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D E C I S I O N
of 6 July 1995-

Case Number: T 0918/94 - 3.3.4

Application Number: 82303667.8

Publication Number: 0070675

IPC: C12N 15/00

Language of the proceedings: EN

Title of invention:
Human calcitonin precursor polyprotein structural gene

Applicant:
CELLTECH THERAPEUTICS LIMITED

Opponent:
-

Headword:
Calcitonin/CELLTECH

Relevant legal provisions:
EPC Art. 83

Keyword:
"Sufficiency of disclosure - main request (no), - auxiliary request (yes)"

Decisions cited:
T 0157/90

Catchword:
-

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Case Number: T 0918/94 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 6 July 1995

Appellant: CELLTECH THERAPEUTICS LIMITED
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Decision under appeal: **Decision of the Examining Division of the European Patent Office dated 5 July 1994 refusing European patent application No. 82 303 667.8 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: R. E. Gramaglia
S. C. Perryman

Summary of Facts and Submissions

I. European patent application No. 82 303 667.8, relating to a human calcitonin precursor polyprotein structural gene, was published with number 0 070 675 with 17 claims.

II. The application had been refused a first time by the Examining Division and the refusal was based on the grounds that the subject-matter of Claims 1 and 8 did not meet the requirements of Article 123(2) EPC.

III. With decision T 157/90 of 12 September 1991 the Board of Appeal decided on the matter of whether an amendment representing a generalisation of a feature was admissible under Article 123(2) EPC. The set of claims according to the first auxiliary request (set A) was found to be allowable with regard to the above Article and the case was remitted to the Examining Division for further prosecution. On 9 March 1992 a Third Party presented observations pursuant to Article 115 EPC citing document Breddam IV (Breddam *et al.* Int. J. Peptide Protein Res. 37, 153-160 (1991)).

IV. The application was refused a second time by the Examining Division with a decision given at the oral proceedings of 18 May 1994. The refusal was based on the grounds that the subject-matter of Claims 1 to 7, 10 to 11 and 13 of set A did not comply with the requirements of Article 83 EPC. Claim 1 of set A read as follows:

"1. A vector including a structural gene encoding a polypeptide comprising the amino acid sequence of human calcitonin, wherein said polypeptide is enzymatically processable to produce human calcitonin using the C-terminal modification activity of the yeast enzyme carboxypeptidase Y."

V. The reasons for the decision were essentially as follows:

The yeast carboxypeptidase Y (hereafter: CPD-Y) catalysed conversion of CYS^{1}-----PRO^{32} and CYS^{1}------PRO^{32}-GLY^{+1} into calcitonin was not feasible. The two possible reactions:

$$\text{(a)}\ \ldots\ldots\ldots\text{Pro-Gly} \xrightarrow[NH_3]{\text{CPD-Y}} \ldots\ldots\text{Pro-}NH_2 + \text{Gly-OH}$$

$$\text{(b)}\ \ldots\ldots\ldots\text{Pro-OH} \xrightarrow[\text{Pro-}NH_2]{\text{CDP-Y}} \ldots\ldots\text{Pro-}NH_2 + \text{Pro-OH}$$

which the Appellant maintained to be explicitly or implicitly disclosed by documents Breddam I (Breddam *et al*. Carlsberg Res. Commun. 45, 237-247 (1980)) and Breddam II (*ibidem*, pages 361 to 367) were either not disclosed (reaction(a)) or were disclosed but did not work (reaction (b)). Reaction (a) was disclosed for the first time by the later document Breddam III (Breddam *et al*, Carlsberg Res. Commun. 46, 121-128 (1981)), while reaction (b) was shown by Breddam IV to yield no calcitonin.

VI. An Appeal was filed, the fees paid and the written statement setting out the grounds of appeal comprised an auxiliary request.

VII. The appeal was essentially substantiated as follows: As regarded reaction (a) Breddam III merely stated that (see Section V *supra*), reaction (a) was not demonstrated in Breddam I: demonstration was not the same as disclosure.

As to reaction (b) Table I of Breddam IV also showed that a synthetic peptide terminating in proline could be hydrolysed by CPD-Y, albeit slowly. Breddam IV was thus to be taken as meaning that calcitonin could not be amidated efficiently using CPD-Y in that the reaction was too slow.

According to Prof. Gilles, an Appellant's expert, it was known at the priority date of the patent application that CPD-Y proceeded via a covalent acyl-enzyme intermediate, deacylation of which could be effected by transfer of the acyl group from the enzyme to the incoming nucleophile (H_2O or an amine such as an amino acid or NH_3). Scheme I of Breddam I supported the above view by illustrating the product distribution obtained with different amino acid amides as nucleophiles and by showing that NH_3 could also act as a nucleophile. With this knowledge the skilled person would have easily established under what conditions reactions (a) and (b) could be performed.

It was also emphasized that the Examining Division had not substantiated its position in any way and in such a situation the benefit of doubt should be given to the Appellant.

VIII. During the oral proceedings held on 6 July 1995 the Appellant submitted an auxiliary request of which Claim 1 read as follows:

"1. A fusion polypeptide comprising a host polypeptide and a polypeptide comprising the amino acid sequence of human calcitonin, characterised in that the polypeptide comprising the amino acid sequence of calcitonin includes the amino acid sequence of human calcitonin-glycine."

IX. The Appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of set A or on the basis of the claims of the auxiliary request filed at the oral proceedings on 6 July 1995.

Reasons for the Decision

1. The appeal is admissible.

2. The only question at issue in these appeal proceedings is the allowability of the claims with regard to Article 83 EPC, ie., whether the disclosure of the application allows a skilled person to effect a post-translational conversion of [desamido]^{32}-calcitonin and [desamido]^{32}-Gly^{+1}-calcitonin into calcitonin by using CPD-Y.

3. The Examining Division split the sufficiency problem in two parts: first it had to be established whether Breddam I (Breddam II is less important since it is concerned with esters) disclosed reactions (a) and (b), either implicitly or explicitly, and in case the answer was yes, it had to be established whether the reactions (a) and (b) worked. It was concluded that reaction (a) was not disclosed, therefore the question of whether it worked was irrelevant, although in fact later published evidence represented by the Sankyo patent application

EP-A-0 197 794 stated that it did not work. As regarded reaction (b), the Examining Division accepted that the reaction was disclosed by Breddam I, however, it had serious doubts about the possibility that it worked, in view of later evidence (Breddam IV) showing that reaction (b) did not yield any calcitonin.

4. As part of the background to the invention, it should be stated that a chemical synthesis of calcitonin was known and used, but that it was necessary to start with proline, convert this to prolinamide, and then add the other amino acid residues. This was because if an amino acid chain ending in proline was formed, no method of amidating the proline to prolinamide was known which did not involve a serious risk that unwanted amidation of other amino acids would also occur. Thus it was appreciated that the provision of the final prolinamide was a critical step.

The application disclosed the preparation of the polypeptides:

$$CYS^{1}------PRO^{32} \text{ and } CYS^{1}------PRO^{32}\text{-}GLY^{+1}$$

Yet these 2 polypeptides differed from authentic calcitonin in that calcitonin exhibited a C-terminal prolinamide, the latter being necessary for the biological activity of calcitonin. Therefore, they needed to be modified in such a way that they terminated with a $Pro^{32}\text{-}NH_2$. There was a statement on page 31 of the application on how the above conversion should have been made: "Conversion of the liberated peptide into authentic calcitonin is possible through the use of the C-terminal modification activity of yeast carboxypeptidase Y (Breddam, K, Widmer, F and Johanson, J.T., Carlsberg Res. Commun. 45, 237-247 and 361-367, 1980)". The Breddam *et al.* articles (Breddam I and

Breddam II) were cited in the patent application and the Board already accepted in decision T 157/90 (supra) that they could be incorporated into the disclosure by reference.

5. In the Board's view, once a given enzyme has been shown, as done in Breddam I and II, to exhibit a spectrum of activities, which are for CPD-Y the amidase, the peptidyl-amino-acid-amide hydrolase and the carboxypeptidase activities and the mechanism of catalytic activity has been elucidated as in the present case, where it was known that CPD-Y proceeded via an acyl-enzyme intermediate which underwent nucleophilic attack, a skilled enzymologist acquires a **theoretical** knowledge about all the possible reactions which the enzyme could potentially catalyse, at least about all those possible reactions for which there is no *a priori* bar, eg, if the nucleophile is manifestedly too weak or too bulky. The Board has come to the above view by considering that the draft of the theoretical Scheme 1 of Breddam III (see page 122) relating to possible CPD-Y catalysed exchanges of C-terminal amino acid residues in peptides did not seem to have required any knowledge other than that already imparted by Breddam I about the amidase, peptidase and peptidyl-amino-acid-amide hydrolase activity of CPD-Y as well as its mechanism of action via the acyl-enzyme intermediate. Breddam III is indeed merely concerned with establishing whether and under what conditions all the reactions of Scheme 1 (with exception of reaction IIb already shown to occur in Breddam I) take place. This means that the Breddam team would not have been able to investigate the reactions listed in Scheme 1 of Breddam III if said Scheme had not previously been set down in the light of theoretical considerations arising from Breddam I and II optionally completed by general knowledge in the art. The Board thus agrees with Prof. Gilles' statements, yet

only in connection with the possibility that Scheme 1 of Breddam I (page 244) merely set the skilled person in a position to draft a list of **possible** reactions such as that of Scheme 1 of Breddam III (page 122) and Scheme 1 of Breddam IV (page 156).

6. Yet writing down a mere reaction scheme is not synonymous with sufficient disclosure, if the skilled person is not also taught by way of experimental verification whether the reaction actually works, and if it does not, which measures should be taken to remedy the failure. The Board has come to this view on the following evidence:

Breddam *et al.* place much emphasis on the provisos that need to be fulfilled in order that a given CPD-Y catalysed reaction be likely to succeed (see Breddam I, page 243, "Discussion" and Breddam III, page 123, under "Results"): (i) it is necessary to work at a pH where the peptide is substrate of CPD-Y, (ii) the nucleophile should be able to perform its attack on the acyl-enzyme intermediate and (iii) the product should accumulate under the conditions used, i.e., the product which forms in the reaction should not be a better substrate for CPD-Y than the starting substrate. Put in other words the rate of acylation should be higher for the peptide substrate than for the reaction product otherwise no product will be observed. In view of the above, it is not surprising that some reactions do not work at all and indeed for example Table V of Breddam I, Table II of Breddam II and Tables II and III of Breddam III, show this.

The same conclusion is forced upon the Board even more strongly when considering the Breddam team's following important statement relating to the criticality of proviso (iii) above (see Breddam III, page 127, r.h.

column, lines 11 to 15): "For the evaluation of the **feasibility** of a given reaction, knowledge about the relative rates of acylation of the initial substrate and the coupling product is essential." (emphasis added)

7. Therefore, in view of the above and especially because a team of highly skilled enzymologists such as the Breddam one has declared *expressis verbis* that a given reaction cannot be considered as **feasible** without experimental verification, the Board must apply the same criterion when dealing with feasibility according to Article 83 EPC.

8. When applying to reactions (a) and (b) (see Section V *supra*) the Breddam team's criterion for feasibility also adhered to by the Board, it is noted that experimental evidence in support of the feasibility has been provided neither by Breddam I and II nor by the Appellant despite the Examining Division and the Board have asked for such additional experimental information.

9. The only experimental evidence in connection with these reactions comes from Third Parties according to Article 115 EPC and is negative in nature. With a view to reaction (a), the Comparative Example on page 17 of the Sanyo patent application EP 0 197 794 demonstrates that no amidation reaction occurs between CBZ-Ala-Pro-Gly-OH and NH_3, rather the reaction occurs only if Gly is replaced by Leu or Ile. The above finding is not inconsistent with the statement found on page 127 of Breddam III, r.h. column, second paragraph: "In less favourable cases, the use of ammonia, amino acids esters and free amino acids as nucleophiles might not be possible."

As regards reaction (b) the same paragraph of Breddam III recites "It should however generally be possible to convert peptides to peptide esters and peptide amide using alcohols and amino acid amides as nucleophiles, respectively, since in this case the rate of acylation is far higher for the peptide substrate than for the product under the conditions of coupling.". There is therefore a suggestion that proviso (iii) of Section 5 supra might be fulfilled. However the Breddam *et al.* papers are concerned with reactions involving Gly, Leu, Val or Phe nucleophiles. The Board finds doubtful that these results might be predictive on whether the reaction would occur with proline nucleophiles as in reaction (b). Even if proviso (iii) were fulfilled for reaction (b), proviso (ii) relating to the necessity that the nucleophile should be able to perform its attack on the acyl-enzyme intermediate (see point 6 *supra*) might not be, especially when one takes into account that proline nucleophiles exhibit an imino group rather than a primary amino group (they are thus less nucleophilic and could also lead to sterical problems). The above Board's view appears to be supported by the fact that Breddam I to III never mention any proline nucleophile. Only Breddam IV does (see page 159, last paragraph), however, for announcing a failure.

10. In his description, the Appellant did not give the protocol of a method of converting a calcitonin predecessor but relied on a reference to two publications (Breddam I and II) by a group working on CPD-Y. Unfortunately for the Appellant, later published works of this group suggest that CPD-Y would not work for the purpose for which the Appellant suggests its use. It has been submitted that the Appellant now uses a different method. The only evidence submitted is that of Prof. Gilles, based on theory and not experiment, that the reactions should work. In these circumstances the

Board can make no assumption in the Appellant's favour that the method would work. If it had been shown that a method exists that does work, the Appellant might have been given the benefit of the doubt, at the application stage at least, on the question of whether the skilled person would have hit on this method. But where, as here, there is no experimental demonstration of success to set against very substantial indications that it does not work, this is not appropriate. The Board has thus to come, through a slightly different line of argument than the Examining Division, to the conclusion that on the evidence presently available neither reaction (a) nor (b) are workable and therefore the requirements of Article 83 EPC are not fulfilled for the subject-matter of the main request.

11. The basis for the Examining Division's refusal of the application was that the main request did not comply with Article 83. The Claims of the auxiliary request not being directed to the CPD-Y catalysed conversion of calcitonin predecessors to calcitonin, this objection does not apply to the auxiliary request, but other aspects have yet to be examined. The Board thus exercises its discretion under Article 111 EPC to refer the application back to the first instance for further prosecution on the basis of the auxiliary request.

12. Claim 1 of the auxiliary request relates to an amino acid sequence including the amino acid sequence of calcitonin-glycine. The only use suggested in the application for calcitonin-glycine is conversion to calcitonin. In view of the Board's finding that the application did not contain an enabling disclosure for the conversion of calcitonin-glycine to calcitonin, this raises the interesting point of whether the claimed

subject-matter of the auxiliary request is susceptible of industrial application as required by Articles 52(1) and 57 EPC. This is one of the points for the first instance to consider in the further prosecution.

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 for further prosecution on the basis of the set of claims of the auxiliary request filed at the oral proceedings of 6 July 1995.

The Registrar: The Chairwoman:

L. McGarry U. Kinkeldey

5.5.95