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D E C I S I O N
of 18 April 2002

Case Number: T 0294/99 - 3.3.4

Application Number: 92102543.3

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Title of invention:
Method for producing cysteine-free peptides

Patentee:
TAKEDA CHEMICAL INDUSTRIES, LTD.

Opponent:
BioNebraska, Inc.

Headword:
Cysteine-free peptides/TAKEDA LTD.

Relevant legal provisions:
EPC Art. 56

Keyword:
"Granted claims - inventive step (yes)"

Decisions cited:
-

Catchword:
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Case Number: T 0294/99 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 18 April 2002

Appellant: (Opponent)
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 14 January 1999 rejecting the opposition filed against European patent No. 0 499 990 pursuant to Article 102(2) EPC.**

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
S. C. Perryman

Summary of facts and submissions

I. The appeal lies from the decision of the Opposition Division to reject the opposition against European patent EP-0 499 990 with the title "Method for producing cysteine-free peptides" which was granted with 10 claims for all Designated Contracting States.

Granted claim 1 read as follows:

"1. A method for producing a cysteine-free peptide, which comprises cultivating a transformant having a vector carrying a gene coding for a fused protein comprising a protein having cysteine at its N-terminal and a cysteine-free peptide ligated to the N-terminal to express said fused protein, and subjecting the expressed fused protein to a reaction for cleaving the peptide linkage on the amino group side of the cysteine residue, wherein the reaction for cleaving the peptide linkage is conducted by a cyanilation reaction by using a S-cyanilation reagent followed by

(i) hydrolysis to produce a carboxypeptide, or by

(ii) aminolysis to produce an amide or substituted amide

derivative at the respective C-terminal."

Independent claims 4 and 7 were directed to further methods of producing a cysteine-free peptide which also involved said peptide being the N-terminal part of the fusion protein and being linked to the rest of the molecule by a cysteine. Dependent claims 2 and 3, 5 and

6 and 8 were directed to further features of the methods of claims 1, 4 and 7 respectively.

Independent claim 9 and claim 10 dependent thereof were addressed to specific peptides.

II. The opposition for lack of inventive step was directed against granted claims 1 to 8. The Opposition Division decided that the teaching of document (2) (see below) on its own did not destroy the inventive step of the presently claimed method. In addition, it was found that the combination of the teaching of document (2) with that of document (11) (see below) could only be argued to render the claimed invention obvious by applying hindsight.

III. The documents mentioned in the present decision are the following:

(2) EP-A-0 301 485,

(11) Uhlén, M. and Moks, T., Gene Expression Technology, Methods in Enzymology, Volume 185, pages 129 to 143, Edited by D. V. Goeddel, Academic Press, Inc., 1990.

IV. The arguments in writing and during oral proceedings by the Appellants (Opponents) insofar as they are relevant to the present decision may be summarized as follows:

- Document (2) which described a process for obtaining a cysteine-free peptide, the first two steps of which involved:

  - expressing said peptide as part of a fusion

protein whereby it was linked to the rest of the molecule by a cysteine (col. 2, lines 11 to 32);

- cleaving the fusion protein at the level of the cysteine intermediary residue (col. 4, lines 41 to 51).

These steps were the same as those of the process in claim 1. That the desired peptide was the C-terminal rather than the N-terminal part of the fusion protein (as now claimed) was not a relevant feature. Indeed, the skilled person would notice that the N-terminal part of the fusion protein, ie. the carrier part, was recovered intact by scission on the amino group of the cysteine residue. He/she would also infer therefrom that any other peptides expressed as the N-terminal part of the fusion protein could also be produced in a native state.

It should also be taken into account that no difference was made in the claims between the N- and C- terminal parts of the fusion protein, which were both called "peptide".

For these reasons, document (2) on its own destroyed the inventive step of the claimed process.

- Alternatively, document (2) being the closest prior art, the problem to be solved could be defined as using the process described therein to provide cystein-free peptides in a different manner.

  The solution provided was that the fusion protein be produced with the desired peptide being the

N-terminal part of said protein and linked by a cysteine to the rest of the molecule.

The combination of the teachings of documents (2) and (11) rendered this solution obvious as document (11) disclosed that a fusion protein could be produced with the desired peptide at the N- or C- terminal end. In the passage bridging pages 134 and 135, it was explained that it was advantageous to produce the desired peptide as the N-terminal part in order to facilitate direct N-terminal sequencing of said peptide. Given this information, the person skilled in the art would understand that if the desired peptide was produced as the N-terminal part of the fusion protein, it would be obtained with an intact N-terminal end. This was precisely the solution proposed in the patent in suit for obtaining the desired peptide in its native state, which solution was, thus, obvious.

- The fact that in the claimed proces, the desired peptide was directly obtained in its native state rather than with a modified cysteine at its NH2 end was not an advantage of said process compared to that described in document (2) because document (2) taught how to get rid of the modified cysteine.

- Finally, as the process of claim 1 amounted to a non-synergistic addition of a known recombinant step for making a fusion protein and a known chemical step for cleaving that protein, the combination of any document describing the first step with any document describing the second step

rendered the claimed subject-matter non-inventive.

V. The arguments in writing and during oral proceedings by the Respondents (Patentees) insofar as they are relevant to the present decision may be summarized as follows:

- Document (2) described a process for the production of cysteine-free parathormone by expressing this peptide as the C-terminal part of a fusion protein linked to the N-terminal part by a cysteine and, then, separating it from said fusion protein by various chemical means. Parathormone was, thus, obtained with a modified cysteine instead of its first amino-acid (serine), which modified cysteine had to be either reconverted into a serine or eliminated in further experimental steps.

  The problem of producing any desired cysteine-free peptide in its native state was neither mentioned nor suggested, let alone was the solution claimed in the patent in suit, given.

  The argument by the Appellants that document (2) taught the skilled person to express the desired cysteine-free peptide as the N-terminal part of the fusion protein, if this was wanted in its native state, because the document disclosed, albeit implicitly, that the carrier part of the fusion protein (the N-terminal part) was obtained in a native state, was not convincing. Firstly, document (2) was clearly limited to the production of parathormone. Secondly, cysteines were present in the carrier part of the fusion protein (cro-â

galactosidase) ie. it was not a cysteine-free peptide. Finally, only the N-terminal subfragment of the carrier protein would be retrieved in its native state by chemical scission, which subfragment was never mentioned in document (2), nor was the desirability of obtaining it.

Contrary to the Appellants' argument, there was no ambiguity as to which part of the fusion protein was intended to be produced by the claimed processes, as the desired peptide was the only one identified as being cysteine-free.

Document (2) on its own did not destroy inventive step.

- Starting from document (2) as the closest prior art, the problem to be solved could be defined as providing a cysteine-free peptide in its native state and in high yield.

None of the cited references alone or in combination gave any indication as to what to do. Document (11) which discussed C- and N-terminal fusion strategies did not disclose that fusion proteins could be produced with a cysteine residue linking the N- and C-terminal parts. It was, thus, only with hindsight that its teaching could be combined with that of document (2). The mention on page 134 that N-terminal fusions were advantageous for direct sequencing of the peptide of choice would be taken by the skilled person as meaning that with such fusions, one would not have to sequence the entire carrier peptide before getting to the sequence of the desired peptide. Thenagain,

it was only with hindsight knowledge of the present invention that the observation about ease of sequencing could be interpreted as suggesting in an obvious manner that positioning the desired peptide at the N-terminal end of the fusion protein with a cysteine link to the C-terminal carrier part of the fusion protein would enable its recovery in a native state.

- It was a distinctive advantage that the claimed process enabled the direct recovery of the desired peptide in its native state, as the methods suggested in document (2) for cleaving the modified cysteine from the N-terminal end of the desired peptide obtained by the method therein described were either cumbersome or did not give satisfactory results.

The claimed subject-matter was inventive over the combination of the teachings of documents (2) and (11).

VI. The Appellants requested that the decision under appeal be set aside and that the European patent No. 0 499 990 be revoked.

The Respondents requested that the appeal be dismissed and the patent be maintained.

Reasons for the decision

1. Document (2) describes three processes for ameliorating the recombinant production in E. coli, of the hormone called parathormone. In one such process, advantage is

taken that parathormone does not contain any cysteine:

- parathormone is expressed as **the C-terminal part** of a fusion protein, which part is linked to the N-terminal part by a cysteine, the linkage involving the substitution of the first amino acid of parathormone (serine) by said cysteine (Fig. 1B2), and,

- parathormone is retrieved from the fusion by known chemical methods (page 3, col. 4, lines 41 to 58), one of them being that the cysteine is cyanilated and, then, the fusion protein is cleaved at the level of the modified cysteine which remains at the N-terminal end of the C-terminal part of the molecule ie. **at the N-terminal end of parathormone**. The modified cysteine needs to be eliminated thereafter.

2. The argument by the Respondents that the N-terminal part of the fusion protein (the carrier part) linked to the C-terminal part (the parathormone) by a cysteine in the above-mentioned process and its subsequent scission from parathormone could be considered as meeting the requirements of claim 1 must fail, because this N-terminal part is not free of cysteine (submission by the Respondents at oral proceedings, not challenged by the Appellants). Cyanilation of the fused protein will "conceptually" result in the N-terminal peptide of the carrier protein (from the first amino-acid in the molecule to the amino-acid preceeding the first cysteine) being recovered in an intact form whereas all subsequent peptides will carry a modified cysteine residue at their N-terminal end. However, document (2) is not concerned and does not describe what happens to

or could be done with the carrier part of the fusion protein. Thus, it does not provide a clear and unambiguous disclosure of a cysteine-free peptide being derivable from the N-terminal end of said carrier part by a process such as claimed. Accordingly, lack of novelty (which was anyhow not cited as a ground of appeal) is not at stake and document (2) is to be considered solely under Article 56 EPC as the closest prior art to the subject-matter of claim 1.

3. Starting from document (2), the problem to be solved may be defined as providing a recombinant process to produce any cysteine-free peptide in a native state.

4. The solution is a process involving two steps:

- expressing the cysteine-free peptide as **the N-terminal part** of a fusion protein, which part is linked to the C-terminal part by a cysteine.

- retrieving the cysteine-free peptide from the fusion by the "cyanilation method" (see above), the modifed cysteine residue thus remaining **at the N-terminal end of the the carrier part** of the fusion protein.

As the desired cysteine-free peptide is the N-terminal part of the fusion protein, it is directly obtained in its native state.

5. Document (2) does not mention *expressis verbis* that parathormone is a cysteine-free peptide. Furthermore, as already mentioned in point 2 above, document (2) provides three processes for producing parathormone which differ from each other by the amino-acids used to

link the hormone to the carrier part in the fusion protein. In the Board's judgment, the skilled person wanting to produce a cysteine-free peptide would, nonetheless, consider the teachings of document (2) and within these teachings, his/her attention would be drawn to the method involving the cysteine link at the expense of the other methods because the sequence of parathormone was known at the priority date (and, therefore, the lack of cysteines within it; patent-in-suit, page 2, col. 2, lines 14 to 17)) and the method of cleaving at the level of a cysteine residue, would be thought especially convenient since it could not take place within the desired cysteine-free peptide. Thus, document (2) points out, albeit in an indirect manner, to cysteine being a suitable link in fusion proteins to be used as intermediary products in the production of cysteine-free peptide.

6. However, there is no direct or indirect disclosure in document (2) that parathormone could be expressed as the N-terminal part of the fusion protein. On the contrary, this document gives detailed information on how to treat the modified parathormone obtained as the C-terminal part of the fusion protein after the cleavage reaction, to recover it in its native state. The skilled person is, thus, clearly directed to this and only this way to proceed. Accordingly, document (2) does not make obvious the reversal of the order of the carrier part and the desired cysteine-free peptide in fusion proteins.

7. The Appellants' argument that the claimed solution (desired peptide expressed as the N-terminal part of the fusion protein, cysteine linkage) was rendered obvious by the fact that the carrier part of the fusion

protein produced according to the process disclosed in document (2) was obtained in a native state, is not accepted for the same reasons as given in point 2 supra: the carrier part of the protein is not the peptide which it is desired to produce, it is not cysteine-free, neither its fate nor that of subfragments thereof are mentioned in document (2). Thus, the Board concludes that it is only with hindsight that one could draw the conclusion that the mere existence of a carrier part at the N-terminal end of the fusion protein renders obvious the claimed solution to the above stated problem.

8. The further argument that it was not clear from the claim wording whether it is the N- or the C-terminal part of the fusion protein which is intended to be produced is also not convincing for the Board because the desired peptide is always clearly identified as the one part of the molecule which is cysteine-free.

9. For these reasons, document (2) on its own does not make obvious the subject-matter of claims 1 to 8.

10. It was also argued that the combination of the teachings of documents (2) and (11) was detrimental to inventive step. Document (11) is a review article on "Gene fusions for the purpose of expression". The advantages and disadvantages of N- terminal fusions are discussed in the passage bridging pages 134 and 135. One disadvantage which is mentioned is that "*when chemical methods are used to release X (*the desired peptide)*, a cleavage rest is usually obtained in the C terminus, thus giving a nonnative protein.*" (locution added by the Board). Conversely, "*the ease with which direct N-terminal sequencing of the gene fusion product*

can be performed" is stated as advantageous.

11. From reading the earlier passage, it is clear that for the authors, the desired peptide is recovered as the N-terminal part of the fusion protein, the amino-acid at the C-terminal end of said desired peptide being modified. Processes such as claimed whereby the cleavage reaction at the level of the cysteine link leaves the modified cysteine as the first amino acid at the N-terminal end of the C-terminal part of the fusion protein are not envisaged. Indeed, in a latter chapter devoted to "Site specific cleavage of fusion proteins" (page 140), chemical methods leaving the modified amino acid at the N-terminal end of the C-terminal part of the fusion protein (such as the cysteine method) are not mentioned. In addition, the authors emphasize the low specificity of the chemical methods and direct the skilled person to enzymatic methods which are said to leave an extra residue at the C-terminal end of the N-terminal part of the fusion protein.

12. In the Board's judgment, as document (2) discloses obtaining the desired peptide in a native state but does not make any mention of retrieving it as the N-terminal part of the fusion protein and document (11) discloses retrieving the desired peptide as the N-terminal part but not in a native state, it is only with hindsight knowledge of the present invention that one would combine these teachings to arrive at the processes such as claimed in claims 1, 4 and 7 whereby the desired peptide is produced in a native state as the N-terminal part of the fusion protein.

13. The Appellants also argued that the advantage identified as the ease of sequencing of the desired

peptide, when expressed as the N-terminal part of the fusion protein would make it implicitly obvious to the skilled person that the desired peptide is obtained in a native state and that this feature when combined with the teaching of document (2) rendered the claimed process obvious. This argument cannot be accepted because amino acid sequencing does not require that the desired peptide be cleaved from the C-terminal carrier protein: as it is done on the fusion protein itself, the disadvantage mentioned in point 10 above with regard to retrieving the desired peptide from the fusion protein remains as well as the resulting observations made in point 11. Thus, the reasoning developed in point 12 applies.

14. The Appellants' argument that the combination of any document relating to the expression of a protein fusion with any document relating to its cleavage would make the claimed invention obvious is also not convincing because, as already pointed out, only the combination of a specific order of the desired peptide and the carrier part in the fusion protein with cleavage reactions leaving the modified amino acid with the unwanted part of the fusion protein is suited to carry out the claimed process.

15. In view of the findings in points 2 to 13 above, no advantageous effect is needed for the acknowledgement of inventive step. Thus, the existence of an advantage in the presently claimed processes over that disclosed in document (2) need not be evaluated.

16. For these reasons the Board concludes that the claimed processes are not rendered obvious by any of the documents on file alone or in combination.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairwoman:

P. Cremona U. Kinkeldey