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**Datasheet for the decision
of 20 August 2009**

Case Number: T 0861/08 - 3.3.08

Application Number: 92900779.7

Publication Number: 0561907

IPC: G01N 33/53

Language of the proceedings: EN

Title of invention:

Proteins with changed epitopes and methods for the production thereof

Patentee:

Novozymes A/S

Opponents:

Genentech, Inc.
GENENCOR INTERNATIONAL INC.

Headword:

Subtilisin changed epitopes/NOVOZYMES

Relevant legal provisions:

EPC Art. 56

Relevant legal provisions (EPC 1973):

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Keyword:

"All pending requests - inventive step (no)"

Decisions cited:

T 0537/02, T 0660/02, T 1067/02, T 1329/04

Catchword:

-



Case Number: T 0861/08 - 3.3.08

DECISION
of the Technical Board of Appeal 3.3.08
of 20 August 2009

Appellant: Novozymes A/S
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Decision under appeal: Decision of the Opposition Division of the
European Patent Office posted 21 December 2007
revoking European patent No. 0561907 pursuant
to Article 102(1) EPC 1973.

Composition of the Board:

Chairman: L. Galligani
Members: P. Julià
B. Günzel

Summary of Facts and Submissions

- I. Three oppositions were initially filed against granted European patent no. 0 561 907, based on the International patent application WO 92/10755, on the grounds as set forth in Articles 100(a), (b) and (c) EPC. In a letter dated 22 October 2001, opponent 01 withdrew its opposition. The patent was revoked by the opposition division on the grounds that the main request as well as the first, second, third and fourth auxiliary requests then on file did not fulfil the requirements of Article 83 EPC. The patentee appealed that decision and, in the subsequent appeal proceedings, the then competent board of appeal decided that the seventh auxiliary request on file fulfilled the requirements of Articles 123(2), (3), 84 and 83 EPC and remitted the case to the first instance for further prosecution on the basis of that auxiliary request (cf. T 1067/02 of 30 November 2004).
- II. In the further prosecution of the case, the opposition division considered that the seventh auxiliary request and auxiliary requests 8 to 10 filed with letter dated 21 September 2007 did not fulfil the requirements of Article 56 EPC and the patent was revoked.
- III. On 20 February 2008, the patentee (appellant) filed a notice of appeal and, on 25 April 2008, the statement setting out its grounds of appeal together with auxiliary requests 11 to 18 (auxiliary request 11 being the main request) which replaced the requests on file.
- IV. In a letter dated 1 October 2008, opponent 03 (respondent II) replied to the appellant's grounds of

appeal. No submissions were made on behalf of opponent 02 (respondent I).

V. On 3 June 2009, the board sent a communication pursuant to Rule 100(2) EPC and Article 17 of the Rules of the Procedures of the Boards of Appeal (RPBA) indicating its preliminary, non-binding opinion on the issue of inventive step. The board informed the parties of its intention to dismiss the appeal on the basis of the written submissions on file. It was pointed out that, since oral proceedings were only conditionally requested by respondent II, the board saw no reason to schedule them. A time period of two months was given to the parties to reply to the board's communication.

VI. On 3 August 2009, the appellant replied to the board's communication stating that it had no further comments or arguments to add to the case. No submissions were filed by the respondents.

VII. The **11th auxiliary request**, which was the **main request**, contained only four claims. Claims 1 and 2 read as follows:

"1. A subtilisin protease variant, wherein the immunological potential has been changed in comparison to the parent protease selected from subtilisin BPN', subtilisin amylosacchariticus, subtilisin 168, subtilisin mesentericopeptidase, subtilisin Carlsberg, subtilisin DY, subtilisin 309, thermitase, aqualysin, Bacillus PB92 protease, and proteinase K, in that, in said protease changes have been performed among the amino acid residues at any one or more of positions 151, 174, 176, 193, and 196, by deletion, substitution, or

insertion (single or multiple) adjacent to the indicated positions, whereby said subtilisin protease has an immunological potential lower than that of said parent protease, and in that it possesses at least one mutation affecting an amino acid residue occupying a position chosen from the group of positions 151, 174, 176, 193, and 196.

2. The protease as claimed in claim 1, further characterised in that it contains at least one or more sets of mutations affecting amino acid residues occupying a position chosen from the group of sets of positions:

36+209, 89+120, 136+170, 36+89, 89+235, 136+195, 181+222, 209+222, 235+251."

Claim 3 was directed to a composition comprising any protein variant according to any of claims 1 to 2 and claim 4 to the composition of claim 3, wherein said composition was a detergent composition.

VIII. The 12th and 13th auxiliary requests were identical to the 11th auxiliary request except for the deletion, respectively, of position 151 and of positions 151 and 196. Claim 1 of the 14th auxiliary request was a combination of claims 1 and 2 of the 13th auxiliary request. The 15th, 16th, 17th and 18th auxiliary requests were identical, respectively, to the 11th, 12th, 13th and 14th auxiliary requests but limited to a variant of the subtilisin 309 protease.

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

- D3: WO-A-89/06279 (publication date: 13 July 1989);
- D5: WO-A-91/00345 (publication date: 10 January 1991);
- D11: H. Zachariae et al., *Allergy*, 1981, Vol. 36,
pages 513 to 516;
- D12: EP-A-0 251 446 (publication date: 7 January 1988);
- D34: WO-A-03/057246 (publication date: 17 July 2003);
- D35: WO-A-03/057713 (publication date: 17 July 2003);
- D36: Page 14 of document D5 with part of Table I.
- X. The appellant's submissions may be summarised as follows:

Article 56 EPC

Document D3 represented the closest prior art and the problem to be solved was the provision of subtilisin variants having lower immunological potential. The positions identified in claim 1 of the various requests were among those listed and grouped by polarity on page 19 of the patent-in-suit. When these positions were ordered by amino acid residue position instead of polarity, they fell into the groups 127-131, 136, 151-154, 161-163, 167-176, 186, 193-197, 247, 251 and 261, which represented epitopic regions of the subtilisin. These residues were selected as candidates for change on the basis of the analysis set out in detail in the patent-in-suit. The description of the

patent-in-suit contained abundant experimental evidence for determining the amino acids implicated in epitopes and it showed how different amino acids could be assigned to their respective epitopes on the basis of the interatomic distances of the C α carbon atoms. The five residues (positions) listed in claim 1 of the 11th auxiliary request (main request) were taken from these epitopic regions, namely position 151 from region 151-154, positions 174 and 176 from region 167-176 and positions 193 and 196 from region 193-197. In that context, the conclusion of the opposition division, namely that the claimed subtilisin variants might not exhibit the desired properties, was mere speculation.

The objection in relation to the significant heterogeneity of subtilisin sequences and to the lack of evidence for subtilisins other than subtilisin 309 was without foundation. The sequences alignment of document D36 showed that the sequence heterogeneity in the positions mentioned in the claim was very limited. Moreover, while a certain sequence heterogeneity was present among subtilisins, it was well-known that the three-dimensional structures of subtilisins aligned extremely well spatially. As a matter of precaution, the 15th to 18th auxiliary requests were limited to a subtilisin 309 protease variant.

Many of the subtilisin residues identified in the 11th to 18th auxiliary requests as candidates for change in order to reduce the immunogenic responses to subtilisin were also identified in two PCT applications filed by opponent 03 in December 2002 (documents D34 and D35). This implicitly acknowledged an inventive step for the claimed subject matter. Even without this evidence, it

was in line with the case law of the Boards of Appeal, that if an opponent disputed the existence of an inventive step, the burden of proof was on him. If the opponent had evidence that the claimed invention did not solve the problem (contrary to the teaching of its own patent applications D34 and D35), this evidence had to be submitted. Failing that, even if there were serious doubts on the persuasiveness of the evidence on the patent, which was not the present case, it could not be concluded that the invention failed to solve the technical problem but at worst only that the evidence was inappropriate.

The set of amino acid residues cited in claim 2 of the auxiliary requests 11th to 13th and 15th to 17th (and in claim 1 of auxiliary requests 15th and 18th) were those that, as indicated in Table VII of the patent-in-suit, were reported to have at least a medium probability, and in many cases a high probability, of being in the same epitope. Thus, they involved an inventive step as well.

XI. The respondent II's submissions may be summarised as follows:

Article 56 EPC

There was no evidence on file disclosing a cause-effect relationship for any of the amino acid positions mentioned in the claims of the pending requests, not even for subtilisin 309. None of the amino acid residues present in those positions was mutated in the experiments described in the patent-in-suit and these positions were not derivable from any of these

experiments. There was no indication in the patent-in-suit that the listing of a number of consecutive residues was of any significance nor could any conclusion regarding conformational epitopes be drawn from the mere linear proximity of residues.

The experiments disclosed in the patent-in-suit failed to demonstrate any position (site) in the subtilisin sequence as being or forming part of an epitope (i.e. bound by an antibody) and they did not demonstrate that the claimed changes led to a reduced immunogenicity. In fact, the data disclosed in the patent-in-suit relating to mutated sites were highly inconsistent and did not allow the skilled person to identify any of the exemplified residues (let alone non-exemplified residues, such as those present in the claims) as leading to a reduced immunogenicity. The interatomic distances provided in Table VI of the patent-in-suit could only be used to identify amino acids that were close to the sites identified in the examples of the patent but not as supporting the claimed subject-matter (leading to a lower immunogenicity).

The fact that the claims extended to a bundle of subtilisins exhibiting significant sequence heterogeneity was only one of all the deficiencies of the patent-in-suit, namely the lack of evidence for the total immunogenic potential (allergenicity) of the claimed subtilisin variants, the absence of any indication on the amino acid that replaced the amino acids in the indicated positions of the claimed subtilisin variants and the fact that the claims did not preclude the presence of further modifications that could affect in an unpredictable way the immunogenic

potential of the claimed subtilisin variants. There were also important technical differences between documents D34 and D35 and the experiments of the patent-in-suit. These documents had also quite different overall disclosures.

There was no evidence whatsoever on file showing that the claimed subject-matter solved the technical problem and there was no nexus between the experiments of the patent-in-suit and the claimed subtilisin variants, which were not derivable from any of those experiments. In line with the case law of the boards of appeal, it was fundamental to assess whether the technical problem addressed by the invention was successfully solved. Advantages that were merely referred to but without having sufficient evidence to support comparison with the closest prior art, could not be taken into consideration for assessing inventive step. The mere allegation that the claimed subtilisin variants had a reduced immunological potential was not enough to conclude that the technical problem was solved. In the present case, it was needed to establish a causal relationship between the mutations and the change in property. The burden of proof was on the patentee to demonstrate that the invention achieved the technical effects alleged in the patent-in-suit. Only when this was *prima facie* established, the burden of proof shifted to the opponent to provide counter-evidence. In the present case, the appellant had not discharged its primary burden of proof.

XII. The appellant (patentee) requested to set aside the decision under appeal and to maintain the patent on the basis of the 11th auxiliary request (main request) or, in the alternative, of any of the auxiliary requests 12th to 18th in that order, all filed with the statement of grounds appeal.

XIII. The respondent II (opponent 03) requested that the appeal be dismissed.

Reasons for the Decision

1. The 11th to 18th auxiliary requests result from a succession of further limitations introduced into the 7th auxiliary request, this latter being the request that this board of appeal had considered to fulfil the requirements of Articles 123(2), (3), 84 and 83 EPC in the decision T 1067/02 (*supra*) and that, upon further prosecution, the opposition division considered to lack an inventive step as in its view there was no evidence on file demonstrating that the technical problem was solved. In these appeal proceedings, the 11th auxiliary requests was made the main request followed in the order by the 12th to 18th auxiliary requests. The respondents have not raised any objection to those requests other than that of lack of inventive step. The board also believes that the sole substantive issue is that of inventive step (Article 56 EPC).

Article 56 EPC (all requests)

The closest prior art and the technical problem to be solved

2. Common to all claim requests on file are variants of subtilisin 309 protease wherein, in comparison with the

parent molecule, changes have been performed at one or more of positions 174, 176, and 193, in combination with at least one or more sets of mutations at other specific positions (cf. claim 1 of the 18th auxiliary request), said variant having an immunological potential lower than that of the parent molecule (cf. points VII and VIII *supra*).

3. The decision under appeal identified document D3 as the closest prior art in relation to the claim requests then on file and formulated the problem to be solved as being the provision of subtilisin enzymes with lower immunogenic potential compared to the parent enzyme (cf. point 2.3.3 of the decision under appeal). This remains so also in relation to the claim requests now pending.

4. Document D3 discloses mutant subtilisin enzymes in which the amino acid residues at one or more of the indicated positions (including positions 153, 172, 175, 194 and 195) are changed by substitution, insertion or deletion and which have altered chemical properties including, but not limited to, increased stability to oxidation, augmented proteolytic activity, and improved wash ability. Most of these positions are identified by comparison of the amino acid sequences of several subtilisins known in the art. In particular, document D3 identifies the residues in the active site of subtilisin 309 (Asp-32, His-64 and Ser-221), some residues within the oxyanion binding site (Asn-155, Ser-221) as well as residues of possible importance due to their conservation in most subtilisins (Trp-6, His-67, Asp-153, Pro-168, Arg-170, Met-175, Asn-218, Gly-219, Arg-275) (cf. pages 12 and 13). Several mutants of subtilisin 309 are produced by site-directed

mutagenesis, such as mutants Arg170Tyr, Gly195Glu and Arg170Tyr Gly195Glu (cf. page 28, last paragraph), and the properties of some of these mutants are further disclosed (oxidation stability, proteolytic activity and washing ability).

5. Evidence on file shows that the immunogenicity of proteins intended for human use was a problem known in the background art, such as the immunogenicity of subtilisin when used in the production of detergents (cf. document D11). Therefore, the underlying technical problem of reducing the immunogenicity of subtilisin as a property to be altered does not *per se* represent an inventive contribution over the known art. A contribution, if any, should be found in the identification of specific positions on the molecule which, when modified, bring about the desired immunogenicity reduction. Whether this is the case for the specific positions proposed in the present claims is indeed the question to be answered.

6. In this respect, the question was raised in decision T 1067/02 (*supra*) as to whether there is a cause-effect relationship between the proposed mutations and the desired lowered immunological potential, i.e. whether it is possible to establish a causal link between the specific mutations and the lowered immunogenicity (cf. T 1067/02, *supra*, point 21 of the Reasons; see also T 537/02 of 19 October 2004, points 17 et seq. of the Reasons and T 660/02 of 9 June 2005, points 4 to 10 of the Reasons). This is in fact the critical issue in the present appeal proceedings in relation to inventive step because the simple proposal of one or more changes

without any demonstration of any effect would amount to an academic exercise involving no inventive step.

The disclosure of the patent-in-suit

7. According to the patent-in-suit, "*epitope mapping is used to locate and characterize the various epitopes functionally present in a protein. Thereafter this information is used for selecting which amino acid residues in the epitopes should be changed*". These changes are analyzed, in particular those leading "*to switches from major to minor epitopicity or even to epitope loss*", and "*this information is again used to decide whether the protein variant(s) produced correspond to demands established for the protein, or, whether more or other changes have to be implemented*" (cf. page 3, lines 49 to 57 of the patent-in-suit).

8. As a result of the two experimental series of analyses A and D disclosed in the examples of the patent-in-suit, four different groups of specific positions (Groups I to IV) are identified (cf. page 19, lines 16 to page 20, line 36) as well as, using the known 3-dimensional (3-D) information on subtilisins, the (high or medium) probability of the amino acid residues in some of these positions for being in the same epitope (cf. page 14, line 56 to page 18, Tables VI and VII). As a result of these analyses, 32 positions are selected for amino acid changes that could **possibly** influence the immunological potential of subtilisin 309, including positions 174, 176 and 193 (cf. page 19, line 1 to 12).

9. However, except for positions 136, 170, 195 and 251, none of the residues of the other selected positions is

exemplified in the patent-in-suit nor any reason given for their selection. According to Table VII, positions 170 and 195 have a high probability of being in the same epitope and they are both classified within Group IV for which "*changes seem to be neutral or even beneficial*" (cf. page 19, lines 30 to 34 and page 20, lines 3 to 8).

Does the claimed subject-matter solve the technical problem?

10. The appellant argues that the positions indicated on page 19 of the patent-in-suit represent 10 epitopic regions of subtilisin, namely 127-131, 136, 151-154, 161-163, 167-176, 186, 193-197, 247, 251 and 261.

11. It might well be possible to estimate which amino acids belong to an epitope by combining the experimental results disclosed in the patent-in-suit with the known high resolution X-ray structures (3D) of subtilisin (cf. *inter alia* document D12 and page 15 of the patent-in-suit). This has been done on page 17 and in Tables VI and VII of the patent-in-suit based on the results obtained by the experimental substitution of several amino acids in **specific positions** of the subtilisin (cf. Tables III to V). However, it is not possible to draw any conclusions from other positions for which no experimental results are disclosed in the patent-in-suit. This is all the more so in the absence of any information concerning the criteria used for their selection, i.e. whether they have been selected for being in the neighbourhood of residues identified as forming (linear) continuous epitopes or, based on the tertiary structure of subtilisin, for their

- proximity to residues identified as forming (conformational) discontinuous epitopes.
12. Although the patent-in-suit refers to the drawbacks of identifying epitopes which are not in their native environment (cf. paragraph bridging pages 2 and 3), positions 174, 176, 193 and 196 are selected based only on the possible presence of continuous epitopes (167-176 and 193-197) near the exemplified positions 170 and 195. There is, however, no evidence for these continuous epitopes in the patent-in-suit nor any reason (should they be present) to expect them to extend to the positions indicated in the claims (epitope boundaries). The patent-in-suit identifies only the **specific** residues 170 and 195 as having a high probability of being in the same (conformational) discontinuous epitope but there is no information on a possible contribution of other nearby residues and certainly not for residue at position 151.
 13. Indeed, as stated in the patent-in-suit, even if residues are identified within an epitope (be it continuous or discontinuous, linear or conformational, structural or functional), they might still have to be differentiated as "essential", "critical" or "present" (cf. page 19, lines 20 to 21). Nothing is derivable from the information disclosed in the patent-in-suit for the specific positions indicated in the claims, should they be present within an epitope.
 14. Since no experimental evidence and no reasons are given in the patent-in-suit for selecting the positions indicated in the claims, the board fails to see any causal link between these positions and a lowered

immunogenicity of the subtilisin protease variant (cf. point 6 *supra*).

15. In respect of this issue, the following points are also of relevance:

i) According to the patent-in-suit the "*simple accumulation of effects cannot be expected in multiple (amino acid) exchange variants*" and "*further analysis is needed to confirm any accumulation of immunological effects*" (cf. page 20, lines 9 to 11), however the requests under consideration are not limited to single exchange variants but embrace multiple exchange variants (claim 1 reads "*in said protease changes have been performed among the amino acid residues at any one or more of positions*", underlined by the board) for which no information is disclosed in the patent.

ii) The patent-in-suit also states in explicit manner that change at position "*181 gives a heteroclitic effect*" and therefore, "*from an immunological point of view a change in this position should be avoided*" (cf. page 19, line 55 to page 20, line 2). Nevertheless, multiple exchange variants are envisaged having a change at position 181 (181+222).

iii) Since a "*simple accumulation of effects cannot be expected*", the effect of these possible multiple exchanges on the immunological potential of the corresponding variants should be assessed for each and every one of them. This assessment is not made in the patent-in-suit.

iv) Except for a deletion at position 36 (*36D), there is no information in the patent-in-suit on the immunological effects of deleting any other residue(s) of a subtilisin protease, not even for those specifically mentioned in the claims (positions 151, 174, 176, 193 and 196). In fact, even the specific immunological effect of deleting the residue at position 36 is not disclosed in the patent-in-suit. Indeed, except for mutant S021 for which no immunological results are provided (cf. page 12, Table IV), this deletion is always found in combination with exchanges at other positions (cf. page 5, Table I and page 10, Table III).

v) There is no information in the patent-in-suit regarding the possible immunological effects derived from a (single or multiple) insertion adjacent to the positions indicated in the claims, let alone regarding the immunological effects derived from a combination of all the possible exchanges contemplated in claim 1, namely deletions, substitutions and/or (single or multiple) insertions.

16. It follows from the above that claim 1 of all the requests on file embraces many possible subtilisin variants for which there is no evidence on file to demonstrate that they actually solve the underlying technical problem (cf. point 3 *supra*).

Post-published evidence and burden of proof

17. According to the established case law, post-published evidence may only be used to support information that is already derivable from the original application

(cf. T 1329/04 of 28 June 2005, Catchword and points 12 to 14 of the Reasons). In the light of the observations made above, the board considers that this information is not derivable from the patent-in-suit. It is also noted that only positions 174 and 176 are mentioned in the post-published evidence referred to by the appellant (cf. pages 62 to 64, Examples 2 and 3 of document D34 and pages 66 to 70, Examples 3 to 6 of document D35).

18. In T 1067/02 (*supra*) the board already drew the appellant's attention to the relevance of a causal link between the positions mentioned in the claims and their alleged immunological effects for inventive step (cf. point 21 of the Reasons and point 6 *supra*). In the further prosecution of the case, the opposition division revoked the patent based on its negative findings on this causal link. Under the circumstances of the present case, the burden was on the appellant to show that such link exists. For the reasons given above, this burden has not been discharged.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar:

The Chairman:

A. Wolinski

L. Galligani