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D E C I S I O N
of 15 September 2005

Case Number: T 0627/04 - 3.3.8

Application Number: 99947259.0

Publication Number: 1124946

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Language of the proceedings: EN

Title of invention:
Glycosylated proteins having reduced allergenicity

Applicant:
Novozymes A/S

Opponent:
-

Headword:
Glycosylated proteins/NOVOZYMES

Relevant legal provisions:
EPC Art. 83, 84

Keyword:
"Support in the description (yes)"
"Sufficiency of the disclosure (yes)"

Decisions cited:
T 0694/92

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0627/04 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 15 September 2005

Appellant: NOVOZYMES A/S
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 22 December 2003 refusing European application No. 99947259.0 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: T. J. H. Mennessier
C. Rennie-Smith

Summary of Facts and Submissions

I. The applicant (appellant) lodged an appeal against the decision of the examining division of 22 December 2003 refusing the European patent application No. 99 947 259.0 with publication number 1 124 946. The application entitled "Glycosylated proteins having reduced allergenicity" originated from an International patent application published as WO 00/26354, to be referred to in the present decision as "the application as filed".

II. Reasons for the refusal were lack of support (Article 84 EPC) and insufficiency of disclosure (Article 83 EPC). Novelty and inventive step had not been discussed in the decision under appeal. The basis for the refusal was the set of twenty seven claims (numbered 1 and 3 to 28) as filed at the oral proceedings held before the examining division on 27 November 2003.

III. The appellant filed a statement of grounds of appeal which was accompanied by a new main request and five auxiliary requests.

IV. The examining division did not rectify its decision and referred the appeal to the Board of Appeal (Article 109 EPC).

V. A communication under Article 11(1) of the Rules of Procedure of the Boards of Appeal presenting some preliminary and non-binding views of the Board was sent to the appellant.

VI. In reply to the Board's communication, on 19 July 2005, the appellant submitted a new main request and three auxiliary requests to replace the corresponding requests on file, together with observations. The former forth and fifth auxiliary requests were withdrawn. The request previously made for reimbursement of the appeal fee was withdrawn.

VII. On 9 September 2005, the oral proceedings scheduled to take place on 13 September 2005 were cancelled by the Board. On 13 September 2005, the outstanding issues in relation to the reasons which had led to the refusal of the application were discussed during a telephone consultation with the appellant.

VIII. On 14 September 2005, the appellant sent a new main request to replace the main request on file.

Claim 1, which was an amended version of claim 1 as refused by the examining division, read as follows:

"1. A method of producing an N-glycosylated protein variant having a reduced allergenicity measured as a reduced IgE antibody production in animals, including man, as compared to parent protein, comprising constructing DNA molecules encoding protein variants, said DNA molecules having at least one sub-sequence encoding an additional N-glycosylation site compared to the parent protein,
selecting a DNA molecule encoding a glycosylated protein variant, having an allergenicity reduced by at least 50% compared to the parent protein, the allergenicity being measured as a reduced IgE antibody

production **in rats** exposed intratracheally to the variant,
introducing the DNA molecule encoding the variant into a suitable host capable of glycosylation,
culturing said host in a suitable medium, whereby said protein variant is expressed and glycosylated in the host,
recovering the glycosylated protein variant from the medium."

(emphasis added by the Board to show the difference in comparison with claim 1 of the request refused by the examining division, the phrase "in animals" having been replaced by the phrase "in rats")

Claim 2 which was an amended version of claim 3 of the request refused by the examining division read as follows:

"The method according to Claim 1, wherein the allergenicity, **as expressed by the IgE antibody response in rats**, of the glycosylated protein variant is lower than 1% of the parent protein."

(emphasis added by the Board to show the difference in comparison with claim 3 of the request refused by the examining division, the phrase "as expressed by the IgE antibody response in rats" having been added)

Claims 3 to 10 corresponded to claims 4 to 7 and 9 to 12 of the request refused by the examining division. They were dependent on claim 1 and were directed to particular embodiments of the claimed method.

IX. The following document is referred to in the present decision:

(D4) US-A-5,585,250 (published on 17 December 1996)

X. The submissions made by the appellant, insofar as they are relevant to the present decision, may be summarised as follows:

The application contained examples of two variants, one containing a single glycosylation site and the other having multiple glycosylation sites. The variant having multiple glycosylation sites achieved the claimed reduction in allergenicity and so constituted an example of the claimed invention, whereas the variant containing a single glycosylation site was not an example of the invention. The person skilled in the art was well aware that if one glycosylation site did not achieve the desired reduction in allergenicity, then additional glycosylation might be necessary, because this was explicitly disclosed in the present application. Moreover, the skilled person could predict from the disclosure in the application that multiple additional glycosylation sites were likely to have a greater effect in reducing allergenicity than a single glycosylation site. Therefore, the fact that one of the variants in the application, having a lower degree of glycosylation of the protein, did not attain the desired degree of reduced allergenicity did not mean that the invention could not be performed by the person skilled in the art. On the contrary, this was part of the technical teaching of how to achieve the desired degree of reduced allergenicity.

Identification of a particular protein was not an issue, because this was simply the protein for which a reduction in allergenicity was required.

Suitable hosts could be identified by the person skilled in the art on the basis of the disclosure in the application together with background general knowledge. He/she clearly knew that, since glycosylation was required, a host cell capable of glycosylation had to be employed.

The glycosylation site or multiple sites could be identified as set out in the application. Thus, epitope patterns were first identified and were localised on the 3-D structure of the protein. The production of variants of the protein having one or more additional glycosylation sites close to these epitopes could then be carried out by standard protein engineering. Screening could then be carried out to determine whether the variants had the required degree of reduction in allergenicity, using techniques involving only routine trial and error investigations.

XI. The appellant requests that the decision under appeal be set aside and the application be remitted to the examining division for consideration of novelty and inventive step on the basis of the main request filed on 14 September 2005 or of auxiliary requests 1 to 3 filed on 19 July 2005.

Reasons for the Decision

Main request

1. Claim 1 differs from claim 1 as refused by the examining division in that the DNA selecting step has been clarified by specifying that the allergenicity of the encoded variant is measured as a reduced IgE antibody production in rats exposed intratracheally to the variant. This is in line with the application as filed which reports in the experimental part of the description (see page 56, lines 15 to 17) that the antigenic and allergenic potencies of the variants and the parent protein referred to therein were compared in a rat model with intratracheal exposure to the antigen. Thus, the skilled person reading the application understands immediately that the "animals intratracheally exposed to the antigen" referred to in the description are the rats of the test.

2. A similar amendment with the same support in the description is contained in claim 2.

3. Therefore, the amendments contained in claims 1 and 2 have a basis in the description of the application as filed and the requirements of Article 123(2) EPC are met.

4. These amendments define unambiguously the test to be performed in the DNA selecting step of the claimed method and, thus, are in compliance with the clarity requirement of Article 84 EPC.

5. In the decision under appeal it was considered that the scope of claim 1 then on file did not correspond to the actual extent of the appellant's contribution to the state of the art. Thus, an objection to lack of support in the description was raised under Articles 83 and 84 EPC. It was found that the skilled person was faced with the task of testing for the claimed effect each and every protein with all possible potential sites where a N-glycosylation site could be introduced, not knowing moreover which host to use. This amounted to "undue burden" for the skilled person especially in view of the fact that a unique protein variant had been exemplified.

6. The present application is concerned with the use of protein engineering techniques in the field of immunology. It relates to the reduction of allergenicity of a protein by N-glycosylation.

7. Claim 1 at issue is generally directed to a method of producing an N-glycosylated protein variant having a reduced allergenicity compared to a parent protein. The claim is not limited as to the choice of the protein from which variants are prepared. The method comprises five steps. In a first step, DNA molecules encoding protein variants are constructed, which have at least one sub-sequence encoding an additional N-glycosylation site in the protein variant compared to the parent protein, no specific details being given in the claim as to the location(s) at which in the sequence of the protein the glycosylation should take place. In a second step, one of the constructed DNA molecules is selected which encodes a glycosylated protein variant having an allergenicity reduced by at least 50%

compared to the parent protein, the allergenicity being measured as a reduced IgE antibody production in rats exposed intratracheally to the variant. In a third step, the selected DNA molecule is introduced into a host capable of glycosylation. Hosts having this capability are not specified. In a fourth step, the transformed host is cultured in a medium in such a way that the encoded protein variant is expressed and glycosylated into the host. In a fifth and last step, the glycosylated protein variant is recovered from the medium.

8. The use of site-directed mutagenesis of a DNA sequence to introduce recognition sites for N-glycosylation into the corresponding encoded protein sequence has been disclosed in the state of the art (see for example document D4 which describes methods for constructing nucleic acid sequences that encode HIV-1 gp120/160 proteins bearing additional N-linked glycosylation consensus sites). It is also unquestionable that the third, fourth and fifth steps of the claimed method involve no more than routine techniques and materials, such as hosts capable of protein glycosylation, which were well known to the skilled person at the priority date.

9. The description also provides general guidance as to the performance of the claimed method. The sites at which N-glycosylation may occur in a protein are discussed in detail on page 7 (see lines 18 to 26). In order that the reduction in allergenicity exhibited by the protein variants be maximized, as being caused by both macrophage scavenging (as explained on page 6, lines 12 to 18 and page 7, lines 1 to 9) and epitope

shielding (as explained on page 6, lines 20 to 33), the skilled reader is taught on pages 8 and 9 to choose the location of the N-glycosylation sites in the vicinity of epitopes on the protein surface, while the identification of appropriate epitopes and the finding of suitable substitutions to introduce N-glycosylation sites in the protein sequence are discussed in detail on pages 9 to 11. General instructions are also given on pages 42 and 43 in respect of the choice of an appropriate host. Numerous hosts capable of protein glycosylation, including a number of fungi and animal cell lines, are referred to.

10. Examples 1 to 6 (see pages 52 to 60) report in detail the successful preparation and testing of an N-glycosylated variant of lipolase, a lipase derived from Thermomyces lanuginosus, having an allergenicity reduced by 58% compared to the parent protein (see Table 2 on page 58 in the description), the allergenicity being measured as a reduced IgE antibody production **in rats** exposed **intratracheally**. This variant, named variant #5, comprises four additional glycosylation sites compared to the parent protein, the glycosylation affecting three epitopes (one glycosylation site being not located within an epitope). It was produced by performing a five step method in accordance with the method of claim 1. Lipolase variants were designed to introduce additional glycosylation sites of the consensus sequence Asn-Xaa-Thr/Ser in such a way that the effects of both **macrophage scavenging** (see page 52, lines 30 and 31 in the description) and **epitope shielding** be maximized. The difference in allergenicity exhibited by the variant #5 when tested in the rat-model with

intratracheal exposure and in the mouse-model with sub-cutaneous exposure (see Examples 4 and 5, on pages 56 to 60 in the description) is regarded (see page 7, lines 1 to 9 in the description) as an indication that resident macrophages, e.g. alveolar macrophages of the lung, contributed to the reduction in allergenicity.

11. The skilled person having read the description can only conclude that, as stated on page 11, lines 8 to 10, **the scope of the invention is by no means limited to the preparation of protein variants of only lipolase but, on the contrary, encompasses the preparation of protein variants whatever the parental protein**.

12. Whereas the method of claim 1 is directed to the production of variants having a reduced allergenicity as the result of the presence of at least one additional N-glycosylation site, in the application only one such a variant is described, namely variant #5, which contains four additional N-glycosylation sites compared to the parent protein. Moreover, comparative variant #1, the only particular variant having one additional glycosylation site, induces a reduction in allergenicity of 29% (see Table 2 on page 58 in the description) and, therefore, does meet the criterion set out in claim 1 (a reduction of at least 50% is required). Nevertheless, in the absence of any serious doubts substantiated by verifiable facts, **there is no reason not to believe that other protein variants with only one or more N-glycosylation sites and having the expected reduced allergenicity might be prepared according to the method of claim 1**.

13. In view of these remarks, it is the Board's judgment that the skilled person would be able to perform the claimed method over the whole area of claim 1 without undue burden. This can be readily achieved by applying the general instructions contained in the description while taking into account the detailed way of carrying out the invention described in the experimental part of the description.

14. Indeed, the broad wording of claim 1 reflects **the actual contribution to the state of the art by the disclosure** in the application (see decision T 694/92, OJ EPO 1997, 408), which essentially consists in using a rat-model as a selecting means for the production by protein engineering techniques of N-glycosylated variants having a reduced allergenicity compared to the parent protein.

15. Thus, the method of claim 1 is supported by the description (see Article 84 EPC) and has been sufficiently disclosed (see Article 83 EPC).

16. The examining division has not raised any other objections as regards compliance with the requirements of Articles 83 and 84 EPC against the claim request then on file. Nor does the Board have any further objections against the claims at issue. Therefore, the main request as a whole meets the requirements of Articles 83 and 84 EPC.

Conclusion

17. The main request may form a basis for further prosecution, namely consideration of novelty and inventive step by the first instance, as requested by the appellant.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance for further prosecution on the basis of the main request filed on 14 September 2005.

The Registrar: The Chairman:

G. Nachtigall L. Galligani