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D E C I S I O N
of 4 May 2006

Case Number: T 1333/04 - 3.3.08

Application Number: 90908882.5

Publication Number: 0472651

IPC: C12N 9/24

Language of the proceedings: EN

Title of invention:
Endo F-Free PNGase

Patentee:
GENZYME CORPORATION

Opponent:
Roche Diagnostics GmbH

Headword:
PNGase/GENZYME

Relevant legal provisions:
EPC Art. 123(2), 56

Keyword:
"Main request - claim 2 - added subject-matter - (yes)"
"Auxiliary request - inventive step - (yes)"

Decisions cited:
T 0187/91, T 0838/97

Catchword:
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Case Number: T 1333/04 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 4 May 2006

Appellant: (Proprietor of the patent)
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 10 September 2004 revoking European patent No. 0472651 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: F. Davison-Brunel
C. Heath

Summary of Facts and Submissions

I. European patent EP-A-0 472 651 with the title "Endo F-Free PNGase" was granted on the basis of the European patent application No. 90 908 882.5 with five claims which read as follows:

"1. A purified nucleic acid comprising a nucleotide sequence encoding an enzyme having PNGase activity produced by the bacterium *Flavobacterium meningosepticum*, wherein said nucleotide sequence has at least 90% homology with the PNGase F gene present in pGB29, ATCC 67987.

2. The nucleic acid of claim 1, wherein said nucleotide sequence is able to :*[sic]* hybridize under stringent conditions with a 20 base pair sequence of said PNGase F gene.

3. The nucleic acid of claim 1, wherein said nucleotide sequence is able to hybridize under stringent conditions with a 30 base pair sequence of said PNGase F gene.

4. A plasmid carrying the nucleotide sequence defined in any of claims 1 to 3.

5. The plasmid pGB29, present in ATCC 67987."

II. An opposition was filed under Article 100(a) EPC (lack of inventive step) and Article 100(c) EPC (added subject-matter). The opposition division concluded that claim 2 of the main request (granted claims) contained added subject-matter, whereas the auxiliary request

(granted claims 1, 3 to 5) failed to fulfil the requirements of inventive step. The patent was thus revoked.

III. The appellant (patentee) filed an appeal, paid the appeal fee and submitted a statement of grounds of appeal together with the same main request (granted claims) and auxiliary request (granted claims 1, 3 to 5) as considered by the opposition division. The grounds of appeal were accompanied by seven new documents or declarations.

IV. The respondent (opponent) filed a submission in answer to the appellant's grounds of appeal.

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 submissions in answer to this communication. The respondent's submission was accompanied by three documents.

VII. Oral proceedings took place on 4 May 2006 whereby the respondent submitted one further document.

VIII. The following documents are mentioned in this decision:

(1): Tarentino, A.L. and Plummer, Jr. T.H., Methods in Enzymology, Vol.138, pages 770 to 778, 1987;

(2): Langer, B.G. et al., Analytical Biochemistry, Vol.166, pages 212 to 217, (1987);

(3): Tarentino, A.L. et al., Biochemistry, Vol.24, pages 4665 to 4671, 1985.

IX. The appellant's arguments in writing and during oral proceedings may be summarized as follows:

Main request; claim 2
Article 123(2) EPC; added subject-matter

By virtue of its dependency on claim 7 via claim 11, originally filed claim 12 related to a nucleic acid sequence encoding an enzyme having PNGase F activity produced by the bacterium *Flavobacterium meningosepticum* wherein said nucleotide sequence had at least 90% homology with the PNGase F gene present in pGB29, ATCC 67987.

Originally filed claim 19 related to a nucleic acid able to specifically hybridize under stringent conditions with a 20 base pair sequence of the PNGase F gene present in pGB29, ATCC 67987, and it was unambiguous from the information given on page 6, lines 13 to 21 of the application that this nucleic acid encoded a protein with PGNase F activity.

These were, thus, two preferred embodiments of the invention and there was no reason why the skilled person would have considered them as mutually exclusive. To the contrary, he/she would have readily understood that they could be combined. Therefore, there was a basis in the application as filed for the subject-matter of claim 2.

Auxiliary request; claim 1
Article 56 EPC; inventive step

– The closest prior art was document (3) which was concerned with the deglycosylation of glycoproteins. Within this framework, it taught two enzymes Endo F and PNGase F with different cleavage specificities of the sugar moieties from the amino acid backbone. Both enzymes were separated and PNGase F was said to have been obtained in a high state of purity (at least 90%). On page 4670 (Footnote), it was also mentioned that highly purified PNGase F was commercially available.

Document (3) did not suggest that it might be of interest to provide such intact oligosaccharides as exclusively resulting from cleavage of the glycoproteins by PNGase F. Nor did it provide a motivation to produce PNGase F following a different approach since, firstly, the enzyme was thought to be pure - a belief comforted by the teachings of document (1) that by slightly modifying the protocol described in document (3), PNGase F could be obtained "95% pure and free of Endo F" - and, secondly, the enzyme would have been considered as isolatable in adequate quantities as indicated by its commercial availability.

There existed no motivation to use a different approach to the production of PNGase F. Inventive step could be acknowledged on this basis alone.

– If the skilled person had nonetheless decided to embark on the project of producing PNGase F by recombinant means, he/she would have been faced with unexpected difficulties while cloning the PNGase F gene. The cloning required that a partial PNGase F protein sequence be determined and this could only be done with a pure preparation of the enzyme. In contrast, document (3) was highly misleading as to the state of purity of PNGase F produced by the method it described, as was confirmed later on. Thus, there would have been no reasonable expectation of success in identifying the partial PNGase F amino acid sequence ie. in cloning the PNGase F gene.

X. The respondent's submissions in writing and during oral proceedings may be summarized as follows:

Main request; claim 2
Article 123(2) EPC; added subject-matter

A DNA exhibiting at the same time the property of hybridising under stringent conditions with a 20 base pair sequence of the PNGase F gene and that of having a 90% homology to the PNGase F gene present in pGB29 was not disclosed anywhere in the application as filed. The subject-matter of claim 2, thus, extended beyond the content of said application. The claim did not fulfil the requirements of Article 123(2) EPC.

Auxiliary request; claim 1
Article 56 EPC; inventive step

The closest prior art was document (3) which taught how to purify PNGase F from *Flavobacterium meningosepticum* to a 90% state of purity and nearly free of the contaminating enzyme Endo F. The purification process involved growing large quantities of the pathogen and took no less than eight days. Furthermore, document (3) pointed out that Endo F present in a PNGase F preparation introduced heterogeneity in the oligosaccharides cleaved from glycoproteins by PNGase F as it was capable of secondary reactions on said oligosaccharides. For these reasons, the skilled person would have been motivated to find a different approach to producing PNGase F.

The problem to be solved was to devise an alternative approach to the production of PNGase F.

The solution provided by the patent in suit was to clone the DNA encoding PNGase F with the aim of producing the enzyme by recombinant means.

At the priority date, it was a matter of common general knowledge that recombinant DNA techniques were particularly suited for the safe production of enzymes in high yields and state of purity. The above mentioned solution was, thus, obvious to try.

The cloning was achieved by standard methods and neither the patent in suit nor any of the documents on file disclosed that any difficulties were encountered while cloning. The appellant had argued that the

partial sequencing of the PNGase F protein which was a necessary first step in the cloning could not be achieved starting from the PNGase F preparation according to document (3) because this preparation was not pure enough. This argument was not convincing. It was true that the apparent specific activities of the PNGase F preparations according to document (3) and to later, post-published work differed by a factor of about five. Yet, this finding reflected differences in the conditions used for assessing specific activity rather than the presence of more protein contaminants in the preparation according to document (3). It was, thus, irrelevant when assessing the level of purity of the protein.

In conclusion, the skilled person had a motivation to find a different approach to the production of PGNase F. The recombinant way would immediately come to mind. The cloning could be achieved in a standard manner starting from existing preparations of PNGase F. For these reasons, there was no inventive step in the claimed subject-matter.

XI. The appellant requested that the decision under appeal be set aside and that the patent be maintained as granted (main request) or, in the alternative, on the basis of the auxiliary request comprising claims 1 to 4 as filed with the statement of grounds of appeal.

The respondent requested that the appeal be dismissed.

Reasons for the decision

Main request; claim 2
Article 123(2) EPC; added subject-matter

1. Originally filed claim 12 relates to a nucleic acid sequence encoding an enzyme having PNGase F activity produced by the bacterium *Flavobacterium meningosepticum* wherein said nucleotide sequence has at least 90% homology with the PNGase F gene present in pGB29, ATCC 67987. Originally filed claim 19 relates to a nucleic acid encoding a protein with PGNase F activity which is able to specifically hybridize under stringent conditions with a 20 base pair sequence of the PNGase F gene present in pGB29, ATCC 67987. However, there is no disclosure of a purified nucleic acid with these two characteristics anywhere in the application as filed.

2. This last point was not challenged by the appellant who simply argued that they were no reason why the skilled person would consider the two characteristics as mutually exclusive. This may well be so but is not relevant when determining whether or not the claimed subject-matter extends beyond the content of the application as filed. In accordance with the case law (eg, T 187/91, OJ EPO 1994, 172), the decisive question is rather whether or not the claimed subject-matter can be directly and unambiguously deduced from said application. As already stated, the application is wholly silent with regard to the existence of a DNA with the two specific characteristics. Nor can such a DNA be implicitly derived from the original disclosure.

The subject-matter of claim 2 extends beyond the content of the application as filed.

3. The main request is rejected for failing to fulfil the requirements of Article 123(2) EPC.

Auxiliary request; claim 1
Article 56 EPC; inventive step

4. The closest prior art is document (3) which is concerned with the degradation of aspargine-linked glycans and teaches that two enzymes will perform the cleavage of oligosaccharides from glycopeptides: Peptide:*N*-glycosidase F (PNGase F) and Endo F. Both enzymes are purified from the culture medium of *Flavobacterium meningosepticum* (page 4667, right-hand column) and separated from each other on the basis of their different hydrophobicity (page 4668, left-hand column). They are shown to have different cleavage specificity, PNGase F releasing full length oligosaccharides from the glycopeptides whereas one glyco-moiety remains on the peptide by cleavage with Endo F (page 4668, right-hand column). On page 4670, it is also mentioned in a footnote that highly purified PNGase F is commercially available.

5. A comparison of this teaching with the subject-matter of the patent in suit reveals that the latter provides a different approach to producing PNGase F, namely by recombinant techniques involving the isolation of PNGase F encoding DNA as the first step.

6. The first question which arises is whether or not document (3) provides any incentive/motivation to

change the hitherto available purification method for PNGase F, otherwise stated whether or not it was obvious to formulate the problem of providing PNGase DNA, starting from the teachings of the closest prior art.

7. It is readily apparent from reading document (3) that it provides no suggestion to this effect. Furthermore, the purification method is disclosed in the following terms on page 4670, left-hand column:

"*Flavobacterium meningosepticum is a relatively easy organism to cultivate, and large amounts of highly purified protease-free PNGase (0.1% Endo F) can be obtained from only 1 or 2 L of cultural filtrate in less than eight days, by simple column chromatography on TSK HW-55(S). The purified enzyme is stable at -70°C and can be kept at 4°C for months without loss of activity.*"

Added to the fact that, as already mentioned, PNGase F was commercially available at the time, these observations do not provide a technical or commercial incentive to produce the enzyme in a different way.

8. Now looking at the prospects of using the enzyme for further scientific developments, one may wonder whether the sentence on page 4666, left hand column:

"In this report, we demonstrate a very simple and reproducible procedure for obtaining PNGase F in good yield (51%) and a degree of purity (>90%), ***nearly free of EndoF."*** (emphasis added by the board)

would not raise some concerns that the enzyme directly purified from *Flavobacterium meningosepticum* may be unsuitable. Yet, in the last paragraph on page 4670, the authors also identify their goal for the immediate future as follows:

"Studies are now in progress to test the ability of PNGase F to release oligosaccharides from the surface of cultured cells."

They, thus, tell the skilled person that the small amount of contamination by Endo F will not cloud the interpretation of any results obtained with the PNGase F enzyme preparation.

9. There are two other prior art documents published in 1987 - after document (3) - which report work done on/with PNGase F. Document (1) discloses the purification of the enzyme to over 95% and free of Endo F (page 775) by a method which is only slightly modified compared to that described in document (3), pages 772 to 775. There again no suggestion is made that it might be advantageous to go the recombinant way for producing PNGase F. Of course, as two of the authors of documents (1) and (3) are the same, it may be reasonable that the same "basic" purification method is used. However, it also means that two years after the publication date of document (3), this method was still considered worth pursuing.

10. In document (2) which originates from a different group of researchers who apparently did not regard the purification as carried out in document (3) as sufficient for their own purpose (deglycosylation of

fibrinogen by PNGase F), the purification of PNGase F is nonetheless essentially carried by this earlier method, further steps (HPLC) being added (pages 214 and 215). The necessity for a radically different purification approach such as the recombinant one is not envisaged.

11. It is, thus concluded that already the formulation of the problem of taking a different approach to the purification of PNGase F was not obvious starting from the closest prior art or even from the prior art as a whole.

12. At oral proceedings, the respondent stated that it was a matter of common general knowledge at the priority date that recombinant DNA techniques were the best means to produce a protein safely, in high yields and high state of purity. The board would agree to this statement but not to the implications which the respondent wants to draw therefrom, namely, that it would be an obvious goal to produce PNGase F in the recombinant way, even in the absence of any suggestion thereof in the art. Indeed, it must be kept in mind that whereas in accordance with the case law, the skilled person is forever occupied with furthering the state of knowledge, he/she is also to be considered as realist and pragmatic (T 838/97 of 14 November 2000). Thus, in the board's judgment, he/she would not spend the time and expenses needed to shift the production method of an enzyme in a completely new direction unless they were good reasons to do so, especially in consideration of the fact that it was already commercially available in highly purified form. There

is no evidence that such reasons (whether scientific or commercial) existed.

13. These findings (points 4 to 11, supra) lead to the conclusion that an inventive step may be acknowledged already on the basis of having formulated the problem which finds its solution in the cloning of the DNA from *Flavobacterium meningosepticum* encoding PNGase F.

14. During the written phase of the proceedings and also at oral proceedings, many arguments were produced in favour/against an inventive step being linked to the cloning procedure per se. However, in view of the above mentioned findings, these need not be addressed here.

15. Finally, the respondent raised the argument in its submissions dated 4 April 2006 that the description did not provide a sufficient disclosure in relation to the cloning of PNGase F DNA - more specifically, in relation to how to obtain the protein in sufficiently pure form to be able to determine a partial sequence thereof, which partial sequence was indispensable to probe for the PNGase F DNA. Two observations must be made in this respect. Firstly, Article 100(b) EPC was not cited as a ground of opposition and, therefore, arguments pursuant to sufficiency of disclosure may not be considered. Secondly, what must be reproducible is the PNGase F DNA and this is readily available from the duly deposited plasmid pGB29, ATCC 67987.

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 the auxiliary request comprising claims 1 to 4 as filed with the statement of grounds of appeal and the description as granted.

The Registrar: The Chairman:

A. Wolinski L. Galligani