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Datasheet for the decision of 14 November 2007

Case Number: T 1380/05 - 3.3.08

Application Number: 88904113.3

Publication Number: 0353250

IPC: C12N 15/00

Language of the proceedings: EN

Title of invention:
The engineering of electrostatic interactions at metal ion binding sites for the stabilization of proteins

Patentee:
Novozymes A/S

Opponent:
GENENCOR INTERNATIONAL INC.

Headword:
Protein stabilization/NOVOZYMES

Relevant legal provisions (EPC 1973):
EPC Art. 87, 54, 56, 83
RPBA Art. 10a

Keyword:
"Main request - priority - no"
"Main request - novelty - no"
"First auxiliary request - allowability of disclaimer - no - lack of clarity"
"Second auxiliary request - novelty - yes"
"Inventive step - yes"
"Sufficiency of disclosure - (yes)"

Decisions cited:
G 0002/98, G 0002/93, T 0391/91, T 0500/91, T 0412/93, T 0207/94

Catchword:
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Case Number: T 1380/05 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 14 November 2007

Appellant: (Opponent)
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 26 August 2005 rejecting the opposition filed against European patent No. 0353250 pursuant to Article 102(2) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: F. Davison-Brunel
C. Rennie-Smith

Summary of Facts and Submissions

I. European patent No. 0 353 250 with the title "The engineering of electrostatic interactions at metal ion binding sites for the stabilization of proteins" was granted with 7 claims on the basis of the European application No. 88 904 113.3 claiming priority from document US 0 349 65 filed on 6 April 1987.

Granted claim 1 read as follows:

"1. A subtilisin variant with increased thermal stability over the corresponding wild-type subtilisin, wherein the subtilisin variant comprises an alteration of the amino acid sequence at the calcium A binding site such that the electrostatic attractive interaction between the amino acids at the calcium A binding site and a calcium ion is increased relative to that of the corresponding wild-type subtilisin."

Claims 2 to 6 related to further features of the subtilisin variant of claim 1. Claim 7 related to a washing preparation comprising the subtilisin variant of any of claims 1 to 6.

II. One opposition was filed on grounds of Article 100(a)and (b) EPC for lack of novelty, lack of inventive step and insufficiency of disclosure. The opposition division rejected the opposition pursuant to Article 102(2) EPC by its decision dated 26 August 2005.

It was concluded, in particular, that the patent in suit enjoyed priority rights from the filing date of the US priority document and, that, therefore, the

requirement of novelty was met. Inventive step and sufficiency of disclosure were also acknowledged.

III. The appellant (opponent) filed a notice of appeal, paid the appeal fee and submitted a statement of grounds of appeal in due time.

IV. The respondent (patentee) filed a submission in answer to the grounds of appeal, which was accompanied by an auxiliary request.

V. The board sent a communication pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal indicating its preliminary, non-binding opinion.

VI. Both parties filed observations in answer to this communication.

VII. By a fax letter dated 5 November 2007, the respondent's representative advised the board that the proprietor would not be represented at oral proceedings. This last submission was accompanied by amended first and second auxiliary requests to replace the auxiliary request on file, as well as by an amended page 4 of the description.

Claim 1 of the **first auxiliary request** read as follows:

"1. A subtilisin variant with increased thermal stability over the corresponding wild-type subtilisin, wherein the subtilisin variant comprises an alteration of the amino acid sequence at the calcium A binding site such that the electrostatic attractive interaction between the amino acids at the calcium A binding site

and a calcium ion is increased relative to that of the corresponding wild-type subtilisin, **provided that, if the alteration is at a position corresponding to one or more of positions 41, 75, 76, 77, 78, 79, 80, 81, 208 and 214 of subtilisin BPN', then the alteration is not a substitution of a negatively-charged amino acid."** (differences from granted claim 1 highlighted by the board)

Claim 1 of the **second auxiliary request** read as follows:

"1. A subtilisin variant with increased thermal stability over the corresponding wild-type subtilisin, wherein the subtilisin variant comprises an alteration of the amino acid sequence at the calcium A binding site such that the electrostatic attractive interaction between the amino acids at the calcium A binding site and a calcium ion is increased relative to that of the corresponding wild-type subtilisin, provided that, if the alteration is at a position corresponding to one or more of positions 41, 75, 76, 77, 78, 79, 80, 81, 208 and 214 of subtilisin BPN', then the alteration is not **a replacement of the amino acid with a negatively-charged amino acid."** (differences from claim 1 of the first auxiliary request highlighted by the board)

In the two auxiliary requests, claims 2 to 7 were identical to granted claims 2 to 7.

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

(1): Bryan, P.N. et al., Proteins: Structure,

Function, and Genetics, Vol.1, pages 326 to 334, 1986;

(3): Voordouw, G. et al., Biochemistry, Vol.15, No.17, pages 3716 to 3724, 1976;

(10): WO 88/ 08033 filed on 28 March 1998 with a priority date of 10 April 1987 and a publication date of 20 October 1988;

(22): Bode, W. et al., The EMBO Journal, Vol.5, No.4, pages 813 to 818, 1986;

(25): Smith, E.L. et al., The Journal of Biological Chemistry, Vol.243, No.9, pages 2184 to 2191, 10 May 1968;

(P1): US 34965, priority document of the patent in suit with the filing date of 6 April 1987.

IX. The appellant's arguments in writing and during oral proceedings insofar as relevant to the present decision may be summarized as follows:

Main request; claim 1
Articles 87 and 88 EPC; entitlement to priority

Claim 1 related to subtilisin variants with increased thermostability comprising an alteration of the amino acid sequence at the calcium A (CaA) binding site. Even if the priority document encompassed the possibility of mutations at Ca binding sites for the purpose of increasing thermostability, as part of a general teaching, it did not provide priority for the claimed

specific embodiment of the alteration of the Ca**A** binding site. In fact, the references to the two Ca binding sites - A and B - of subtilisin were at a purely informational level. The sites were identified but the reader was not taught whether either or both was suitable for mutation. There was no teaching in the priority document that an improvement in thermal stability could be obtained by mutating the CaA site. For these reasons, the document did not provide priority for subtilisin variants specifically mutated at the CaA site.

Article 54(3)(4) EPC; novelty

As the claimed subject-matter only enjoyed priority from the filing date of the patent in suit (6 April 1988), document (10), a PCT application filed on 28 March 1988 with priority of 10 April 1987 and published on 20 October 1988 was to be taken into consideration under Art.54(3) EPC. This document disclosed subtilisin variants comprising an alteration in the amino acid sequence at the CaA binding site, eg. of Leu^{75}. Thus, claim 1 of the main request did not fulfil the novelty requirement.

First and second auxiliary requests; claim 1
Article 123(2) EPC; allowability of the disclaimer

The disclaimer which had been added to claim 1 of each of these requests to avoid the teachings of document (10) was not allowable for the following reasons:

– More was disclaimed than needed insofar as amino acids 208 and 214 did not belong to the CaA binding site.

– Less was disclaimed than was needed insofar as document (10) disclosed changes in **the region of** a Ca binding site rather than **at** the Ca binding site - itself defined as involving the specific amino acids which were disclaimed. It was this region which should have been disclaimed to take proper account of the disclosure in document (10).

– The disclaimer failed to fulfil the requirements of Article 84 EPC:

  – it mentioned the substitution of a negatively-charged amino acid without it being clear whether substitution **by** or substitution **with** was intended.

  – what constituted the Ca ion binding site was rendered uncertain by the fact that amino acid positions were disclaimed which did not belong to this site (see supra) and the replacement of amino acid 41 by a negatively-charged amino acid was disclaimed when this amino acid already was negatively-charged.

Second auxiliary request; claim 1
Article 56 EPC; inventive step

The closest prior art needed not to be a document but could be seen as the specific situation that the skilled person working with subtilisins was well aware

of their use, in particular in laundry detergents, and, also, of course, of the necessity for the proteins to be resistant when used, for example, to be thermostable.

The problem to be solved could then be expressed, as for any industrial application, as finding a means for improving the relevant property, here the thermostability.

The skilled person had a choice either looking in nature for enzymes more stable than those already in use or improving the stability of already known enzymes. When choosing the latter course of action, he/she had two possibilities: mutagenising at random the DNAs encoding the known subtilisins or, alternatively, engineering subtilisin variants in a targeted manner on the basis of the relationship between their secondary structure and their function. This second approach would have been obvious as shown, for example, in document (1) which emphasized its usefulness. Yet, document (1) on its own was not extremely informative since the thermostable subtilisin which it described had been obtained by random mutagenesis and, furthermore, it gave no suggestion as to what the appropriate changes might be.

The skilled person would, thus, have turned to further prior art for guidance and, when doing so, would necessarily have taken document (3) into consideration. This taught that calcium ions contributed to the stability of subtilisins and, most importantly, that subtilisin Carslberg was more stable than subtilisin BPN'. This observation would have led him/her to

compare the amino acid sequences of the two proteins ie. to document (25) which taught that there were amino acid differences between them, most of them but not all being conservative substitutions. On this basis, the skilled person would have come to the obvious conclusion that the differences which were not conservative had to be responsible for the differences in the properties of the enzymes. Document (22) disclosed the location of the Ca binding sites in subtilisin Carlsberg and BPN'. Comparison of the amino acids in these sites showed that four of them were different, one of these differences being non-conservative. For the skilled person, it would have been obvious to alter this position in BPN' - the enzyme which was least thermostable - by substituting a negatively-charged amino acid. When doing so, he/she would have obtained a more thermostable enzyme, thus confirming the validity of the protein engineering approach to increasing thermostability.

Accordingly, altering the CaA binding site for the purpose of increasing thermostability was an obvious measure and the claimed subject-matter did not fulfil the requirements of Article 56 EPC.

Article 83 EPC; sufficiency of disclosure

When considering the tri-dimensional structure of the CaA binding site, it was readily apparent that Asn^{77}, an amino acid which was polar but uncharged, was closer to the calcium ion than any other amino acid residues. Thus, it would be expected that replacing Asn^{77} by the negatively-charged amino acid, aspartic acid, would result in stronger calcium ion binding and, thus,

better thermal stability. But the experimental evidence showed the contrary.

On the face of it (only two out of three mutations in the Ca binding site resulting in the required effect), the concept of increasing thermostability by altering the Ca binding site did not work predictably, ie it was not fit for generalisation. The observed failure with the Asn^{77} to Asp substitution could not be taken as an exceptional failure of the kind regarded in the case law as "tolerable", because it represented one third of all exemplified embodiments. For this reason, sufficiency of disclosure was not achieved over the scope of the claim.

X. The respondent's arguments in writing insofar as relevant to the present decision may be summarized as follows:

Admissibility of documents (22) and (25) in the proceedings

Document (22) was refused admission into the first instance proceedings by the opposition division on the basis it was late filed whereas document (25) was a completely new document. They should not be admitted into the proceedings.

Main request; claim 1
Articles 87 and 88 EPC; entitlement to priority

At page 4, the priority document of the patent in suit referred to altering metal ion binding sites (plural), then both calcium ions binding sites of subtilisin were

discussed. The legend to figure 2 showed detailed structural information for the CaA binding site. This, was, of course, with the same purpose as for the CaB binding site, namely that it could be altered to enhance thermostability. The final European application showed that the instructions and predictions concerning the CaA site were correct.

The priority document contained no suggestion that only weak calcium binding sites would be appropriate for beneficial alteration. The fact that the inventors chose to mutate the CaB site first did not amount to ignoring the CaA site.

It was not necessary for the priority document to contain worked examples of all that was ultimately claimed in the European patent.

For these reasons, entitlement to priority should be acknowledged.

Article 54 EPC; novelty

On the basis that the claims were entitled to the claimed priority date, document (10) was not part of the state of the art under Art.54(3) EPC. No other document was alleged to be detrimental to the novelty of the claimed subject-matter. Novelty had to be acknowledged.

First and second auxiliary requests, claim 1
Article 123(2); allowability of the disclaimer

The objections raised against the admissibility of the disclaimer in claim 1 of each of these requests were not convincing for the following reasons:

– The disclaimer did not render the claim unclear. Document (10) clearly envisaged such an alteration as a means to increase thermostability and in any case, no evidence had been provided that the replacement of Asp^{41} with another negatively-charged amino acid, eg. Glu, would not increase thermostability.

– The disclaimer was not limited to defined positions in BPN' but rather covered alterations to all subtilisins since the alteration was defined in the disclaimer as being at a position **corresponding** to the defined positions in BPN'. This feature was adequate to remove from the claim any subject-matter disclosed in document (10).

– To take into account possible findings by the board that the wording of the disclaimer in claim 1 of the main request lacked clarity, the disclaimer in both auxiliary requests now defined the alteration as being "at a position corresponding to **one or more of** positions....". In addition, in the second auxiliary request, the alteration was defined as being "..a **replacement of the amino acid with** a negatively-charged amino acid".

The disclaimer was clearly drafted. It delimited the claimed subject-matter from the disclosure in document (10) and, thus, established novelty.

Second auxiliary request; claim 1
Article 56 EPC

Document (1) could be regarded as the closest prior art as it disclosed the subtilisin mutant Ser^{218} to Asn^{218} as being more thermally stable than the wild-type enzyme. Position 218 was not in the CaA binding site and there was no suggestion in document (1) that this position had anything to do with calcium binding. Instead, the stability was attributed to increased hydrogen bonding. There was, thus, nothing in document (1) making it obvious to alter the CaA binding site to acquire thermal stability.

As for document (3), it showed that subtilisin Carlsberg had a higher thermal stability than BPN'. Yet, it was only with hindsight that this property could be attributed to an increased strength in calcium binding. Indeed, it was remarked at page 3717 that "the reasons for its kinetic thermal stability are not at all clear". There was nothing in document (3) that would lead the person skilled in the art to alter the polypeptide structure of a subtilisin at any calcium binding site, let alone at the specific site to which the current claim was limited.

For these reasons, the claimed subject-matter was inventive (Article 56 EPC).

Article 83 EPC; sufficiency of disclosure

The appellant had drawn attention to the fact that an Asn77/Asp mutation in subtilisin led to a reduction in

thermal stability. This variant was not within the scope of the claim since the claim was limited to variants with increased thermal stability, and so it was not an example of a failure of the present invention.

The only issue was whether it would be an undue burden to create mutants at the CaA binding site that had increased stability. The appellant had not provided any evidence to show that it would have been an undue burden. One negative result did not show that the patent was insufficient under Article 83 EPC. Furthermore, it was inappropriate to suggest that "one third" of the experimental results represented a failure simply on the basis of two successes and one (alleged) failure. This was not statistically significant. The patent provided a sufficient disclosure of the invention which was claimed.

XI. The appellant requested that the decision under appeal be set aside and that the patent be revoked.

The respondent requested that the appeal be dismissed (main request) or, as auxiliary request, that the patent be maintained on the basis of the first or second auxiliary requests filed on 5 November 2007.

Reasons for the decision

Admissibility of documents (22) and (25) in the proceedings

1. Article 10a of the Rules of Procedure of the Boards of Appeal states, in particular, that:

(1) Appeal proceedings shall be based on
(a) the notice of appeal and statement of grounds of appeal pursuant to Article 108 EPC;
...
(2) The statement of grounds of appeal and the reply shall contain a party's complete case. They shall set out clearly and concisely the reasons why it is requested that the decision under appeal be reversed, amended or upheld, and should specify expressly all the facts, arguments and evidence relied on. All documents referred to shall be

(a) attached as annexes insofar as they have not already been filed ...
...
(4) ..., everything presented by the parties under (1) shall be taken into account by the Board if and to the extent it relates to the case under appeal and meets the requirements in (2).

2. Documents (22) and (25) were filed with the statement of grounds of appeal. They undeniably relate to the case under appeal as they are concerned with the sequence or structure of subtilisins. They are, thus, admitted in the proceedings.

Main request; claim 1
Article 87 EPC; entitlement to priority

3. Pursuant to Article 87(1) EPC, a person who has duly filed in or for any state party to the Paris Convention for the Protection of Industrial Property an application for a patent shall enjoy for the purpose of

filing a European patent application **in respect of the same invention** a right of priority during a period of twelve months from the date of filing of the first application.

4. In accordance with the Enlarged Board decision G 2/98 (OJ EPO 2001, 413), the requirement for claiming priority of **the same invention** means that the priority is to be acknowledged in respect of a claim of a European patent only if the skilled person can, using his common general knowledge, derive the same subject-matter of the claim **directly and unambiguously** from the previous application as a whole.

5. Thus, to assess whether or not the subtilisin variant of claim 1 which bears a mutation in the calcium ion binding site A (**CaA**) enjoys priority, the content of the priority document (P1) (US 34965) filed on 6 April 1987 must be analysed. General considerations on protein structure including potential stabilisation by metal ion binding are found on pages 1 to 4. Starting on page 4 (Summary of the invention), it is envisaged that, if a protein contains more than one metal ion binding site, then more than one site may be altered and, also, that any one site may carry more than one altered amino acid residue. There follow detailed explanations of how to proceed to target the relevant sites - identifying their natural intrinsic thermostability by a number of methods and determining the changes to be performed. Serine proteases in general are disclosed as suitable substrates for alterations on page 16. Increases in the stability of subtilisin BPN' by altering the Ca^{++} ion binding site B are mentioned in the passage bridging pages 18 and 19.

6. From page 19 onwards, a description is provided of how to apply the method disclosed on page 4 to subtilisin BPN'. Thus, the detailed structure of the two calcium ion binding sites present on the molecule is given on the basis of crystallographic data. Then, a chemical analysis of the two sites is carried out to evaluate their intrinsic calcium ion binding strength. It is, thus, found that the calcium ion binds more strongly to the subtilisin CaA binding site than to EDTA, whereas the calcium ion may be replaced by other ions at the calcium ion binding site CaB. Taken together with thermal inactivation kinetics data, these data lead the inventors to conclude on page 23, last paragraph, that:

"In the light of all the available chemical and physical information described above, it is possible to nominate the calcium site B as the most likely candidate responsible for the weak calcium binding demonstrated in Figure 4 for wild type subtilisin BPN'."

The rest of the application is exclusively concerned with altering site B in such a way as to achieve enhanced thermal stability.

7. In the board's judgement, the overall teaching of the priority document (P1) amounts to a direct and unambiguous disclosure of a subtilisin variant at the **Ca^{++} ion binding site B**. Of course, the presence of another calcium ion binding site, namely CaA, is mentioned. The mere knowledge of its presence provides the "theoretical possibility" that it may be altered. But taking into account, on the one hand, that the

experiments relating to it show that it is a strong calcium binding site and, on the other hand, that it is never identified as a possible target for mutagenesis, this theoretical possibility does not amount to **a direct and unambiguous** disclosure of subtilisin variants with increased thermostability carrying changes at CaA binding site.

8. For this reason, it is concluded that the priority date of the claimed subject-matter is the filing date of the patent application, namely, 6 April 1988.

Article 54(3)(4)EPC; novelty

9. As the claimed subject-matter enjoys priority from 6 April 1998, document (10), a PCT application filed on 28 March 1988 with priority of 10 April 1987 and published on 20 October 1988 constitutes prior art under Art.54(3)(4) EPC. This document (page 9) discloses a subtilisin variant wherein one or more of the following amino acids of a calcium binding site: Leu^{75}, Asn^{76}, Ser^{78}, Ile^{79}, Gly^{80}, Val^{81} is replaced with a "negatively charged" amino acid.

This variant molecule falls within the scope of claim 1 which, therefore, lacks novelty.

10. The main request is refused for failing to fulfil the requirements of Article 54 EPC.

First and second auxiliary requests; Admissibility of the disclaimer

11. Claim 1 of each of the first and second auxiliary requests contains a disclaimer aimed at restoring novelty over the teachings of document (10) (see point 9 supra). The two disclaimers are very much alike (see section VII supra) so that they will both be considered concomitantly and, unless otherwise stated, they will be referred to as "the disclaimer".

12. The Enlarged Board's decision G 02/03 (OJ EPO 2004, 448) states that a disclaimer may be used to delimit a claim against the state of the art under Article 54(3)(4) EPC and also establishes the requirements which it should fulfil in point 3 of the decision ("The drafting of disclaimers"):

"... the disclaimer should not remove more than is necessary to restore novelty" and,

"... the requirements of conciseness and clarity under Article 84 EPC are also applicable to claims containing disclaimers."

13. The relevant section of document (10) is found on page 9, lines 1 to 18 which reads as follows:

"The present invention relates to the modification of the calcium binding site of the subtilisin molecule to increase calcium binding. As used herein the term "modification of the calcium binding site" refers to replacement of one or more amino acids **in the region of** a calcium binding site present in the amino acid sequence of subtilisin with a negatively charged amino acid thereby enabling the resulting subtilisin analog to have an additional negative charge. It has been

found that one calcium binding site in a subtilisin involves the following amino acids: Asp^{41}, Leu^{75}, Asn^{76}, Asn^{77}, Ser^{78}, Ile^{79}, Gly^{80}, Val^{81}, Thr^{208} and Tyr^{214} relative to the amino acid sequence set forth in Table 1. The present invention preferably involves replacement of one or more of the amino acids present in the calcium binding site with a "negatively charged" amino acid such as Asp and Glu, and more preferably Asp."

14. From the wording of this passage (eg. "the subtilisin"), it is clear that the term subtilisin is used in its widest sense, ie. that it refers to subtilisin irrespective of its bacterial origin. This is reflected in the present disclaimer by the fact that the disclaimed subtilisin variants are those with alterations at a position **corresponding** to one or more of the specifically mentioned positions in subtilisin BPN'. The person skilled in the art would have no difficulty to understand which amino acids are involved - which may at any one position be different from those mentioned in the disclaimer - because the correspondance between the various subtilisin amino acid sequences is a matter of common general knowledge - a finding which has not been challenged. Thus, the disclaimer removes from claim 1 subject-matter which would be detrimental for novelty.

15. The argument was brought that the disclaimer removed less than was necessary because document (10) disclosed subtilisin variants with alterations **in the region of** a calcium binding site rather than at the calcium binding site. The phrase "... **in the region of** a calcium binding site..." undoubtedly appears in the above mentioned passage. It is not clear in itself and, when

considering the document as a whole, the following statements are found:

– page 6, lines 7 to 12: "The subtilisin analogs ... are characterized as having an amino acid sequence ... that has been modified by having (1) one or more amino acid residues **in a calcium binding site** .... replaced by a negatively charged amino acid...",

– page 7, lines 6 to 8: "In addition, the present invention provides a method for improving the thermal and pH stability of subtilisins by modifying **the calcium binding site...",**

– page 10, lines 11 to 14: "..., the replacement of one or more of the amino acids **in the** above potential **calcium binding sites** will result in a subtilisin having improved thermal and pH stability."
(emphasis added by the board)

Claims 1 and 3 also refer to modifications occurring in **a calcium binding site** (claim 1) or **in the calcium binding site** represented by Asp^{41}, Leu^{75}, Asn^{76}..."

16. For this reason, the board has no doubt that the teaching of document (10), in fact, relates to subtilisin variants with alterations **in the calcium binding site** and that, therefore, the disclaimer is adequate to remove all embodiments that would destroy novelty.

17. It was also objected that in contravention of the principles enounced in G 02/03 (supra), the disclaimer removed more than was necessary insofar as positions 208 and 214 were not in the Ca binding site and replacement of Asn 77 did not bring an increase in thermostability. These features were not known at the filing date of the patent in suit. As the assessment of novelty must be carried out as of this date, the proper course of action is to remove that which was in the prior art at that date. It does not imply that the claimed subject-matter per se is unclear because the skilled person would inevitably understand the claim as being directed towards the calcium binding site irrespective of whether some of its earlier characteristics failed to remain through time.

18. Evaluating clarity also requires to take into account that Asp at position 41 is a negatively-charged amino acid and disclaiming its replacement by another negatively-charged amino acid was said to be confusing. The board does not see why it should be. The skilled person would know of other negatively-charged amino acids and its replacement by Glu is mentioned expressis verbis in document (10).

19. Finally, the phrase "... the alteration is not a substitution of a negatively-charged amino acid" in the disclaimer of the first auxiliary request was said to be ambiguous as it was not certain whether it should be understood as "substituted by" or "substituted with". In answer to this, the respondent filed the second auxiliary request where the phrase was amended to: "... the alteration is not a replacement of the amino acid with a negatively-charged amino acid.". It may be that

the term "substitution of ..." is as ambiguous as was alleged. In any case, the wording of the disclaimer in claim 1 of the second auxiliary request is perfectly clear and faithfully reproduces that which is found in document (10), page 9:

"The present invention preferably involves replacement of one or more of the amino acids present in the calcium binding site with a "negatively charged" amino acid ...".

For this reason, claim 1 of the second auxiliary request provides a more straightforward identification of the subject-matter which needs to be disclaimed. Thus, the board rejects the first auxiliary request for lack of clarity and further prosecution is carried out on the basis of the second auxiliary request.

Second auxiliary request
Article 56 EPC; inventive step

20. The closest prior art is document (1) which is concerned with providing subtilisins with enhanced thermal stability. In its introductory part, it is specifically mentioned that "one important parameter of potential commercial utility that should be alterable by protein engineering is the thermal stability of proteins" and various approaches are discussed which had been used earlier on to achieve this goal. The document itself teaches the isolation of the subtilisin variant Asn^{218} to Ser with increased thermostability, obtained by random mutagenesis of the encoding DNA. The authors are interested in finding out the impact of this alteration on the tertiary structure of the enzyme.

They offer as an explanation for the increase in thermal stability that the hydrogen bonding between amino acids 202 and 219 must have been tightened by the change (page 332). This observed correlation between physical properties and structure leads them to hypothesize that a number of possible further variations of the subtilisin primary sequence would be probably capable of increasing the free energy of unfolding (paragraph bridging pages 332 and 333).

21. Taking into consideration the ever existing need - alluded to in document (1) itself - for improving industrial processes, the problem to be solved may be defined as providing further subtilisins with enhanced thermal stability.

22. The solution provided is subtilisin variants which are altered at the CaA binding site.

23. Whereas the skilled person would obviously be aware of industrial needs such as mentioned in document (1), the technical teaching of this document falls well short of a suggestion as to which alterations to introduce in the subtilisin molecules to enhance thermostability. And, for this reason, document (1) on its own does not affect inventive step.

24. Thus, in order to solve the above mentioned problem, the skilled person would take into account other documents. The document on file which has been considered relevant in this respect is document (3).

25. Document (3) discloses that calcium ions are one of the factors which contribute to the thermal stability of

subtilisins, the other one being intrinsic stability due to the polypeptide chain itself. In the "Results" section dealing with this latter cause for thermostability (page 3719, bottom of right-hand column), it is mentioned that subtilisin Carlsberg is more stable than subtilisin BPN'. When discussing the calcium ion contribution to thermal stability (page 3722, left-hand column), it is said that "the calcium ion contributions are very similar for these three enzymes in this temperature range", meaning subtilisin Carlsberg, subtilisin BPN' and thermomycolase. In the last paragraph on page 3723, the authors state that: "We do not want to speculate on the origins of these differences on a molecular level...".

26. At no point is it suggested that altering hydrogen bonding or Ca binding or both may improve thermal stability. Indeed, one would not expect it since the document dates from before the "era of protein engineering". If anything one would take from the document (page 3719, supra) that intrinsic stability may be responsible for the differences observed.

27. In short, neither document (1) nor document (3) is even remotely concerned with altering thermostability through increasing calcium ion binding. It is only with hindsight that their combination could be regarded as rendering obvious the now claimed subject-matter.

28. A few further remarks may be made. Contrary to the appellant, the board cannot read into document (3) that the thermostability of subtilisin Carlsberg is higher than that of subtilisin BPN' **because of** a stronger binding of the calcium ion to the former molecule and

is consequently unable to follow the reasoning which led the appellant to a conclusion of lack of inventive step based on this premise.

29. It should also be added that the next step in the reasoning - based on the understanding of document (3) just mentioned - relies on the skilled person finding it obvious to compare the Ca binding sites of both subtilisins. In fact, the Ca binding sites are identified "in passing" (page 817) in document (22) concerned with a totally different matter, namely the tertiary structure of a complex between subtilisin and an inhibitor. From there on, the appellant argues, it would have been obvious to develop a research program to find out whether the differences in thermal stability of the two enzymes correlate with differences in the amino acid sequences of the two Ca binding sites and to deduce therefrom that altering the Ca binding sites is a way to increase thermostability.

30. It is true that the notional "skilled person" can be understood as a team of workers in the fields of relevance to the invention (eg. T 412/93 of 21 November 1994). Yet, this is intended to mean that the sum total of knowledge and technical experience of such a team is considered to be "as a matter of course" when assessing inventive step. On the contrary, it does not mean that the skilled person has the "creativity" of this team. This is fully confirmed in further case law dealing with the abilities of the skilled person (T 391/91 of 22 November 1993, capable of routine experimentation; T 500/91 of 21 October 1992, conservative in attitude, T 207/94 (OJ EPO 1999, 273), lacking in imagination and creativity). Thus, a sophisticated

reasoning such as developed by the appellant may be that of a research worker, in particular if one is to admit that the advancement of science takes place through a series of logical steps. It is not however the reasoning expected of the "skilled person" envisaged by the case law.

31. To conclude, whereas document (1) undoubtedly suggests the possibility of obtaining "more" thermostable subtilisin variants by protein engineering, it does not in itself or in combination with document (3) make it obvious that the protein engineering should be carried out at a Ca binding site, let alone at the CaA binding site. For these reasons, inventive step is acknowledged.

Article 83 EPC, sufficiency of disclosure

32. It was not challenged that at the filing date of the patent in suit, techniques were available for altering one specific amino acid within a protein. The argument as regards lack of sufficient disclosure was rather that the desired effect (increase in thermal stability) could not be achieved over the scope of the claim, ie. the concept of altering the Ca binding site for the purpose of increasing thermostability was not fit for generalisation. This argument was brought on the basis that a replacement by a negatively-charged amino acid at position 77 had the opposite effect, namely to decrease thermostability.

33. The board's opinion is that, at the filing date, the skilled person would have been well aware of the high sensitivity of the three-dimensional structure of a protein to, in particular, any changes in its basic

constituents (see eg. patent in suit [section 006] and document (3)) and, consequently, he/she would have accepted that the effects of any one of the theoretically possible changes in amino acid residues on the protein properties could not be evaluated beforehand with absolute certainty. He/she would, thus, start on the isolation of variants with increased thermostability expecting that a significant amount of mutants may need to be tested, yet not considering it an undue burden as the relevant experiments would be achievable as a matter of routine (see point 32, supra). A negative result such as obtained by replacement at position 77 would not be regarded as a lack of reproducibility of the claimed invention but as to be expected from a routine trial and error experiment. A last remark in this respect is that one cannot deduce from the fact that "only" two out of three exemplified alterations resulted in increased thermostability that failure would occur 33% of the time. The data have no statistical value.

34. For these reasons, sufficiency of disclosure is acknowledged.

Adaptation of the description

35. The respondent filed an amended page 4 to put the description in accordance with claim 1 of the second auxiliary request. The appellant did not argue against this modified version of the description. The board is satisfied that the amendment is allowable and sufficient to take account of the introduction of the disclaimer in claim 1.

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 7 of the second auxiliary request and page 4 of the description both filed on 5 November 2007, pages 3 and 5 to 13 of the description and the figures as granted.

The Registrar | The Chairman

A. Wolinski | L. Galligani