BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS

BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE

CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

Internal distribution code:
(A) [ ] Publication in OJ
(B) [ ] To Chairmen and Members
(C) [X] To Chairmen
(D) [ ] No distribution

D E C I S I O N
of 14 May 2002

Case Number: T 0782/97 - 3.3.4

Application Number: 84902738.8

Publication Number: 0149654

IPC: G01N 33/50

Language of the proceedings: EN

Title of invention:
Detecting agent carrying polymer having multiple units of visualization monomer

Patentee:
YALE UNIVERSITY

Opponent:
Roche Diagnostics GmbH

Headword:
Detecting agent/YALE UNIVERSITY

Relevant legal provisions:
EPC Art. 123(2), 84, 54, 56

Keyword:
"Main request: novelty (yes), inventive step (no)"
"Auxiliary request: formal allowability (yes); novelty (yes), inventive step (yes)"

Decisions cited:
-

Catchword:
-

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0782/97 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 14 May 2002

Appellant: (Proprietor of the patent)
YALE UNIVERSITY
260 White Avenue
P.O. Box 6666
New Haven
Connecticut 06511 (US)

Representative:
VOSSIUS & PARTNER
Postfach 86 07 67
D-81634 München (DE)

Respondent: (Opponent)
Roche Diagnostics GmbH
- Patentabteilung -
D-68298 Mannheim (DE)

Representative:
Weiss, Wolfgang, Dipl.-Chem. Dr.
Weickmann & Weickmann
Patentanwälte
Kopernikusstrasse 9
D-81679 München (DE)

Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 25 April 1997 revoking European patent No. 0 149 654 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: R. E. Gramaglia
V. Di Cerbo

Summary of Facts and Submissions

I. The appeal is against the decision of the opposition division revoking European patent No. 0 149 654 (application No. 84 902 738.8), which had been opposed by the respondent (opponent) on the grounds of lack of novelty and inventive step. The patent had been granted on the basis of 52 claims for the non-AT designated Contracting States and 52 claims for AT.

II. The decision was based on the claims of the main request and of the first and second auxiliary requests submitted at oral proceedings.

III. The following documents are cited in the present decision:

(D2) Wisdom G.B., Clin. Chem., Volum 22, No. 8, pages 1243 to 1255 (1976);

(D3) US-A-4,002,532;

(D4) Engvall E. in "Biomedical Applications of Immobilized Enzymes and Proteins", Volum 2, pages 87 to 96, Edited by Thomas Ming Swi Chang, Plenum Press, New York, London (1977);

(D5) Engvall E., Scand. J. Immunol., Volum 8, Suppl. 7, pages 25 to 31 (1976);

(D6) Engvall E. et al., Immunochemistry, Volum 8, pages 874 to 879 (1971).

IV. Oral proceedings were held on 14 May 2002, during which the appellant submitted amended claims in the form of a

new main request and an auxiliary request in replacement of all preceding requests. Claim 1 of the new main request read as follows:

"1. A method for visualizing the presence of an inorganic or organic target molecule in a biological material, which comprises:
combining said target with a detecting agent for said target wherein the detecting agent carries a visualization polymer through an intermediate ligand binding complex, the visualization polymer comprising multiple units of a visualization monomer covalently bonded together directly or through a coupling agent by means of chemical groups or backbone moieties of said units;

said visualization monomer having at least one visualization site and being selected from an enzyme, a tagged natural or synthetic polypeptide, a tagged polyol, a tagged polyolefin or a tagged carbohydrate;

said chemical group being an amine group, an oxidized 1,2-diol group, a carboxy group, a mercaptan group, a hydroxy group or a carbon-hydrogen bond, said backbone moiety being an amide bond, a carbon-carbon bond, a carbon-oxygen bond or a carbon-hydrogen bond;

said chemical group or backbone moiety being located within said monomer at a position which is at least one atom away from the visualization site of said monomer; and

said coupling agent being derived from coupling agent-chemical groups selected from:

(1) a diacyl or di(iminoester) derivative of an aliphatic dicarboxylic acid of from 4 to 20 carbon atoms which will form amide or amidine bonds with epsilon or primary amine groups of monomers functioning as units of the polymer;

(2) reactive diacyl or dihydrazine derivative of an aliphatic dicarboxylic acid of from 4 to 20 carbons or an aliphatic dihydrazine of from 4 to 20 carbons which will form amide or hydrazone groups with 1,2-diol groups of monomers functioning as units of the polymer when the 1,2-diol is oxidized to a dialdehyde, or which will form amide groups with carboxylic acid groups of monomers functioning as units of the polymer;

(3) a reactive olefin derivative of an N-alkyl bis(maleimide) of from 4 to 20 carbons in the alkyl group which will form disulfide groups with mercaptan groups present in monomers functioning as units of the polymer;

(4) a reactive aliphatic heterobi[o]functional reagent substituted with an N-maleimide group an[d] either an iminoester or an N-(carbonyloxy)imide group wherein the aliphatic chain length is from 4 to 20 carbons which will form a sulfide group with a mercaptan group of a monomer functioning as a unit of the polymer and will form an amidine or amide group with an amine group an adjacent monomer functioning as a unit of the polymer;

(5) a reactive aliphatic heterobi[o]functional reagent substituted with Schiff base protected amine group and an acyl or iminoester derivative group of a

carboxylic acid wherein the aliphatic chain length is from 4 to 20 carbons which will form an amide or amidine bond with an amine group of a monomer functioning as a unit of the polymer, and after removal of the Schiff base protecting group, will form an amide bond by carbonyl dimidazole or diimide coupling with a carboxyl group of an adjacent monomer functioning as a unit of the polymer; and

(6) a trifunctional lysyl lysine reagent which will form imine or amide bonds with oxidized 1,2-diol groups or carboxylic acid groups respectively which are present in monomers functioning as units of polymer.

Claims 2 to 24 of the main request were adressed to specific embodiments of the method of claim 1. Claims 25 to 38 related to a detection-visualization arrangement carrying a visualization polymer, whereas claims 39 to 44 covered a visualization polymer complex. Claims 45 to 47 related to a detection kit.

Claim 1 of the auxiliary request read as follows:

"1. A method for visualizing the presence of a target DNA molecule in a biological material, which comprises:

combining said target with a detecting agent for said target wherein the detecting agent carries through an intermediate ligand binding complex a visualization polymer comprising multiple units of a visualization monomer covalently bonded together through a coupling agent derived from DSS by means of chemical groups of said units;

said visualization monomer having at least one visualization site and being alkaline phosphatase;

said chemical group being an amine group ; and

said chemical group being located within said monomer at a position which is at least one atom away from the visualization site of said monomer;

wherein said detecting agent is a complementary polynucleotide sequence and wherein said intermediate ligand binding complex comprises as a first ligand biotin covalently bonded to said agent, as a second ligand biotin covalently bonded to said vizualisation polymer and as a ligand binding compound avidin or streptavidin wherein said first and second ligands are complexed with said compound."

Claims 2 to 4 of the auxiliary request were adressed to specific embodiments of the method of claim 1. Independent claim 5 related to a detection-visualization arrangement carrying a visualization polymer, whereas claim 6 covered a detection kit comprising the detection-visualization arrangement of claim 5.

V. The submissions by the appellant, insofar as they are relevant to the requests still on file, can be summarised as follows:

(i) With respect to the main request

Novelty

- Claim 1 had been restricted to a method for

visualising the presence of an inorganic or organic target molecule in a biological material, wherein the detecting agent carries a polymer through an intermediate ligand binding complex (see page 45, lines 15 to 17 and claim 9 of the application as filed) and wherein the coupling agent belongs to groups (1) to (6). This specific embodiment differed from the method disclosed in documents (D5) and (D6) involving no such intermediate ligand binding complex and making use of glutaraldehyde as coupling agent.

Inventive step

- The closest prior art was represented by documents (D5) and (D6). The visualisation method according to these documents involved the use of glutaraldehyde as coupling agent. However, the following drawbacks arose: (i) a 30-70% loss of enzymatic activity (see document (D5), page 28, under the heading "Yield of enzyme activity during conjugation") and (ii) glutaraldehyde reacted with amino groups of proteins giving raise to unstable Schiff bases (-N=CH-) which needed to be reduced with $NaBH_4$ to give stable linkages (-NH-CH_2-) (see document (D2), page 1245, r-h column).

- The visualization method according to document (D5) was only suited to determining IgG.

- The technical problem to be solved vis-à-vis this state of the art was to devise a visualization method with improved sensitivity and flexibility, which overcame the drawbacks of using glutaraldehyde as cross-linking agent. This problem

was solved by the use of visualisation polymers prepared by means of the coupling agents (1) to (6) listed in claim 1 at issue and by the use of an intermediate ligand complex. The improved sensitivity and versatility could be deduced from the Examples and the experimental results of Table 2 of the patent in suit.

- Although documents (D5) and (D6) disclosed an immunoassay wherein the label was polymerised alkaline phosphatase, there was no teaching in these documents that a polymerised enzyme exhibited a better enzymatic activity or achieved a higher sensitivity than the monomeric enzyme, let alone that the signal delivered by each monomer was multiplied by the number of monomers in the polymer. In fact, it was stated on page 28 of document (D5) (see under the heading "Yield of enzyme activity during conjugation") that "there was no direct relationship between the efficiency of a conjugate and its total enzyme activity". The authors of documents (D5) and (D6) merely noted an increase in "binding efficiency" of protein A or rabbit IgG linked to polymerised alkaline phosphatase. They believed this effect to originate from a more favourable sterical situation of protein A and rabbit IgG.

- Document (D6) was concerned with a similar investigation using glutaraldehyde for linking alkaline phosphatase to rabbit IgG.

- As for document (D3), it did not disclose polymers of enzymes bound to an antibody.

(ii) With respect to the first auxiliary request

Novelty

- Compared with claim 1 of the main request, claim 1 of this request had been further restricted as follows:

(a) the target molecule being visualised was DNA; (b) the visualisation monomer was alkaline phosphatase; (c) the chemical group on the visualisation monomer was an amino group; (d) the coupling agent was derived from DSS; (e) the detecting agent was a complementary polynucleotide sequence; and (f) the intermediate ligand binding complex comprised as a first ligand biotin covalently bonded to said agent, as a second ligand biotin covalently bonded to said visualisation polymer and as a ligand binding compound avidin or streptavidin, wherein said first and second ligands were complexed with said compound.

- No prior art disclosed the above specific embodiment.

Inventive step

- The method according to claim 1 of this request rendered possible the detection of 1-2 pg of DNA (see line 11 of Table 2 of the patent in suit). This level of detection was 10 to 15 times better than the most sensitive detection procedure used in diagnostic laboratories before the priority date of the patent in suit, namely the "ABC detection procedure" referred to on page 3, lines 32 to 46 of the patent in suit.

VI. The submissions by the respondents, insofar as they are relevant to the requests still on file, can be summarised as follows:

(i) With respect to the main request

Inventive step

- As for the versatility of the claimed visualisation method, the techniques disclosed in documents (D5) and (D3) were also universal (see eg document (D3), column 2, lines 9 to 16). Furthermore, the avidin-biotin-based technique for increasing the versatility was already known (see the literature cited in the patent in suit on page 3, lines 16 to 31).

- As regarded the problem of overcoming the drawbacks of glutaraldehyde as cross-linking agent, document (D3) proposed a great many coupling agents in alternative to glutaraldehyde (see claim 1: "p.p'-difluoro-m,m'-dinitrophenylsulfone and dimethyladipimate").

- Glutaraldehyde had a remarkable stability when used as cross-linking agent. This spoke against a Schiff base formation (see document (D4), page 89, second full paragraph).

- Claim 1 still covered cross-linking agents involving the formation of a Schiff base (see claim 1(2): "dihydrazine derivatives ... dialdehyde").

- As for the sensitivity of the claimed visualisation

method, document (D5) taught an increase in sensitivity (5 times) by using a polymerised alkaline phosphatase instead of monomeric alkaline phosphatase (see page 27, r-h column).

- Therefore, the problem to be solved was to provide an obvious alternative, not a better visualisation method.

(ii) With respect to the auxiliary request

Article 84 EPC

- The expression in claim 1 "coupling agent **derived from** DSS" was ambiguous.

- Claim 1 lacked the critical technical feature that the phosphatase substrate had to be a mixture of nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP), a feature necessary for the claimed visualisation method to render possible the detection of 1-2 pg of DNA.

Article 123(2) EPC

- The feature in claim 1 "coupling agent derived from DSS" found no basis in the application as filed.

VII. The appellant (patentee) requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request or the auxiliary request, both filed during the oral proceedings.

The respondent (opponent) requested that the appeal be dismissed.

Reasons for the Decision

1. The appeal is admissible.

Main request
Formal admissibility (Articles 84, 123(2)(3) EPC) and novelty (Article 54 EPC)

2. The formal admissibility and novelty of the claims of this request is not disputed by the respondent and the board also sees no objections, so that there is no need for further detailed substantiation of this matter.

Inventive step
Closest prior art

3. For the purpose of formulating the technical problem to be solved in the light of the closest state of the art, the claimed visualisation method has to be compared with the art concerned with a similar visualisation method which differs therefrom by a minimum of structural and functional modifications. The visualisation method disclosed by document (D5) satisfies this requirement. It involves an alkaline phosphatase polymer cross-linked with glutaraldehyde ("the visualization polymer comprising multiple units of a visualization monomer covalently bonded together through a coupling agent by means of chemical groups of said units") bound to protein A ("a detecting agent for said target"), used to detect a mouse antibody to

alpha-fetoprotein ("the target"). Therefore, it merely differs from the visualisation method of claim 1 by the absence of the "intermediate ligand binding complex" and by the fact that the cross-linking agent ("coupling agent") belongs to groups (1) to (6) listed in claim 1 instead of being glutaraldehyde.

Problem to be solved

4. The appellant argues that the visualization method of claim 1 achieves the following advantageous effects vis-à-vis the visualization method of document (D5): (i) it overcomes the drawbacks of using glutaraldehyde as coupling agent (formation of an unstable Schiff base and 30-70% loss of enzymatic activity); (ii) improved versatility; (iii) improved sensitivity. Therefore, the problem to be solved in the light of document (D5) was an improvement of the visualisation method described there.

5. As regards technical effect (i), the board observes that claim 1 still covers cross-linking agents which form a Schiff base (see claim 1 (2) in conjunction with Scheme I, Reaction 2B: "dihydrazine which will form hydrazone groups with 1,2-diol groups of monomers functioning as units of the polymer when the 1,2-diol is oxidized to a dialdehyde", and claim 1 (6): "a trifunctional lysyl lysine reagent which will form imine bonds with oxidized 1,2-diol groups"). Moreover, a loss of enzymatic activity upon cross-linking occurs with almost any cross-linking agent (see document (D3), column 1, lines 24-26 and document (D2), page 1245, last line: "70%"). For all these reasons, the board is not prepared to acknowledge that the coupling agents (1) to (6) listed in claim 1 at issue (ie almost the whole

cross-linking chemistry) obviate the drawbacks of using glutaraldehyde as coupling agent (formation of an unstable Schiff base and loss of enzymatic activity).

6. Nor can technical effect (ii) ie, improved versatility be taken into account by the board. It is acknowledged on page 3, lines 16 to 31 of the patent in suit that the presence of an "intermediate ligand binding complex", which can be an antibody, a lectin, avidin, streptavidin (page 15, line 19) and preferably a biotin/avidin complex is a known expedient for increasing flexibility (ibidem, line 30: "this allows for greater flexibiliy"). Adopting this measure would have been obvious for the skilled person looking for a visualization method endowed with greater versatility.

7. As for technical effect (iii) above, an increase in sensitivity is indeed mentioned in the patent in suit, page 3, lines 47 to 49. Table 2 (see experiments 10 and 11) and Table 3 (see experiments 3 and 4) of the patent in suit compare inter alia two visualisation methods differing only by the detector, namely monomeric alkaline phosphatase (ABAP) versus polymeric alkaline phosphatase (poly ABAP). The latter performs better than ABAP (see also page 23, line 44). The board is thus satisfied that the claimed visualisation methods, involving polymeric alkaline phosphatase is more sensitive than that involving monomeric alkaline phosphatase.

8. However, the board does not share the appellant's view that this technical effect could not be derived from document (5). Table I on page 27 thereof compares inter alia two visualisation methods differing only by the detector, namely monomeric alkaline phosphatase (first

two experiments) versus polymeric alkaline phosphatase (last three experiments). It can be deduced from the figures under "Efficiency" that **at least** 7 times (210/31) more enzyme (monomer)-labeled protein A than enzyme (polymer)-labeled protein A is required to obtain the same solid-phase bound enzymatic activity after incubation in microtitre plates coated with rabbit IgG. Therefore, the technical teaching, which can be derived from document (D5) is that the immunoassay with the polymerised enzyme is at least 7 times more sensitive than that with the monomeric enzyme. Adopting the measure of polymerising the enzyme would have thus been obvious for the skilled person trying to device a more sensitive visualization method according to claim 1.

9. The board also disagrees to the appellant's argument that there is no teaching in document (D5) that a polymerized enzyme achieves a higher sensitivity. This is because the "Efficiency" as defined in the legend to Table I correlates with the enzymatic activity of the conjugate and hence with the sensitivity of the assay (less enzyme (polymer)-labeled protein A is needed for detecting eg 1 ng rabbit IgG because more signal is released).

10. The appellant relies on the passage on page 28 of document (D5) under the heading "Yield of enzyme activity during conjugation" ("there was no direct relationship between the efficiency of a conjugate and its total enzyme activity") for arguing that the author of document (D5) did not realise that the increase of sensitivity was due to the fact that the signal delivered by each monomer was multiplied by the number of monomers in the polymer.

Whatever the reason for the increase in sensitivity noted in the experiments carried out according to document (D5), be it of sterical nature (as believed by its author) or otherwise, the document does convey, in the board's judgement, the technical teaching that it is worth polymerising the enzyme in order to increase sensitivity of a visualisation method.

11. Since for the reasons given in this decision it was obvious for the skilled person to arrive at the subject-matter of claim 1, the appellant's main request cannot be considered to be acceptable under the terms of Article 56 EPC.

Auxiliary request
Article 84 EPC

12. The respondent argues that the expression in claim 1 "coupling agent **derived from** DSS" is ambiguous. However, the board observes that the wording "derived from" in relation to a coupling agent was already present in claim 36 as granted and in claim 5 as filed. The term "derived from" is clear to the skilled person in the light of page 4, lines 37 to 40 of the patent in suit, according to which a coupling agent is "derived from" a cross-linking reagent after the latter binds with the appropriate chemical group. In the embodiment of claim 1, DSS (disuccinimidyl suberate) binds to amino groups ("the appropriate chemical group") and becomes the "coupling agent" -CO-$(CH_2)_6$-CO- upon loss of the two succinimidyl leaving groups (see page 10, line 13: "N-oxasuccinimide" and reaction 1A on page 12). This objection has thus to be dismissed.

13. The respondent also maintains that claim 1 lacks the

critical technical feature that the phosphatase substrate has to be a mixture of nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP), a feature necessary for the claimed visualisation method to allow the detection of 1-2 pg of DNA. Yet, this respondent's argument is in contradiction with the disclosure on page 24, lines 28 to 29 of the patent in suit of a mixture of naphthol AS phosphate and fast red TR salt as a substrate for the phosphatase. The board assumes that the latter substrate performs no worse than NBT/BCIP, since poor substrates do not seem to be appropriate in experiments aiming at improving the most sensitive detection procedure then available (see paragraph 19 below).

Article 123(2) EPC

14. Contrary to the respondent's view, the expression in claim 1 "coupling agent derived from DSS" find a basis in claim 5 in combination with page 57, line 25, both of the application as filed.

Novelty (Article 54 EPC)

15. The novelty of the claims of this request is not disputed by the respondent and the board also sees no objections, so that there is no need for further detailed substantiation of this matter.

Inventive step

Closest prior art

16. For the purpose of formulating the technical problem to be solved in the light of the closest state of the art, the claimed visualisation method, restricted to include features (a) to (f) (see paragraph IV (ii) supra, under "novelty") has to be compared with the art concerned with a similar one which differs therefrom by a minimum of structural and functional modifications.

17. Although the technique of document (D5) implies an alkaline phosphatase polymer, it involves neither a biotin-avidin-biotin-based intermediate ligand binding complex nor a DNA/complementary DNA as the target molecule/detecting agent and merely allows a sensitivity in the ng range (see document (D5), page 28, l-h column: "1-1,000 ng/ml"). Therefore, it is unrealistic that the skilled person would depart from this remote prior art as starting point.

18. In the board's judgement, the prior art closest to the claimed subject-matter is the "ABC detection procedure" referred to on page 3, lines 32 to 46 of the patent in suit, ie the most sensitive detection procedure used in diagnostic laboratories before the priority date of the patent in suit. This technique involves the use of an avidin-biotinylated horseradish peroxidase complex (ABC) as a "detector" (ibidem, lines 33 to 24) and exhibits a limit of sensitivity of 30-100 pg (ibidem, lines 45 to 46) or 75-150 pg in the case of biotin-labeled DNAs (Bio-DNA probes) (ibidem, lines 6 and 7 of Table 2 on page 21). This technique involving an intermediate ligand binding complex (biotin-avidin-biotin) has thus to be regarded as the closest prior

art. Compared with this technique, the claimed visualisation method differs therefrom in that the "detector" ("poly ABAP")(see page 18, line 45) comprises an alkaline phosphatase polymer (claim 1) instead of monomeric horseradish peroxidase (ABC).

19. The problem to be solved lies with providing a visualisation method which substantially improves the sensitivity over the "ABC detection procedure" (see page 3, lines 47 to 49 of the patent in suit) and renders possible the detection of 1-2 pg DNA (ibidem, page 21, line 11 of Table 2 and lines 49 and 53). This problem is successfully solved (see eg Table 2 and page 23, line 44: "0.8 pg") by the visualisation method of claim 1, characterised by features (a) to (f).

20. In the board's judgement, nothing in the prior art points towards "fine-tuning" the above features (a) to (f) the way now stated in claim 1, in order that 1-2 pg DNA can be detected. This unexpectedly lower detection range around the pg goes beyond the measure known from document (D5) of polymerising the alkaline phosphatase in order to increase sensitivity (see paragraph 8 supra). In fact, it can be deduced from Table 2 of the patent in suit that a three times increase in sensitivity already occurs from passing from the "ABC" (lines 6 and 7; 75-150 pg) to the ABAP (line 10; 20-30 pg), ie **before** the polymerisation of the enzyme (line 11; poly ABAP) (page 20, lines 55 to 46: "Complexes made with avidin-DH and biotinylated intestinal alkaline phosphatase (ABAP complexes) were even more sensitive than ABC complexes made with peroxidase"). A further support lies with the 13 pg detected by means of the "ABC detection procedure" (see page 23, line 43 of the patent in suit), compared

with the 3-6 pg detected via the "ABAP" detector (ibidem, line 44). It is reasonable to assume that this improvement follows from using alkaline phosphatase instead of horseradish peroxidase and DSS as a cross-linking agent, both being features not hinted at by any prior art document.

21. In view of the foregoing, the visualisation method of claim 1 satisfies the requirements of Article 56 EPC. This conclusion also applies to claims 2 to 4, addressed to specific embodiments of the method of claim 1 and to claims 5 and 6, relating to means specifically designed for carrying out the visualisation method of 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 6 (auxiliary request) filed at the oral proceedings, and a description to be adapted thereto.

The Registrar: The Chairwoman:

P. Cremona U.M. Kinkeldey