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D E C I S I O N
of 6 February 2002

Case Number: T 0029/99 - 3.3.4

Application Number: 85905953.7

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Language of the proceedings: EN

Title of invention:
Recombinant methods for production of serine protease inhibitors and DNA sequences useful for same

Patentee:
Amgen Inc.

Opponent:
Teijin Limited

Headword:
Serine protease inhibitors/AMGEN

Relevant legal provisions:
EPC Art. 56, 83

Keyword:
"Inventive step - yes"
"Sufficiency of disclosure - new objection put forward too late"

Decisions cited:
T 0032/81, T 0455/91, T 0412/93, T 0386/94, T 0367/95

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0029/99 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 6 February 2002

Appellant: (Proprietor of the patent)
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 14 September 1998 revoking European patent No. 0 205 475 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: F. L. Davison-Brunel
S. C. Perryman

Summary of Facts and Submissions

I. The appeal lies from the decision of the Opposition Division issued on 14 September 1998 whereby the European patent No. 0 205 475 with the title "Recombinant methods for production of serine protease inhibitors and DNA sequences useful for same" which was granted with 30 claims for all Designated Contracting States other than Austria (non-AT States) and for Austria respectively was revoked pursuant to Article 102(1) EPC.

Independent product claim 16 as granted read as follows:

"16. A synthetic DNA sequence capable of directing microbial synthesis of a serine protease inhibitor comprising a single unfragmented polypeptide chain, said inhibitor having at least one active site possessing serine protease inhibitor activity and exhibiting at least 40% homology to a native serine protease inhibitor isolated from parotid secretions, said native serine protease inhibitor having the following amino acid sequence:

(here follows the amino acid sequence of the claimed inhibitor)."

Independent claim 1 related to a method for the production of a recombinant protease inhibitor defined as in claim 16 and claims 2 to 15 related to further features of the method of claim 1.

Independent claim 20 was addressed to an isolated DNA sequence coding for a serine protease inhibitor defined

as comprising a single unfragmented polypeptide chain having the amino-acid sequence recited in claim 16. Claims 17 to 19 and 21 to 24 related either to further features of the DNA sequences of claims 16 and 20 respectively, or to a recombinant vector comprising them or to a host cell transformed with said recombinant vector.

Independent claim 25 related to a method for the synthesis of recombinant protease inhibitors having some selected modifications compared to the sequence recited in claim 16 or fragments thereof. Claim 26 related to a particular embodiment of said method wherein the amino-acid sequence was as recited in claim 16.

Independent claim 27 was directed to DNA sequences encoding protease inhibitors having some selected modifications compared to the sequence recited in claim 16 or fragments thereof. Claim 28 was addressed to a DNA sequence encoding a serine protease inhibitor comprising the amino sequence recited in claim 16 or a fragment thereof. Claims 29 and 30 were addressed to a recombinant vector comprising the DNA sequences of claims 27 or 28, and to a host cell transformed with said recombinant vector, respectively.

The corresponding claims were granted for AT, all being formulated as method claims.

II. The Opposition Division decided that the subject-matter of claim 16 and claim 1 lacked inventive step. In addition, it was found that the requirement of sufficiency of disclosure was not fulfilled in relation to DNA sequences capable of directing the microbial

synthesis of serine protease inhibitors exhibiting at least 40% homology to the native serine protease inhibitor defined by its amino-acid sequence.

III. At oral proceedings which took place on 6 February 2002, the Appellants (Patentees) submitted a new request as sole request for consideration by the Board.

The claims of this request differed from the granted claims in that the homology language was deleted.

Independent product claim 16 thereof read as follows:

"16. A synthetic DNA sequence capable of directing microbial synthesis of a serine protease inhibitor comprising a single unfragmented polypeptide chain having at least one active site possessing serine protease inhibitor activity, said inhibitor having the following amino acid sequence:

(here follows the amino acid sequence of the claimed inhibitor)."

The corresponding claims were filed for AT, all being formulated as method claims.

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

(1): Schiessler, H. et al., Hoppe-Seyler's Z.Physiol. Chem., Vol. 357, pages 1251 to 1260, September 1976,

(3): Fritz, H., in "Protein Degradation in Health and Disease", Ciba Foundation Symposium 75 (new

series), Excerpta Medica, pages 351 to 379, 1980,

(5): Roberts, B. et al., "Molecular Biology of the Cell", Garland Publishing Inc., pages 185 to 194, 1983,

(6): Seemüller, U. et al., FEBS, Vol. 199, No. 1, pages 43 to 48, April 1986,

(8): Heinzel, R. et al., Eur.J.Biochem., Vol. 160, pages 61 to 67, 1986,

(9): Schiessler, H. et al., in "Neutral Proteases of Human Polymorphonuclear Leukocytes", K. Havemann and A. Janoff, Eds, Urban & Swarzenberg Inc. Baltimore-Munich, pages 195 to 207, 1978.

V. The arguments in writing and during oral proceedings by the Appellants insofar as they are relevant to the present decision can be summarized as follows:

Article 56 EPC: Inventive step

The closest prior art was document (3) which disclosed a partial sequence of the serine protease inhibitor found in seminal plasma.

The problem to be solved was to provide the tools and means for the production of a serine protease inhibitor in high yield and purity.

The solution given in the claims was to clone the DNA encoding a serine protease inhibitor and express it by recombinant means.

At the priority date, there were no documents suggesting the recombinant DNA (rDNA) route as a solution for producing the serine protease inhibitor, all approaches to its purification making use of biochemical methods. The skilled person was, thus, a protein chemist and, in accordance with the case law relative to the notion of the "skilled person", he/she would not be expected to switch easily from one field to another (T 455/91, OJ EPO 1995, 684). For these reasons, the rDNA approach would not even be tried.

The skilled person who nonetheless attempted to clone the DNA encoding the serine protease inhibitor would have to choose from which tissue to start this cloning. Document (3) taught human seminal plasma as the source of the inhibitor protein. Yet, as was readily apparent from post-published work (documents (6) and (8)), tissues other than seminal vesicles had to be used for isolating the serine protease inhibitor gene.

Having constructed a DNA library from a tissue producing the serine protease inhibitor, the skilled person would not have known which part of the partial aminoacid sequence of the protease inhibitor from seminal plasma to choose for devising the oligonucleotide probes necessary to screen this library. And, besides, he/she could not be sure that a probe devised on the basis of this amino acid sequence would be suitable to screen for the DNA encoding the serine protease inhibitor from other tissues than seminal vesicles. Indeed, at the priority date, the amino acid compositions of the various serine protease inhibitors had been found very similar but not exactly the same (document (9)). This cast doubts on whether the DNAs encoding the inhibitors would have had the

same sequence ie on whether a short oligonucleotide probe derived from any one of them would be able to hybridize to the others.

The combination of the teachings of document (3) and the common general knowledge represented by document (5) would not have helped in solving these problems.

For these reasons, the claimed subject-matter was inventive.

Article 83 EPC: Sufficiency of disclosure

The objection under Article 83 EPC was originally raised against claims 1 to 19 then on file because these claims made reference to DNA sequences having various degrees of homology to the DNA sequence encoding the serine protease inhibitor from seminal plasma. The claims filed on appeal did not contain this reference anymore. For this reason, the Respondents had withdrawn their allegation of insufficiency in their answer to the grounds of appeal. There was, thus, no basis for discussing whether the requirements of Article 83 EPC were fulfilled.

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

Article 56 EPC: Inventive step

The closest prior art, document (3), was concerned with the isolation and characterisation of the human serine protease inhibitor from seminal plasma. On page 361, it

was emphasized that this task was rendered difficult by the fact that the molecule existed in a low amount and in multiple forms in vivo. In contrast, the potential therapeutical use of the inhibitor was highlighted on page 360, which use the skilled person would understand as requiring large quantities of the inhibitor. Thus, document (3) provided a strong incentive to look for efficient ways to produce it.

Document (5), a text book representing the common general knowledge at the priority date mentioned on pages 189 and 192 that the easiest way to sequence a protein (ie to obtain it in pure form and large quantities) was to clone and express the corresponding gene. Taking the cDNA route for the production of the serine protease inhibitor would, thus, have been obvious to the person skilled in the art who, according to the case law of the Boards of Appeal, was to be seen as a team of specialists in the fields of expertise relevant to rDNA technology (T 412/93 of 21 November 1994).

The skilled person could use seminal vesicles as a source of tissue from which to obtain the genetic material encoding the serine protease inhibitor. Alternatively, he/she would have contemplated using some other tissues such as cervical mucus, bronchial fluid, tears etc... mentioned in document (3) as producers of the serine protease inhibitor. Document (1) (abstract, pages 1252 and 1259) made it clear that the serine protease inhibitors from all these sources were the same molecule.

The probe used for screening the positive recombinant clones would be devised in an obvious manner from the

partial amino acid sequence of the inhibitor disclosed in document (3) on the basis of the common general knowledge (document (5), page 194). The skilled person would not be discouraged in this task by the fact that Table 2 of document (9) showed that the amino acid **compositions** of inhibitors from different sources varied slightly because it would have been obvious that Table 2 presented a compilation of data from different laboratories which were not directly comparable. Moreover, the proteins, the amino acid compositions of which had been characterized in document (9), did not necessarily contain the region described in document (3).

- The present case was similar to that encountered in previous case T 386/94 (OJ EPO 1996, 658) insofar as the skilled person would have expected to perform the cloning and expression of the serine protease gene in a fairly straightforward manner and would not have encountered any difficulties on the way. Therefore inventive step could not be acknowledged.

Article 83 EPC: Sufficiency of disclosure

The claims did not anymore refer expressis verbis to DNAs encoding serine protease inhibitors homologous to that encoding the serine protease inhibitor from seminal plasma. Yet, they still comprised homologous DNAs in the form of fragments (cf claims 25 to 28). The patent specification provided no evidence that fragments encoding an active serine protease inhibitor could be made. The requirements of Article 83 EPC were not fulfilled.

VII. The Appellants requested that the decision under appeal be set aside and that the patent be maintained on the basis of the sets of claims filed at the oral proceedings on 6 February 2002, page 7 of the description as filed at the oral proceedings on 6 February 2002, pages 3 to 6 and 8 to 37 of the description as granted and the Figures as granted.

The Respondents requested that the appeal be dismissed.

Reasons for the Decision

Formal requirements

1. Claims 1 to 30 filed on appeal are derived from granted claims 1 to 30 by deletion of the homology language. The remaining subject-matter was already disclosed in the application as filed. The amendment does not affect clarity and amounts to a restriction of the scope of the granted claims. The requirements of Articles 123(2)(3) EPC and 84 EPC are fulfilled.

Article 56 EPC: Inventive step

2. All claims on file directly or indirectly relate to DNA sequences encoding a serine protease inhibitor, the amino-acid sequence of which is defined by, or derived from that recited in claim 16. If the DNA of claim 16 is found to be inventive, then the subject-matter of all other claims will also fulfill the requirements of Article 56 EPC. It is, therefore, expedient firstly to assess the inventive step of the subject-matter of claim 16.

3. The closest prior art to the subject-matter of this claim is document (3). This document (pages 360 to 363) presents a study of the serine protease inhibitor from human seminal plasma (HUSI-I: acid stable elastase-cathepsin G inhibitor). On page 361, it is disclosed that the inhibitor exists in several multiple forms, which complicates both its purification to homogeneity and the determination of the amino acid sequence. The sequence of two stretches of the molecule is nonetheless shown. It is also mentioned on page 362 that other types of secretion such as cervical mucus, bronchial fluid etc... contain serine protease inhibitors with HUSI-I like biochemical and/or immunological properties. On pages 360 and 363, the potential therapeutic use of serine protease inhibitors is emphasized, which is said on page 363 to be hampered by the limited amount available from natural sources.

4. Starting from the closest prior art, the objective problem to be solved can be defined as the provision of means and tools for the production of a human serine protease inhibitor in high yield and purity.

5. The solution given in claim 16 is a synthetic DNA encoding a serine protease inhibitor defined, in particular, by the amino acid sequence of the latter.

6. In the Board's judgment, the disclosure in document (3) of the existing difficulties in purifying the serine protease inhibitor by biochemical methods and, of the need to obtain sufficient amount of it to test its therapeutic value was an incentive to try and find out efficient ways of producing it. In view of the common general knowledge already prevailing in 1983 and expressed in document (5) (page 189) that *"At present,*

the easiest and most accurate way to sequence the amino acids in a protein is by sequencing its gene and then using the genetic code as a dictionary to convert the nucleotide sequence back to a protein sequence.", it was obvious to the person skilled in the art in a situation such as that encountered here, to turn to rDNA technology.

7. The Appellants' argument in this respect that the person skilled in the art would be a protein chemist who would not think of switching from one field (protein chemistry) to another (rDNA technology) is not found convincing. Regarding the role of the skilled person, the Board in case T 32/81 (OJ EPO 1982, 225) gave the following ruling: *"If the problem prompts the person skilled in the art to seek its solution in another technical field, the specialist in that field is the person qualified to solve the problem. The assessment of whether the solution involves an inventive step must therefore be based on that specialist's knowledge and ability.".* In the present case, it was obvious on the basis of document (5) to use rDNA technology to try and solve the problem at hand. Thus, in accordance with the case law of the Boards of Appeal relative to the notion of the skilled person in the field of biotechnology (cf eg T 412/93, supra), the person skilled in the art must be seen as a team comprising specialists in carrying out rDNA experiments.

8. Producing the serine protease inhibitor by rDNA technology requires a source of tissue which produces that inhibitor. The Respondents argued that seminal vesicles would be thought as adequate in this respect. Yet, it appears that this route was not considered

practical since this tissue was never used, not even in latter published work such as presented in documents (6) and (8), where use is made of cervix tissue. In document (8) (to be taken as an expert document), it is emphasized that *"cervix uterus is one of the few human tissues available for such experimental manipulations".*

9. At the priority date, in contrast, it was known from document (3) (page 362) that cervical mucus, bronchial fluid, tears etc... were sources of human seminal plasma inhibitor-**like** substances. This information was also given in document (1) where it is stated on page 1258 that *"The acid-stable inhibitor from the mucous secretion of human cervix uteri shows a high degree of similarity to HUSI-I..."* and in the passage bridging pages 1258 and 1259: *"The given characteristics of HUSI-I and CUSI are also common to an acid-stable inhibitor isolated from bronchial mucus...The high degree of similarity of these acid-stable inhibitors of human mucous secretions indicates that they are the same or very similar proteins."* On the basis of these teachings and given the lack of availability of tissue from seminal vesicles, the skilled person would have found it obvious to turn to one or the other of sources just mentioned to construct the cDNA library containing the DNA encoding the serine protease inhibitor.

10. The question which remains to be answered is whether he/she would have had a reasonable expectation of success that the DNA encoding the serine protease inhibitor from a tissue other than seminal vesicles could be isolated.

11. Document (5) (page 194) teaches that *"..., it is possible, in principle, to work backward from **a protein to a gene that encodes it**: by using a short stretch of amino acid sequence from the protein, specific DNA probes can be synthesized that will hybridize with the mRNA and DNA encoding the protein."* (emphasis added). Thus, the skilled person may have expected to retrieve the DNA encoding the serine protease inhibitor from seminal plasma on the basis of the partial amino acid sequence of said serine protease inhibitor (document (3), page 361) by screening the DNA **isolated from seminal vesicles** with short oligonucleotides probes representing part of said amino acid sequence.

12. Yet, the situation is different here since the skilled person would have had to screen the cDNA library constructed from a tissue **different from seminal vesicles** with the probe derived from the seminal plasma inhibitor. The degree of confidence he/she might have had that this would succeed was entirely dependent on whether or not the serine protease inhibitors from different tissues would have been expected to have the same amino acid sequence. At the priority date, serine protease inhibitors had not been identified by their amino acid sequences. Only amino acid compositions were available for some of them (document (9), Table (2)). These amino acid compositions although quite similar are nonetheless not identical. Consequently, no one knew that the amino acid sequences of the various protein inhibitors would be the same, or otherwise stated there was no reason to believe, though there might be a hope, that a probe derived from any one of these sequences would hybridize to the DNA encoding another. Therefore, on the basis of the scanty

knowledge then available, there being no sequence information for a protease inhibitor from a readily available tissue, the person skilled in the art would not have had a reasonable expectation that the isolation of recombinant clones containing the DNA encoding the serine protease inhibitor from any of the available tissues would succeed.

13. In this respect, the Respondents argued that the amino acid compositions shown in document (9) would not have been regarded as relevant by the person skilled in the art because, on the one hand, there was no evidence that these compositions were those of the whole serine protease inhibitor molecules and, on the other hand, Table 2 showing these amino acid compositions was a compilation of data from different laboratories, which, therefore, could not reliably be compared. The Board cannot accept these arguments. Table 2 provides the information that the molecular weight of the three serine protease inhibitors, the amino acid compositions of which are compared, is of about 11000. This is the same molecular weight as given in document (3). There is, thus, no reason to believe that the amino acid compositions shown in document (9) are those of fragments of serine protease inhibitors. In the same manner, and in accordance with the finding in T 367/95, of 27 October 1998 that *"if by comparing the amino acid compositions of two peptides it is found ... that some amino acids are either absent or present in a different molar percentage, it can be concluded that the two peptides, although being possibly similar, are not identical.",* the Board considers that the amino acid compositions shown in document (9) would have been taken at their face value, ie as an indication that the protease inhibitors were not necessarily identical.

14. In summary, the skilled person wanting to clone the DNA encoding a serine protease inhibitor could not carry out a straightforward cloning experiment whereby the sequence of the DNA probe and the DNA to be cloned would be derived from the same tissue. He/she had to resort to a "cross screening" (DNA from tissue other than seminal vesicles)/probe from seminal plasma), the successful outcome of which could not reasonably be expected since there was evidence that the primary structure of protease inhibitors from various sources may not be the same.

15. Contrary to the Respondents' view, the situation herein encountered is thus different from that in decision T 386/94 (supra) whereby the then competent Board decided that the skilled person at the onset of the project (there, the cloning of the DNA encoding chymosin) would be fairly confident that the combination of the teachings of the state of the art relative to chymosin and standard knowledge on biotechnological protocols would lead to the successful cloning of the genes encoding preprochymosin and its maturation forms.

16. For the reasons given in points 2 to 14 above, inventive step is acknowledged to the subject-matter of claims 1 to 30 of the request for non-AT States as well as to the subject-matter of claims 1 to 30 for AT filed on appeal.

Article 83 EPC: Sufficiency of disclosure

17. In the first instance, the Respondents' objection in relation to lack of sufficient disclosure was that the patent in suit did not contain sufficient instructions

for the production of **active** serine protease inhibitors **homologous** to the specific inhibitor defined by its aminoacid sequence (claims 1 to 19 as granted; a view which was followed by the Opposition Division. The homology language has been omitted from the claims on appeal and, as a consequence thereof, the Respondents withdrew their objection for lack of sufficient disclosure during the written part of the appeal procedure. At oral proceedings, they argued for the first time that sufficiency of disclosure failed in relation to DNA encoding fragments of the serine protease inhibitor (claims 20 to 28 as granted). This argument was never presented before and there was no evidence at all in support of it on file. The issue could only be considered properly if both parties were given an opportunity to file evidence. However, the Board considers that once a case has got to oral proceedings before the Board of Appeal, to give further time for filing evidence on an objection which potentially already was open against the claims as granted, would be inappropriate. Thus, the Board considers the objection as being put forward too late, and refuses to allow it into the proceedings.

Adaptation of the description

18. At oral proceedings, the Appellants adapted the description to the request on the basis of which it was intended to maintain the patent, by filing a new page 7. The Respondents agreed to this amendment and the Board also considers it suitable to bring the description in conformity with the claims.

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 Claims 1 to 30 of the sets of claims for the Contracting States other than Austria and for Austria, respectively as filed at the oral proceedings on 6 February 2002, pages 3 to 6 of the description as granted, page 7 of the description as filed at the oral proceedings on 6 February 2002, pages 8 to 37 of the description as granted and the Figures as granted.

The Registrar: The Chairman:

P. Cremona L. Galligani