decision T 13/84, OJ EPO 1986, 253). However, in the present case, the skilled person could not have considered the step of separating the protein alone as the problem to be solved. The conclusion is, thus, reached that the reformulation of the problem and its solution by claim 1 amounts to changing the subject-matter to the application as filed in a way which the skilled person would not have considered. This is contrary to the gist of Article 123(2) EPC that the public must not be taken by surprise by claims which it could not directly and unambiguously have expected on the basis of the original disclosure of the application as filed. Claim 1 is not allowable under Article 123(2) EPC.

8. In view of this finding, the objection by the Appellant that the expression " non-ionic detergents" in claim 1 finds no basis in the application as filed need not be investigated.

Auxiliary request

Article 123(2)(3) EPC

9. The features of the process of claim 1 are disclosed on page 5 of the application as filed. Compared to claim 1 as granted, present claim 1 is restricted to the use of Triton X-100 in a process which includes the recovery of an active protein. The scope of the claim has, thus, been limited. The requirements of Article 123(2)(3) EPC are fulfilled.

Article 83 EPC

10. No objections were raised by the Appellant that the requirements of this article were not fulfilled. In the Board's opinion, the circumstances of the case are such that they deserve to be discussed.

11. How to perform the invention is shown by only one specific example on pages 4 to 9 of the application as filed, where a process is described which leads to the production of active chymosin. The starting material in this process is prochymosin i.e. the very substrate which is disclaimed in claim 1 to distinguish the invention over the teaching of the prior art document (4). An unusual situation has, thus, occurred whereby the only specific teaching which is provided to illustrate the reproducibility of the claimed invention does not fall within the scope of the claim.

12. On a very formal basis, the skilled person reading the description of the application as filed is not given detailed guidance, how to perform the claimed invention

for any desired protein. Nonetheless, the successive general process steps to be taken to carry out the invention are described in detail. Therefore, following the example, the reproducibility of the claimed invention depends on whether the material which is recovered and activated in the exemplified process (prochymosin) is a key feature of said process.

13. In the Board's judgment, each of the steps of bacterial lysis, disruption of DNA, solubilisation, renaturation, denaturation, which are necessary to recover chymosin in active form can be performed without undue burden by the person skilled in the art of handling proteins and DNA, also for other proteins to be activated. No evidence has been provided that this process would not lead to the recovery of an active protein other than chymosin. For these reasons, there is sufficiency of disclosure of the process for recovery of a desired protein.

Article 54 EPC

14. Document (4) is relevant prior art under Article 54(3)(4) EPC. It discloses the cloning and expression in E.coli of the cDNA encoding chymosin or its precursor. After cell lysis, the enzyme is found in the insoluble membrane components of the bacteria and it is recovered in active form after solubilisation of said components with Triton X-100, denaturation with urea and renaturation of the recombinant protein.

15. To circumvene a possible objection for lack of novelty, the Respondent disclaimed the process when carried out for the recovery of recombinantly produced chymosin or

its precursors. The Appellant argued that this disclaimer was inadequate to establish novelty because the skilled person would have understood from document (4) that the process could be carried out with any proteins.

16. The invention disclosed by document (4) is defined on page 1 as "the synthesis using recombinant DNA techniques of a polypeptide derived from calf rennin which displays milk clotting ability". Nine out of the eleven examples describing the experimental features of the invention are concerned with the isolation and recovery of recombinant clones expressing rennin (chymosin). The last two examples describe the recovery of rennin in active form from the recombinant clones. It is not suggested anywhere in the document that the methods described in these examples would be applicable to other proteins. Thus, document (4) solely provides the disclosure of one embodiment of the claimed process. In accordance with the case law of the Board's of Appeal (see decision T 433/86 of 11 December 1986), a disclaimer is admissible to establish novelty over document (4).

17. This is not in contradiction with the findings under points 10 to 13 above that the requirements of Article 83 EPC are fulfilled in the patent in suit, in the absence of any example, because the teaching in said patent is not restricted to chymosin (see page 1, lines 5 to 10)

18. No other documents on file disclose subject-matter which could destroy novelty. The requirements of Article 54 EPC are fulfilled.

Inventive step

19. Document (3), which, in the Board's view, is the closest prior art, discloses a process for the recovery from E.coli of inclusion bodies containing a recombinant fusion protein comprising the capsid protein VP3 of picornaviruses. The step of lysing the bacteria is carried out with a lytic enzyme in the presence of the non-ionic detergent NP-40. The viscosity of the lysate is decreased enzymatically. The inclusion bodies are thereafter separated from the cellular impurities by centrifugation. The VP3 hybrid protein is solubilized in denatured form in 8M urea and renatured at pH 8.3. It is shown to possess the immunogenic properties of viral VP3.

20. Starting from this prior art, the technical problem to be solved can be defined as the provision of a process for the recovery in soluble form of an active recombinant protein.

21. On the basis of claims 1 to 3, the Board is satisfied that this problem has been solved.

22. The process disclosed in document (3) differs from the claimed process in one fundamental aspect which is that no specific step is taken to solubilise the cellular debris. The non-ionic detergent is used in the context of lysing the bacterial cells. It is only with hindsight that this disclosure could be argued to suggest that the non-ionic detergent concomitantly serves to solubilise the cell debris. Thus, in the Board's judgment, document (3) on its own, does not render obvious the claimed process.

23. Document (2) is concerned with the isolation of inclusion bodies naturally occurring in plant cells after viral infection. On page 395, right hand column, it is disclosed that inclusion bodies which are composed of an aggregate of a 55.000 daltons matrix protein can be recovered in insoluble form after the plant cells have been lysed and the cellular debris have been solubilised with Triton X-100. The matrix protein is said to have an extreme tendency to aggregate (page 395). It is essentially insoluble at pH values near neutrality or higher (up to pH 10.5) (page 397). In fact, the best solvent for the unaggregated insoluble matrix protein is the non-ionic detergent Triton X-100 (which seems to imply that the protein does not have the same properties as its own aggregate)(page 398).

24. In the Board's judgment, the skilled person reading document (2) would not take the properties of the matrix protein as representative of the properties of proteins in general (see point 19, for example, the solubility of the VP3 at pH.8.3). Thus, there would be no reason to expect that the properties of the aggregates of the matrix protein would be shared by the aggregates of other insoluble proteins.

25. Accordingly, also the combination of documents (3) and (2) does not suggest a process for the recovery of active recombinant proteins whereby a non-ionic detergent would be used to solubilise the cell debris while the inclusion bodies remained intact. Inventive step is, thus, acknowledged.

Order

For these reasons it is decided that:

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:

   (a) claims 1 to 3 filed during oral proceedings as auxiliary request

   (b) cover page and pages 2, 2a and 2b of the description submitted during oral proceedings

   (c) page 3 and page 4, lines 1 to 50 of the description as granted

   (d) drawings, Figures 1 to 4 as granted

The Registrar: The Chairwoman:

U. Bultmann U. Kinkeldey