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D E C I S I O N
of 21 July 1998

Case Number: T 0202/95 - 3.3.4

Application Number: 86304723.9

Publication Number: 0206769

IPC: C12P 21/02

Language of the proceedings: EN

Title of invention:
A process for the production of alpha-human atrial natriuretic polypeptide

Patentee:
Fujisawa Pharmaceutical Co., Ltd.

Opponent:
Suntory Limited/ Shiratori Pharmaceutical Co., Ltd.

Headword:
Alpha-h ANP/FUJISAWA

Relevant legal provisions:
EPC Art. 56, 112

Keyword:
"Main request, auxiliary requests 1 and 2 - inventive step (no)"
"Auxiliary request 3 - inventive step (yes)"
"Referral of a question to the Enlarged Board of Appeal (no)"

Decisions cited:
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Catchword:
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Case Number: T 0202/95 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 21 July 1998

Appellant: (Proprietor of the patent)
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 23 January 1995 revoking European patent No. 0 206 769 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: L. Galligani
C. Holtz

Summary of Facts and Submissions

I. The appeal lies from the decision of the opposition division issued on 23 January 1995 whereby the European patent EP-B-0 206 769, which had been opposed under the terms of Article 100(a) and (b) EPC by one party, was revoked under Article 102(1) EPC. The earliest priority date of the patent was 20 June 1985.

The decision was based on claims 1 to 4 as granted which read as follows:

"1. A synthetic gene encoding an amino acid sequence of a protective peptide-fused α-hANP (α-human atrial natriuretic polypeptide) wherein said synthetic gene comprises a DNA sequence encoding lysine as the C-terminal residue of the protective peptide.

2. An expression vector comprising the synthetic gene of claim 1.

3. A microorganism transformed with the expression vector of claim 2.

4. A protective peptide-fused α-hANP wherein said protective peptide has lysine as the C-terminal thereof."

The opposition division considered that the claimed subject-matter did not involve an inventive step having regard to the prior art knowledge about the nucleotide sequences encoding α-human atrial natriuretic polypeptide (α-hANP) as represented by the following documents:

(2) Nature, Vol. 309, 1984, pages 724 to 726;

(3) Nature, Vol. 312, 1984, pages 656 to 658;

(4) Nature, Vol. 312, 1984, pages 654 to 656;

in combination with the general teaching about the expression of heterologous proteins in a recombinant system in a fused, cleavable form found in the following document:

(11) EP-A-0 001 929,

and in the light of either one of the further documents:

(5) Bioch. Biophys. Acta, 1981, Vol. 660, pages 51 to 55;

(6) FEBS, Vol. 170(1), 1984, pages 135 to 138,

which described the lysine (Lys)-specific Achromobacter protease I (API).

As regards the Article 83 EPC objection raised by the opponents, the opposition division considered it to be more of a "rhetorical nature" and left it aside. The interpretation of claim 1 put forward by the opponents that, due to the word "comprises" in claim 1, more codons could be present than those required for a lysine residue, was considered possible, but "rather improbable".

II. On 23 May 1995, with the statement of grounds of appeal, the appellants (patentees) filed an auxiliary claim request with an amended claim 1 which read as follows:

"A synthetic gene encoding an amino acid sequence of a protective peptide-fused α-hANP (α-human atrial natriuretic polypeptide) wherein said synthetic gene comprises a DNA sequence encoding lysine as the C-terminal residue and intended cleavage site of the protective peptide from the α-hANP."

The appellants filed further documents in support of their case, among them the following:

(19) EP-A-0 159 943, published on 30 October 1985;

(22) G. Allen et al., J. Cell Sci. Suppl. 3, 1985, pages 29 to 38, Proceedings of the British Society for Cell Biology - The Company of Biologists Limited Symposium in Glasgow, April 1985.

III. The appellants filed later further documents, among them the following:

(23) English translation of three articles published in Nikkei Biotech, respectively, in 1985 (9.9 issue, pages 8 to 9), 1989 (4.24 issue, page 3) and 1995 (3.27 issue, pages 8 to 9);

(25) J.A. Knott et al., Eur. J. Biochem., Vol. 174, 1988, pages 405 to 410;

(26) F. Sakiyama et al., Methods in Enzymology, A.J. Barrett ed., Vol. 244, 1994, pages 126 to 137, Academic Press, San Diego, CA, USA;

(27) English translation of the section B-72 of page 45 of programm and abstract of the 7th Annual Meeting of the Molecular Biology Society of Japan held on 4 to 7 December 1984 at Kobe International Conference Hall and Kobe Culture Hall, Japan.

The appellants submitted also an additional experimental report (document (28)).

IV. The board outlined the points to be discussed at oral proceedings in a communication pursuant to Article 11 of the Rules of procedure dated 20 March 1998.

V. With letter dated 8 July 1998, the respondents (opponents) withdrew their opposition against the patent.

VI. Oral proceedings took place on 21 July 1998. Auxiliary requests 2 and 3 were filed.

Claims 1 and 4 of **auxiliary request 2** read as follows:

"1. A synthetic gene encoding an amino acid sequence of a protective peptide-fused α-hANP (α-human atrial natriuretic polypeptide) wherein said synthetic gene comprises a DNA sequence encoding lysine as the C-terminal residue and intended cleavage site by API of the protective peptide from the α-hANP."

4. A protective peptide-fused α-hANP wherein said protective peptide has lysine as the C-terminal thereof and intended cleavage site by API of the protective peptide from the α-hANP."

Claims 1 and 4 of **auxiliary request 3** read as follows:

"1. A synthetic gene encoding an amino acid sequence of a protective peptide-fused α-hANP (α-human atrial natriuretic polypeptide) wherein said synthetic gene comprises a DNA sequence encoding lysine as the C-terminal residue and intended cleavage site by API of the protective peptide from the α-hANP, said protective peptide being the peptide encoded by the DNA sequence given in Fig. 4."

4. A protective peptide-fused α-hANP wherein said protective peptide has lysine as the C-terminal thereof and intended cleavage site by API of the protective peptide from the α-hANP, said protective peptide being the peptide having the amino acid sequence given in Fig. 4."

In both requests claims 2 and 3 were identical to claims 2 and 3 as granted.

VII. The appellants argued in essence as follows:

(a) In judging the patentability of the claimed subject-matter, the board should be wary of ex post facto analysis. The decision under appeal was based on hindsight as it did not make a "real life" assessment of the overall state of the art at the priority date and of the appellants' contribution thereto.

(b) As well documented by (23), before the appellants' invention, work on α-hANP by several companies, in particular by the former respondents, was based on the chemically synthesised product, not on a product obtained by the so-called "genetic route". Only after the appellants had disclosed the successful use on a commercial scale of this route and, in particular, of fusion protein technology, many competitor companies adopted the same approach.

(c) At the priority date there was no teaching or suggestion by anyone that intact α-hANP could be produced through the "genetic route" by means of fusion protein technology. In fact:

- Although document (27) disclosed a fusion protein of α-hANP similar to that disclosed in Example 3 of the later document (19), it had to be appreciated that this fusion protein could not be cleaved (and cannot be cleaved even today) to obtain intact α-hANP by using any commercially available enzymes or chemicals;

- document (11), which was concerned with the fusion protein technology, described three specific methods, namely the Met-CNBr system, the trypsin system and the chymotrypsin system, none of which would be suitable for the production of intact α-hANP. At the priority date there were no proteins being produced on a commercial scale by a method which entailed the formation and then enzymatic cleavage of a fusion protein. Even today there are only few recombinant proteins on sale that are prepared by this technology.

- The teaching of document (22) was limited to a protein which is neither homologous nor analogous to α-hANP. The document indicated the unpredictability of the fusion protein technology, firstly, because it reported the ineffectiveness of some batches of endoproteinase LysC in cleaving the fusion protein, and, secondly, because the passage headed "Alternative Fusion Protein Construction" reported a series of failures. This document had no real bearing on the inventive step analysis in the present case as the skilled person occupied with the production of α-hANP would not have attached any degree of significance to it.

If this view was not shared by the board, a question in accordance with the following should be referred to the Enlarged Board of Appeal:

"When assessing inventive step, as distinct from novelty, it is a desirable legal principle that the significance attached to a prior art document (or other disclosure) in proceedings before the EPO should reflect the degree of significance that would in reality have been attached to it by a person skilled in the art at the relevant date. Specifically, a document containing a limited teaching published only a short time before the priority date of the subject patent should not automatically or necessarily be given the same weight as a document published years earlier."

- the enzyme API described in documents (5) and (6) in relation to the hydrolysis of peptides had a degree of specificity for lysine residues. However, as documented by (26), it had been reported to non-specifically cleave also at least other four bonds, which were actually present in α-hANP. Consequently, the choice of this enzyme was not obvious for the skilled person;

- Later document (25) showed that, when other companies independently tried to produce α-hANP from fusion proteins, they chose a completely different approach and that they were not as successful as the appellants.

(d) Thus, for a skilled person, faced with the problem of producing intact α-hANP on a large scale, the selection of the "genetic route", in particular of the approach of fusion proteins, and the choice of

the Lys-API cleavage system would not have been an obvious course, and, thus, given the state of the art at the priority date, there would not have been a reasonable expectation of success.

VIII. The appellants requested that the decision under appeal be set aside and the patent be maintained on the basis of the claims as granted (main request) or the auxiliary request filed with the statement of grounds on 23 May 1995 or either of auxiliary requests 2 and 3 as submitted in the oral proceedings.

The appellants further requested that a question be referred to the Enlarged Board of Appeal in accordance with their submission in the oral proceedings (cf. Section VII, item c, supra).

Reasons for the Decision

The main request

1. The board sees no problems in the interpretation of claim 1 which is unambiguously directed to a synthetic gene wherein the DNA sequence encoding the protective peptide, which is fused to α-hANP, has a codon encoding Lys at the C-terminus.

2. The board considers that the disclosure in the patent specification is sufficient for a skilled person to carry out the invention as claimed. Thus, the requirements of Article 83 EPC are satisfied.

3. The novelty of the claimed subject-matter has never been in dispute. The board has no reasons to put novelty into discussion as none of the documents on file, including those filed during the appeal phase, affects the novelty of the claims.

4. It remains to be established whether the claimed subject-matter involves an inventive step. In this respect, the board has to decide whether the arguments and evidence put forward by the appellants are sufficiently convincing to confute the reasons given in the decision under appeal so as to lead to its setting aside.

4.1 In the board's judgement, the closest prior art is represented by the disclosure at the 7th Annual Meeting of Molecular Biology Society of Japan of the expression of hANF (human-Atrial Natriuretic Factor, which corresponds to α-hANP of the patent in suit) as a fusion protein with TrpE in E.coli (document 27). The abstract reports that the production of the TrpE-fused hANF reached about 5% (10 mg/l) of the cell proteins, 15% of insoluble proteins of the cell and it was able to react with an antibody against hANF. It is indicated that solubilisation of this protein and a physiologically active fragment thereof were under investigation.

4.2 The appellants argue that, because the fusion protein described in document (27) cannot be cleaved to obtain intact α-hANP by use of any enzymes or chemicals, this document cannot be considered to suggest using the "genetic route", in particular fusion protein technology, for producing intact α-hANP on a large scale.

4.3 The board cannot share this view. This is because:

- The abstract does not state that cleavage of the fusion protein was not achievable. Rather the information given by the abstract is that work was under way to solubilise the fusion protein and obtain a physiologically active fragment thereof. The skilled person had no reason to doubt about

the possibility of completing the work as indicated. Whether or not this was then achieved (later document (19) provides no data in this respect), is information which was not part of the state of the art at the priority date and is thus of no relevance to the issue of inventive step;

- the abstract does not contain any dissuasive statement of any kind in respect of the applicability of the "genetic route" and of the fusion protein approach to α-hANP. On the contrary, the good yields reported therein would have encouraged further experimentation in this area, also on a large scale.

4.4 Thus, in the board's view, the disclosure of document (27) is a suitable starting point for an analysis of the inventive step issue in the present case.

4.5 In the light of the disclosure of document (27), the problem to be solved was the finding of an alternative fused form of α-hANP to be used as intermediate in the production of intact α-hANP.

4.6 As a solution thereto, the claims propose a protective peptide-fused α-hANP wherein said protective peptide has Lys as its C-terminal, and a gene encoding it, said intermediate polypeptide being then cleavable by means of a Lys-specific protease such as e.g. API. As shown in the patent specification, the use of this approach resulted in the production of intact α-hANP.

4.7 The key question in the present case is whether the skilled person, starting from the knowledge that α-hANP could be prepared in fused form by the "genetic route" (document (27)), needed more than ordinary skill in order to arrive at the claimed solution.

4.8 At the priority date, the skilled person was quite familiar with the techniques for expressing a heterologous protein of interest in a host in fused, cleavable form. The skilled person knew that this approach was useful especially when expressing small peptides (NB: α-hANP is such a peptide) as their temporary conjugation with additional protein could preserve them against e.g. in vivo degradation by endogenous enzymes (cf. document 11, page 12). The skilled person knew that a selective cleavage site could be created between the peptide of interest and its fusion partner in order to effect the conversion of the fused precursor protein to the active peptide (ibidem, pages 12 to 13). He or she was of course aware of the fact that the creation of such a **selective** cleavage site implied the use of a sequence not present in the desired peptide in order to ensure the integrity of the latter. Chemical cleavage, e.g. with cyanogen bromide in the case of methionine-free peptides, and enzymatic cleavage, e.g. with a protease, were possible options (ibidem, loc.cit.).

4.9 When faced with the problem to be solved as defined in point 4.5 supra, the skilled person, in view of his/her knowledge of the art of preparing fusion proteins (cf. point 4.8 supra), would have designed a chimera protein with α-hANP which could be selectively cut by means of a proteolytic enzyme. In fact, due to the presence of a methionine residue in α-hANP, chemical cleavage would not have been a reasonable choice. Having thus opted for an enzymatic cleavage, the skilled person had a number of equally suited alternatives to choose from. Document (11), for example, directed his or her attention to the possibility of enzymatically cleaving arginine (Arg) and lysine (Lys)-free proteins with trypsin or chymotrypsin at Arg-Arg, Lys-Lys or like cleavage sites (cf. page 12). As α-hANP contains the sequence Arg-Arg, this cleavage site would not have

been taken into consideration. The skilled person would have noted that α-hANP lacked inter alia Lys. This observation would have prompted the skilled person to take document (22) into consideration, as it related to the expression of a fusion protein, namely TrpE protein - mouse epidermal growth factor (EGF), with a lysine link, which was cleaved by lysine-specific proteolysis, EGF lacking lysine and thus being resistant to the protease.

4.10 Document (22), although possibly published after the priority date of the patent in suit, is considered to give a true account of a presentation delivered in April 1985, ie before the priority date (20 June 1985). Its contents constitute thus prior art within the meaning of Article 54(2) EPC. The appellants, who have not attempted to prove the contrary, have accepted this finding (cf. also letter dated 4 January 1995, page 3, paragraph 3). However, they dispute the significance of this prior art document because in their view it contains a limited teaching made available only a short time before the priority date. In this respect, they request that a question be referred to the Enlarged Board of Appeal (EBA) (cf. Section VII, item c supra).

4.11 According to Article 112 EPC questions referred to the EBA should concern important points of law or necessitate a decision by the EBA in order to ensure uniform application of law. The question proposed by the appellants does not satisfy any of the stated criteria as, in spite of the attempted general formulation, it concerns the assessment of evidence, a matter which normally does not lend itself to questions of a general legal nature. In the present case, the board must assess the evidence, ie the documents on file, having regard to the particular circumstances, e.g. the weight to be attached to each of them. Consequently, the request for referral of a question to

the Enlarged Board of Appeal is refused.

4.12 As regards the weight to be attached to the disclosure of document (22), it is observed that:

- When evaluating inventive step, the skilled person is assumed to be aware of the totality of the prior art pertinent to the relevant area of technology, ie of everything made available to the public by any means (cf. Article 54(2) EPC);

- Document (22) is indeed pertinent to the technical area of gene technology, in particular to fusion protein technology, and contains information addressed to the person skilled in the art. Thus, the skilled person would have had knowledge of it and would have referred thereto when trying to achieve the same or a similar technical effect;

- Although dealing with the production of EGF, thus with a molecule different from α-hANP, and not being in the form of a general review article, the document represented for the skilled person a good example of the successful application of the known fusion protein approach, whereby a known class of proteases was used for the selective cleavage (cf. point 4.8 supra).

4.13 As already stated (cf. point 4.9, last sentence), the skilled person in designing another chimera protein with α-hANP would have directed his or her attention inter alia to document (22). The appellants argue that a study of the document would have convinced the

skilled person that the fusion protein approach as described therein was not feasible because of the unreliability of the enzyme preparations and because of the reported failures in constructing alternative fusion proteins.

The board cannot share the appellants' view. This is because:

- According to document (22), a peptide-fused EGF, wherein said peptide has Lys as a link to EGF, was expressed in a host and cleaved with endoproteinase LysC to yield biologically active EGF;

- The observation that some batches of the enzyme were ineffective at specifically cleaving the fusion protein would not have prevented the skilled person from using the method as described. It would have simple alerted the skilled person to the use only of controlled enzyme batches;

- The reported failures with alternative fusion protein constructions would have neither diminished the validity of the successful Lys-specific proteolysis approach nor put the fusion protein approach as a whole into doubt. If anything, they would have rather convinced him or her to further pursue the successful Lys-cleavage approach.

4.14 In the board's judgement, the skilled person looking for an alternative fused form of α-hANP did not need more than ordinary skill in order to arrive at the solution proposed in the claims at issue. His or her knowledge of the protein fusion technology and the disclosure of document (22) would have readily suggested choosing Lys as a link to α-hANP and Lys-

specific proteolysis as a feasible means for obtaining an intact α-hANP from the fused intermediate protein. The construction of such fused protein and of the corresponding gene implied merely the association of known elements, all working in a normal way, according to a known scheme with a reasonable expectation of success.

4.15 For these reasons, an inventive step is to be ruled out, and, consequently, the main request cannot be allowed.

Auxiliary request 1

5. There is no difference of substance between the subject-matter of this request and that of the main request. Claim 1 of the present request (cf. Section II supra) has been reformulated only in order to overcome any possible doubts about the interpretation of its scope. Thus, the reasons for ruling out an inventive step given above (cf. points 4.1 to 4.15) apply equally to this request which consequently cannot be allowed.

Auxiliary request 2

6. In comparison with the main request, this claim request specifies that the Lys at the C-terminus of the protective fused peptide is the intended cleavage site by API, ie the known Achromobacter protease I (cf. e.g. documents (5) and (6)).

7. The appellants maintain that the choice of this specific enzyme would not have been obvious for the skilled person, due to reservations about its specificity. In support of this contention, they referred to document (26) as an expert opinion (cf. page 131), which allegedly reports that the enzyme can cleave also bonds which are present in α-hANP.

8. The board notes that document (26), states on page 131: "For both protein and polypeptide substrates, very pure lysyl endopeptidase is **highly specific for the lysyl peptide bond, and cleavage of other bonds is rare or non existent**. Hydrolysis at Arg-Ser,[16] Arg-Ala,[17-19] Gly-Ala[11] and Phe-Lys[20] has been reported, although the latter two cases are quite exceptional." (emphasis added). Thus, non-specific cleavage of other bonds is the exception rather than the rule and a simple, routine control of the enzyme batch in use easily overcomes possible problems.

9. In the board's judgement, the skilled person, having designed a fused form of α-hANP with a Lys cleavage site on the basis in particular of the combined prior art teachings of documents (27) and (22) (cf. points 4.1 to 4.14), was faced with the choice of a Lys-specific protease for cleaving the said intermediate product and obtaining intact α-hANP. Document (22) described the use of endoproteinase LysC. The skilled person knew that this was a suitable candidate in view of the results presented in the said document. However, as Lys-specific proteolysis was known in the art, especially in relation to the hydrolysis of peptides (cf. e.g. documents (5) and (6)), this being a neighbour technical domain, the skilled person would have readily taken other Lys-specific proteases into consideration, among them certainly also API, as the latter was known to be highly specific (cf. e.g. document (5), in particular page 54 "Discussion", first paragraph). Thus, the feature in relation to the choice of API which now characterises the claimed subject-matter in comparison with the subject-matter of the main request already discussed, does not contribute to inventive step.

10. For these reasons, also auxiliary request 2 cannot be allowed.

Auxiliary request 3

11. This request is limited to the particular embodiment which has been exemplified in the description of the patent in suit, ie to a fused form of α-hANP wherein the protective peptide, which has Lys at the C-terminus as the intended cleavage site by API, is the peptide having the amino acid sequence given in Figure 4, (claim 4) and a gene encoding it (claim 1).

12. There are no formal objections under Articles 123(2)(3) and 84 EPC against the new claim request, as none of the amendments introduced result either in an extension of the subject-matter or in an extension of the protection conferred or in any unclarity.

13. According to the results presented in the description of the patent in suit and to the supplementary results submitted during the course of the appeal procedure (document (28)), this particular construction of the intermediate product ensures a particularly high yield of intact α-hANP.

14. As nothing in the available prior art provides any hint or suggestion towards this very specific construction, no information being available about the peptide having the amino acid sequence given in Figure 4, the board, also in the light of the advantageous results thereby achieved, considers the claimed subject-matter as resulting from the non-obvious and thus inventive selection from a broad range of possibilities.

15. Thus, the claim request at issue involves an inventive step and can be allowed.

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 auxiliary request 3 and a description to be adapted thereto.

3. The request for referral of a question to the Enlarged Board of Appeal is refused.

The Registrar: The Chairwoman:

D. Spigarelli U. M. Kinkeldey