BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS

BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE

CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

A		B		C	X

File Number: T 114/91 - 3.4.2

Application No.: 85 114 835.3

Publication No.: 0 184 701

Title of invention: A method for determining a ligand

Classification: G01N 33/543, G01N 33/532, G01N 33/76

D E C I S I O N
of 17 December 1992

Proprietor of the patent: HOECHST CELANESE CORPORATION

Headword:

EPC Articles 54(1), 56

Keyword: "Novelty, inventive step - yes"

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number : T 114/91 - 3.4.2

D E C I S I O N
of the Technical Board of Appeal 3.4.2
of 17 December 1992

Appellant : HOECHST CELANESE CORPORATION
Route 202-206 North
Somerville NJ 08876 (US)

Representative : Hoechst Aktiengesellschaft
Zentrale Patentabteilung
Postfach 80 03 20
W - 6230 Frankfurt am Main 80 (DE)

Decision under appeal : **Decision of the Examining Division 061 of the European Patent Office dated 17 September 1990 refusing European patent application No. 85 114 835.3 pursuant to Article 97(1) EPC.**

Composition of the Board :

Chairman : E. Turrini
Members : C. Black
L. Mancini

Summary of Facts and Submissions

I. European patent application No. 85 114 835.3 (publication No. 0 184 701) was refused by the Examining Division on the grounds that the subject-matter of independent Claims 1 and 16 then under consideration was not novel having regard to the disclosure in

D1: EP-A-0 124 366

and that to the extent that the claims could be said to contain novel subject-matter, this did not involve an inventive step having regard to a combination of the teachings of D1 and D2 (US-A-4 228 237). A further independent Claim 9 was not considered in detail in the decision, the Examining Division merely noting that it essentially reiterated the method steps of Claim 1.

II. The present appeal lies from this decision. The Appellant (Applicant) with the Grounds for Appeal submitted an amended set of claims, of which independent Claims 1, 8 and 14, correspond to the above-mentioned originally filed Claims 1, 9 and 16 respectively.

These claims contained some obvious errors and omissions and, in response to a telephone call from the Board, an amended set of claims was filed (letter dated 27 November 1992, received 2 December 1992).

Of these, independent Claims 1, 8 and 14 read as follows:

"1. A method of determining a ligand in a medium suspected of containing same, which comprises:

(a) providing an insoluble phase containing a specific binding substance for the ligand;

(b) incubating said insoluble phase with the following reagents,

(i) the medium suspected of containing the ligand,

(ii) a soluble reagent selected from the group consisting of

(a') a specific binding substance for the ligand which is covalently linked with avidin reagent;

(b') said reagent (a') complexed with biotin labeled enzyme;

(c') ligand which is linked to avidin; or

(d') reagent (c') complexed with biotin labeled enzyme; and

(iii) soluble biotin labeled enzyme when said reagent is (a') or (c');

(c) separating unreacted reagents from said insoluble phase after incubation; and

(d) determining the enzyme activity of either said separated unreacted reagents or said separated insoluble phase to determined the ligand.

8. A method for determining a ligand in a medium suspected of containing the same, which comprises:

(a) providing an insoluble phase containing a specific binding substance for said ligand;

(b) incubating said insoluble phase with the following reagents;

(i) (A) liquid medium suspected of containing said ligand and (B) a known quantity of a soluble reagent selected from the group consisting of

(a') a specific binding substance for the ligand which is covalently linked with an avidin reagent;

(b') said reagent (a') complexed with biotin labeled enzyme;

(c') ligand which is linked to avidin; or

(d') reagent (c') complexed with biotin labeled enzyme; and

(ii) soluble biotin labeled enzyme when said reagent is (a') or (c');

(c) separating unreacted reagents from said insoluble phase after incubation, and

(d) determining the enzyme activity of either said insoluble phase or separated unreacted reagent whereby said activity is related to the amount of ligand in said liquid medium.

14. A reagent for determining a ligand which comprises

(a) a specific binding substance for the ligand

(b) an avidin labeling group covalently bonded to said specific binding substance by means of a coupling reagent reacted therewith; and

(c) a biotin labeled enzyme group coupled to said covalently bound avidin labeling group."

In Claim 8 "soluble" has been inserted before "biotin" in step (b)(ii) by the Board. Claims 2 to 7, 9 to 13 and 15 and 16 are dependent on Claims 1, 8 and 14 respectively.

III. The Appellant requests that the decision under appeal be set aside and a patent granted on the basis of the claims referred to in paragraph II above. The gist of the Appellant's argumentation in support of the request is in substance as follows:

The claimed method requires only one immobilised entity whereas that disclosed in D1 requires two distinct ones. D1 further measures residual enzyme or enzyme inhibitor

activity whereas the application in suit measures total activity of bound or free supernatant enzyme. The application in suit moreover uses a biotinylated enzyme which is not the same as the biotinyl enzyme disclosed in D1. Accordingly the claimed subject-matter is novel.

The process disclosed in D2 uses an avidin-enzyme conjugate, not a biotin-enzyme conjugate and D2 gives no motivation to use a biotin labelled enzyme (biotinylated enzyme). Further, a combination of the teachings of D1 and D2 would lead inter alia to the use of a biotinyl enzyme. Therefore neither D1 nor D2 nor a combination of these would lead to the subject-matter of the invention.

Reasons for the Decision

1. The appeal is admissible.

2. As compared with Claim 1 of the claims as originally filed and which formed the basis of the appealed decision, Claim 1 now has the word "soluble" inserted before "reagent" in step (b)(ii) and before "biotin" in step (b)(iii). While the description at no time specifically states that the reagent is soluble, it is implicit that it must be so. Throughout the description the immobilised components are distinguished from the other components as being the insoluble phase, indicating that the other phase must be a solution - cf. page 8, lines 3, 4; page 8, lines 26, 27 (unreacted reagent is then removed); page 9, lines 4, 5 (unreacted reagent is separated from the insoluble phase); page 12, lines 7 to 9 (both the insoluble phase containing specific binding substance and avidin labelled specific binding substance). Also page 8, lines 13 to 15 (mixing reagents (ii) and (iii) together in appropriate quantities prior to incubation with ligand

bound to the insoluble phase) indicates that apart from the insoluble phase, solutions are involved. Accordingly, the amendment has a basis in the description and meets the requirements of Article 123(2) EPC. Moreover, it is clear that "soluble" refers to the liquids present in the assay conditions.

3. The most significant difference sought to be drawn by the Appellant between the subject-matter of the application in suit and the disclosure in D1 is that the application uses a biotinylated enzyme (in the claims called a biotin labelled enzyme) whereas D1 uses a biotinyl enzyme. In the Board's view this is a valid distinction. It is clear from D1, page 4, lines 15 to 19 that by a biotinyl enzyme is intended the group of carboxylases in which biotin functions as a co-enzyme and is essential for enzyme activity. In the application in suit (see page 7) biotin is coupled to a variety of enzymes (but not carboxylases) via a coupling agent and does not function as a co-enzyme. It functions rather as the means for linking the enzyme to which it is coupled to the avidin-labelled ligand (competitive process) or avidin-labelled specific binding substance (non-competitive process). The assay therefore does not depend on avidin inhibition of enzyme activity of either the separated insoluble (immobilised) phase or of the supernatant liquid. D1 on the other hand, while also utilising the bonding between avidin and biotin, depends on the inhibition of biotinyl enzyme activity because of said bonding, the extent of inhibition being a measure of the amount of suspected ligand. The question arises as to whether this distinction is brought out in the wording of the claims. In the Board's opinion, the wording "biotin-labelled enzyme" alerts the reader to the fact that something other than the biotin-carboxylase enzyme combination is intended, and this is considered to be all

that is required, the description on page 7 identifying examples of what is intended by biotin-labelled enzymes.

A further distinction is that Claim 1 only requires the specific binding substance to be immobilised as the insoluble phase, whereas D1 discloses the immobilisation of two entities either on separate supports or on separate regions of the same support. This distinction could be said to be implicit in Claim 1 as originally filed and is now emphasised by the insertion of the word "soluble" as set out in paragraph 2 above. The Examining Division's argument to the effect that Example 3 in D1 disclosed one of the embodiments covered by Claim 1 therefore does not apply. Accordingly, the subject-matter of Claim 1 is novel.

4. Moreover, the subject-matter of Claim 1 cannot be said to be obvious having regard to the disclosure in D1 because, as set out in paragraph 3 above, D1 depends on the inhibition of a biotinyl enzyme by avidin, whereas the method of the application in suit does not. Further, D1 requires two entities to be immobilised, so that these cannot effectively react with one another. Without going into detail, whatever one of the four possibilities covered in D1 is chosen, the ligand (1) to be assayed competes with ligand (2) present in a known amount for binding sites on the specific binding substance, and as a result the more ligand (1) in the test solution, the more avidin is available to inhibit the biotinyl enzyme activity. In view of the basic differences between the assay method disclosed in D1 and that according to the application in suit, there is nothing to lead the person of average skill in the art from the disclosure in D1 to the subject-matter of Claim 1.

The Board can therefore agree with the Appellant that, in investigating inventive step, D2 represents the more appropriate prior art. This discloses an assay method utilising an insoluble phase containing a specific binding substance for the ligand to be detected and enzyme-labelled avidin and either biotin-labelled specific binding substance (non-competitive method) or biotin-labelled ligand (competitive method). Starting from this prior art, the problem which is the basis of the application in suit can be seen as that stated on page 1, lines 12 to 14 of the description, that is, to achieve a method in which the specific activity of the enzyme is higher.

As compared with D2, in the method according to Claim 1, the roles of avidin and biotin are reversed. The main effect of this is that, because one avidin molecule can bind four biotin molecules, four enzyme molecules can be coupled per avidin molecule thereby contributing to achieving the desired increase in specific activity. There is nothing in D2 to suggest reversing the said roles, even though it can be assumed that the average skilled person knows of the existence of biotin-labelled enzymes. In this respect the Board notes that the description in the application in suit acknowledges that biotin-labelled enzymes were commercially available at its priority date (see Examples I, III and the corresponding parts of the earlier priority document).

The Examining Division, in the paragraph bridging pages 5 and 6 of its decision, expressed the opinion that the method illustrated in Example 3 of D1 would allow, or not rule out, four biotin labels to be bound to one avidin molecule. The Board cannot follow this argument. In the first place in D1 biotin is not acting as a label but as an essential part of the biotin-apo-enzyme combination. Further, the biotinyl enzyme is immobilised prior to

contact with avidin so that the likelihood of one avidin molecule being able to combine with four biotinyl enzyme groups is remote. There is therefore no teaching here which could be combined with that of D2 with a view to solving the above-mentioned problem. Accordingly, the subject-matter of Claim 1 is seen as involving an inventive step.

5. As compared with original Claim 9, Claim 8 has been amended in the same manner as Claim 1 by insertion of the word "soluble" in step (b)(i)(B) and (b)(ii). In the latter case, the insertion has been made by the Board and clearly reflects the Appellant's intentions - cf. the letter of 27 November 1992 "the respective wording of Claim 1 is used ..." in conjunction with the Grounds of Appeal, page 4:- "soluble" has been added ... before "biotin". The unclear expression "where appropriate" has been deleted and the wording of step (b)(ii) consequentially amended to correspond to that employed in step (b)(iii) of Claim 1. The subject-matter of Claim 8 differs from that of Claim 1 in that it is restricted to a method using a known quantity of reagent a', b', c' or d'. As regards novelty and inventive step however, the same considerations apply as set out in paragraphs 3 and 4 above so that Claim 8 is allowable for the same reasons.

6. The reagent to which Claim 14 relates includes as one of its constituents a biotin-labelled enzyme group. For the same reasons as set out in paragraph 3 above, the subject-matter of Claim 14 is novel over D1 and D2 because these do not disclose a biotin-labelled enzyme group.

In the first complete paragraph on page 5 of its decision, the Examining Division considered that in carrying out Example 1 of D1, the skilled person, in step (3), would have prepared a reagent such as is claimed in Claim 14 of the application in suit. The Board cannot agree with this

argument. Apart from the distinction which has been drawn between a biotin-labelled (or biotinylated) enzyme and a biotinyl enzyme, the said example does not disclose the independent existence of something which can be called a reagent for determining a ligand and which is constituted as required by Claim 14. In step (3) of said Example 1 there are brought together immobilised biotinyl enzyme, immobilised ligand (2) (using the terminology of Claim 1 of D1), non-immobilised test ligand (1) and a conjugate of avidin and specific binding substance for the ligands (which in Example 1 are the same). Ligands (1) and (2) compete for binding sites on the conjugate, and the more ligand (1) there is present, the more conjugate remains non-immobilised and free to take part in the avidin-biotin reaction. At this point there will be present ligand (1) bound to specific binding substance conjugated with avidin bound to immobilised biotinyl enzyme. This is not a reagent for determining the ligand (1) but a product of the assay reaction whose residual enzyme activity is related to the amount of ligand (1).

Moreover, for substantially the same reasons as set out in paragraph 4 above, the average skilled person has no reason or incentive to prepare a reagent as claimed in Claim 14, so that its subject-matter also involves an inventive step.

7. Claims 2 to 7, 9 to 13 and 15 and 16 are dependent claims related to particular embodiments of Claims 1, 8 and 14 respectively and are allowable for the same reasons.

8. The application accordingly meets the requirements of Articles 52(1), 54(1) and 56.

9. As regards the description, the Board observes that the passage on page 2, corresponding to Claim 1, requires to be similarly amended by insertion of the word "soluble"

before "reagent" on line 5 and before "biotin" on line 11, again clearly reflecting the Appellant's intention.

10. In view of the foregoing, the Appellant's auxiliary request for oral proceedings does not require to be considered.

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 grant a patent on the basis of the following documents:

Claims 1 to 16 received on 2 December 1992 with the Appellant's letter of 27 November 1992, amended in that in Claim 8, the word "soluble" is inserted before "biotin" in step (b)(ii).

Description pages 1 to 20 as originally filed amended in that on page 2, the word "soluble" is inserted before "reagent" on line 5 and before "biotin" on line 11.

The Registrar: The Chairman:

P. Martorana E. Turrini