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Bezeichnung der Erfindung: Reagent for assaying cholinsterase
Title of invention:
Titre de l'invention :

Klassifikation / Classification / Classement : C12Q 1/46

ENTSCHEIDUNG / DECISION

vom / of / du 17. March 1989

Anmelder / Applicant / Demandeur : UNITIKA LTD.

Patentinhaber / Proprietor of the patent /
Titulaire du brevet :

Einsprechender / Opponent / Opposant :

Stichwort / Headword / Référence : Assaying reagent

EPÜ / EPC / CBE Article 56

Schlagwort / Keyword / Mot clé : "Inventive step (yes) - Improvement - non obvious use of a recently developed enzyme in an assaying reagent"

Leitsatz / Headnote / Sommaire

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Case Number : T 181 /88 - 3.3.2

D E C I S I O N
of the Technical Board of Appeal 3.3.2
of 17. March 1989

Appellant : UNITIKA LTD.
No. 50, Higashihonmachi 1-chome
Amagasakik-shi Hyogo/JP
Japan

Representative : Pearce, Anthony Richmond
MARKS & CLERK
Alpha Tower
Suffolk Street
Queensway
Birmingham B1 1TT
GB

Decision under appeal : Decision of Examining Division 023 of the European Patent Office dated 15 December 1987 refusing European patent application No. 83 301 381.6 pursuant to Article 97(1) EPC

Composition of the Board :

Chairman : P. Lançon
Members : U. Kinkeldey
R. Schulte

Summary of Facts and Submissions

I. European patent application 83 301 381.6, filed on 14 March 1983, and published on 21 September 1983 with publication number 89 210, was refused by the decision of the Examining Division of 15 December 1987. The decision was based on Claims 1-10 as originally filed. Claim 1 reads as follows:

"1. A cholinesterase-assaying reagent comprising:
acetylcholine;
a thermostable acetate kinase; and
adenosine triphosphate".

Claims 2-10 are dependent sub-claims, relating to preferred embodiments of the above cited main claim.

II. The ground for refusal was that the subject-matter of Claims 1-10 did not involve an inventive step within the meaning of Article 56 EPC and hence the said claims were not regarded as relating to a patentable invention in accordance with Article 52(1) EPC.

In its decision the Examining Division stated that a cholinesterase reagent which differed from that of Claims 1-4 and 10 only in that the acetate kinase used therein was a non-thermostable enzyme isolated from E. coli, was described in the prior art document JAP. J. Clin. Chem. Vol. 4, No. 2, 1975, page 186 (Document II).

The European patent application EP-A-29 976 (Document I) had disclosed and made available a thermostable acetate kinase from Bacillus stearothermophilus and had drawn

attention to the fact that the acetate kinase isolated from E. coli was very unstable and not suitable for use on an industrial scale. Further, French patent application FR-A-2 457 321 (Document III) had pointed to the high residual activity of the thermostable acetate kinase isolated from Bacillus stearothermophilus.

A skilled man dealing with a cholinesterase-assay according to Document II would proceed without any inventive effort in substituting an acetate kinase by a more stable and advantageous one, as for instance described in Documents I or III. After all, the subject-matter of Claims 1-10 solely consisted of a substitution of a known reagent by a recently found enzyme, whose properties made it suitable for use in said reagent ("analogous substitution"; Guidelines, Part C, Ch. VI, item 9.8, A1, IV).

III. Notice of appeal against this decision was filed on 4 February 1988; the appeal fee was paid on 16 February 1988 and the statement of grounds was filed on 18 April 1988.

IV. In the notice of appeal the Appellant requested complete cancellation of the decision. The arguments put forward by the Appellant can be summarised as follows:

1. There was no teaching or suggestion in Document I that the thermostable acetate kinase disclosed therein was suitable for any enzyme assaying technique, still less for cholinesterase-assaying. The alleged instability of acetate kinase isolated from E. coli as used for instance in the assay of that document was clearly mentioned in relation to suitability for use on an industrial scale. There would thus be no motivation for a person skilled in

the art to utilise a thermostable acetate kinase in a cholinesterase-assaying reagent since such use could hardly be considered to be on an industrial scale.

2. In an assay system like the present one, comprising a number of different components, each single component might be replaced by a more convenient one. There was clearly no indication in any of the prior art documents mentioned by the Examining Division, as to the substitution of which component would lead to an improved assay system. Rather investigation of the assay system described in Document II would have revealed that the activity of the acetate kinase after assay was unaffected. This would have led the skilled person away from the thought that the inconsistent results achieved by the assay system of Document II could be improved by using a more stable type of acetate kinase. Instead, the skilled person would have been led into thinking there were other factors causing the problem or that the assay of Document II was basically unreliable so that other completely different methods of assay should be tried.

3. After all, the Examining Division had reached its decision on the basis of foreknowledge of the present invention. Such a foreknowledge would not have been available to a person skilled in the art at the relevant date.

V. The Appellant requested that the decision under appeal be set aside and that the patent be granted on the basis of Claim 1 as amended by letter of 30 September 1988 and Claims 2-10 as originally filed.

Claim 1 reads as follows:

"A reagent useful in a cholinesterase-assay, said reagent comprising:

acetylcholine;
a thermostable acetate kinase; and
adenosine triphosphate."

Reasons for the Decision

1. The appeal complies with Articles 106 to 108 and Rule 64 EPC and is admissible.

2. There are no objections according to Article 123(2) EPC to the amendments of the main claim now on file. On page 5 of the description of the present application as published the reactions for assaying cholinesterase are shown. According to these, the three components acetylcholine, acetate kinase and adenosine triphosphate, as mentioned in Claim 1, are involved in an enzymatic and thus specific reaction which finally produces adenosine diphosphate. In subsequent reactions the amount of this adenosine diphosphate can be determined and thus the assay of cholinesterase is completed. The amendment of the originally filed Claim 1, required with regard to Article 84 EPC is thus allowable under Article 123(2) EPC.

3. The application relates to a reagent comprising acetylcholine, a thermostable acetate kinase and adenosine triphosphate, which is useful to assay cholinesterase.

4. As already recognised correctly by the Examining Division, the closest state of the art is Document II which relates to a cholinesterase-assaying reagent comprising

acetylcholine, an acetate kinase and adenosine triphosphate and further compounds which finally allow the measurement of cholinesterase activity in a sample.

The principle of both assays is that a cholinesterase present in a sample hydrolyses acetate from the acetylcholine, present in the assaying reagent. The liberated acetate then reacts with adenosine triphosphate and acetate kinase, which is the third compound present in the said reagent. As a result of that reaction adenosine diphosphate is produced which subsequently is converted to adenosine triphosphate and pyruvate by reaction with phosphoenolpyruvate and pyruvate kinase. The resulting pyruvate is then coloured with 2,4-dinitro-phenyl hydrazine. Thus, one mole of the acetate, liberated by the enzymatical action of one mole cholinesterase, is stoichiometrically converted into one mole of hydrazine of pyruvate.

5. In the description of the present application it is stated on page 3, lines 2-9 that the cholinesterase assay, of the state of the art as described in Document II has disadvantages as the usable concentration range of the acetate kinase is extremely narrow and therefore the values obtained fluctuate, making reproducibility very poor. The accuracy of the process was not good, making the process impractical.

6. Starting from Document II, the ojective technical problem underlying the present application can be seen in providing an improved assay for determining cholinesterase in a sample.

7. The solution proposed lies in a reagent as claimed in Claim 1 whereby instead of the acetate kinase of the known assay reagent a thermostable acetate kinase is selected.

The examples of the description and the experimental data filed by the Appellant with letter of 29 September 1986 show that the problem was indeed solved by this proposal. Whereas the usable concentration range of an acetate kinase according to the cholinesterase-assaying reagent of Document II is narrow and therefore the values obtained from this method fluctuate greatly making reproducibility poor (Figure 1 of the mentioned experimental data), it can be seen from Figure 2 of these experimental data that the use of a heat-stable acetate kinase allows more reproducible determination of cholinesterase. Thus, as already stated in the description, the particular improvement of the assaying-reagent is based on the fact that an extremely wide concentration range can be used so that it is possible to assay cholinesterase with good reproducibility and accuracy, giving a definite value in the wide concentration range of 3 to 10 U/ml. In comparison with this, the cholinesterase activity when using a non-thermostable acetate kinase yielded by E. coli sharply rises to and falls from a peak at 5 U/ml of the enzyme (comparative Example 1, page 11).

8. After examination of all cited documents, the Board has concluded that none of them discloses a cholinesterase assaying reagent as claimed in the present application and thus the subject-matter of Claim 1 is novel.

9. It has now to be examined whether the main claim meets the requirements of Article 56 EPC, i.e. whether, having regard to the state of the art, it was obvious to a person skilled in the art to select a thermostable acetate kinase in order to improve the known assay reagent.

10. In Document II, which was published in 1975, there is no indication whatsoever about the character of the mentioned acetate kinase. On page 186, right column, under "Materials and Methods" it is stated that the acetate

kinase by this firm was purchased from Boehringer Mannheim. At that time the acetate kinase was produced by this firm from the bacterium Escherichia coli, as has already been stated in the specification of the present application (page 3, lines 2-5). In analysing the teaching of Document II with the aim of examining inventive skill for the use of a thermostable acetate kinase in the present application, it is of great importance whether or not there was any indication in Document II or any other cited document as to which action had to be taken to improve the known assaying reagent, whether by substitution of any of the components, by changing of other parameters of the whole reaction or even by replacing the whole reaction as such by something else.

11. In fact in Document II, for optimising the assay described, a few parameters have been tested, namely the concentration of the substrate on cholinesterase activity in serum, the concentration of acetate kinase, the concentration of ATP, $MgCl_2$ and PEP and the effect of the enzyme concentration on cholinesterase activity (Figures 2-5).

It is further mentioned on page 189, left column in the paragraph dealing with "Enzyme Concentration" that the assaying reagent described works satisfactorily up to a cholinesterase activity of 2.5 units. Activities above 2.5 units were said to occur only very rarely. Remarkably in such cases it is proposed to dilute the serum containing the high cholinesterase activity.

Thus, Document II does not provide assistance to solve the problem described in the present application.

12. It is the Board's view that the skilled person, confronted with this problem, would have thought about replacing any one of the components involved in the whole assaying reaction or, even more likely than more or less randomly replacing one of the components, would have thought about changing the method for assaying cholinesterase activity.

13. Even if the skilled person were to try to improve the method by substituting the enzyme used in the assay, namely acetate kinase, however, there is again no indication at all in Document II that especially a thermostable acetate kinase would provide a remarkable improvement of the assay. Indeed, no single step of the complex reaction is conducted under heat stress. As can be seen from Document II and the present application as well, the reaction temperatures are 37°C or room temperature (page 187, left column of Document II; page 9, second paragraph of the present application). In the Board's view it was a surprising and unexpected effect that just selecting from the known acetate kinases a thermostable acetate kinase would lead to a significant improvement as shown by the experimental data filed during the Examination Procedure with letter of 29 September 1986.

14. The Examining Division stated that the new availability of a thermostable acetate kinase and the advantage of stability shown in Document I was a clear hint for the skilled person to substitute the known one by the new one.

In Document I the isolation and use of a thermostable acetate kinase is described and compared in its stability features with acetate kinase obtained as a purified enzyme from Escherichia coli, which is said to be very unstable and not suitable for use on an industrial scale. It has to

be examined whether it was obvious to use the knowledge of Document I to improve an assay such as the present one, used in laboratory scale. The disclosure of Document I has to be understood in connection with further information given in Document I about other labile enzymes, for example polynucleotide phosphorylase (page 2, third paragraph) which is said to be so labile that it is even unsuitable for use in laboratory scale research. Further, the enzyme pyruvate kinase is described (page 4, second paragraph) which is also said to be labile and thus not suitable for industrial use. This enzyme is also comprised in the final and complete assay for cholinesterase as described in the present application and in Document II and could equally have been substituted to improve the assay.

15. The Board cannot concur with the Examining Division's view that the invention expressed in Claim 1 "solely consists of a substitution in a known reagent of a recently found enzyme whose properties make it plainly suitable for use in said reagent" ("analogous substitution"). At the time of filing the present application there was no indication whatsoever in the state of the art as to what might have been the reason for the problems arising with the cholinesterase assay described in Document II and the proposed solution to use a thermostable substance was not derivable from any other circumstances, like temperature stress etc. In the example given in the Guidelines, the "analogous substitution" was carried out knowing the reason for the problem and knowing that the improvement was based on analogous features of the substituted compounds. The present invention is different from that situation.

16. It is therefore the opinion of the Board that in the absence of any indication in the prior art Documents I and II of which compound in a multicompound composition or which reaction in a multistep assaying system possibly could be substituted or changed to improve the whole method, inventive skill is required to change that feature of the whole system which solves the problem, especially when the working conditions for the assay which has to be improved are such that the features of the finally selected compound were not to be expected to solve the problem.

17. The further document cited in the search report, namely FR-A-2 457 321 (Document III) relates to an improved method for the isolation of acetate kinase from Bacillus stearothermophilus. The acetate kinase described in this document still shows, after a heat-treatment test, an activity of 90-100% compared to the initial activity, without however, there being any indication of how to use this heat stable acetate kinase. The disclosure of this document thus does not contain any information going beyond that of Document I discussed above.

Thus, the reagent of Claim 1 involves an inventive step.

18. Claims 2-10 are directly or indirectly dependent on Claim 1, and refer to preferred embodiments of Claim 1. There are no objections to these claims.

Claims 1-10 on file are therefore patentable.

Order

For these reasons, it is decided that:

(1) The decision of the Examining Division is set aside;

(2) the case is remitted to the First Instance with the order that a patent be granted on the basis of Claim 1 as originally filed with amendments as requested in the Appellant's letter of 30 September 1988, Claims 2-10 as originally filed and the description as originally filed with amendments requested by the Appellant with letters of 25 January 1985 and 30 September 1988.

The Registrar: The Chairman:

Ulm, 16.3.89
Schmid 14.3.89

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