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Datasheet for the decision of 13 November 2014

Case Number: T 1234/10 - 3.3.02

Application Number: 99954403.4

Publication Number: 1052510

IPC: G01N33/53

Language of the proceedings: EN

Title of invention:
NOVEL COMPLEXES CONTAINING CROSSLINKED AVIDIN, PROCESS FOR PREPARING AND ANALYTICAL METHOD USING THE SAME

Patent Proprietors:
Mitsubishi Chemical Medience Corporation
KIKKOMAN CORPORATION

Opponent:
Roche Diagnostics GmbH

Headword:
Crosslinked avidin/Mitsubishi

Relevant legal provisions:
EPC Art. 54, 83, 123(2), 111(1)

Keyword:
Added subject-matter - main request (no)
Sufficiency of disclosure - main request (yes)
Novelty - main request (no)
Remittal (yes)

Decisions cited:

Catchword:

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Case Number: T 1234/10 - 3.3.02

D E C I S I O N
of Technical Board of Appeal 3.3.02
of 13 November 2014

Appellant: (Opponent)	Roche Diagnostics GmbH Sandhofer Strasse 116 68305 Mannheim (DE)
Representative:	Herzog, Martin Herzog Fiesser & Partner Patentanwälte PartG mbB Dudenstrasse 46 68167 Mannheim (DE)
Respondent: (Patent Proprietor 1)	Mitsubishi Chemical Medience Corporation 2-8, Shibaura 4-chome Minato-ku Tokyo 108-8559 (JP)
Respondent: (Patent Proprietor 2)	KIKKOMAN CORPORATION 250 Noda Noda-shi, Chiba 278-8601 (JP)
Representative:	Vossius & Partner Siebertstrasse 4 81675 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 12 April 2010 concerning maintenance of the European Patent No. 1052510 in amended form.**

Composition of the Board:

Chairman H. Kellner
Members: K. Giebeler
R. Cramer

Summary of Facts and Submissions

I. European patent No. 1 052 510, based on European patent application No. 99 954 403.4 and on international application No. PCT/JP1999/06172, published as WO 2000/028326, was granted with 10 claims.

II. According to the translation filed with the European Patent Office on 3 August 2000 together with the "Request for entry into the European phase" (hereinafter referred to as the "application as filed") claim 1 reads:

"A biotin-avidin-biotin complex comprising at least two biotin-introduced products which are the same or different, and a crosslinked avidin sandwiched therebetween."

III. An opposition was filed against the granted patent under Article 100(a) EPC (lack of novelty and inventive step), Article 100(b) EPC (insufficiency of disclosure) and Article 100(c) EPC (added subject-matter).

IV. With its interlocutory decision posted on 12 April 2010, the opposition division decided that the patent in amended form on the basis of the main request met the requirements of the EPC (Articles 101(3)(a) and 106(2) EPC). In particular, the opposition division considered that the claims of the main request fulfilled the requirements of Rule 80 EPC and Articles 84, 123(2), 83, 54 and 56 EPC.

V. Independent claim 1 of the main request as held allowable by the opposition division reads:

"A biotin-avidin-biotin complex consisting of at least two biotinylated substances which are the same or different, and a crosslinked avidin sandwiched therebetween, wherein the crosslinked avidin is a crosslinked avidin monomer composed of an avidin molecule or a crosslinked avidin polymer composed of plural avidin molecules which are bound to each other by intermolecular crosslinkages, wherein the crosslinked avidin possesses crosslinkages at least between its subunits."

VI. The following documents are mentioned in this decision:
D2: Nature Biotechnol., 1996, vol. 14, p. 1007-1011
D8: BIO/TECHNOLOGY, 1995, vol. 13, p. 1198-1204
D9: WO 97/11183
D10: Bioconjugate Chem., 1997, vol. 8, p. 819-832

VII. The opponent (appellant) filed an appeal against the decision of the opposition division and requested the revocation of the patent in its entirety. With the statement of grounds of appeal, the appellant submitted new evidence (documents D8 to D10).

VIII. With letter dated 15 December 2010, the respondents requested that the patent be maintained on the basis of the main request or, alternatively, the first to third auxiliary requests, which had previously been presented to the opposition division and which were resubmitted with said letter.

With letter dated 6 April 2011, the respondents submitted four additional sets of auxiliary requests (numbered as the first, third, sixth and seventh auxiliary requests), which had not previously been presented to the opposition division; the auxiliary requests as submitted with letter dated

15 December 2010 were resubmitted and renumbered as the second, fourth and fifth auxiliary requests.

IX. The board summoned the parties to attend oral proceedings and issued a communication in which it gave its preliminary opinion.

X. Oral proceedings before the board were held on 13 November 2014.

XI. The submissions made by the appellant, as far as they are relevant to this decision, may be summarized as follows:

Documents D8, D9 and D10 filed with the grounds of appeal were *prima facie* highly relevant and should therefore be admitted into the proceedings.

Main request

The replacement of the term "comprising" used in the application as filed by the term "consisting of" used in claim 1 violated Article 123(2) EPC, since the "consisting of" language was neither explicitly nor implicitly disclosed in the application as filed. Page 12, lines 6-8 made it clear that the complex could also be prepared by processes other than the one specified. Similarly, the process of claim 1 as originally filed could "comprise" additional steps.

The claimed invention lacked sufficiency of disclosure (Article 83 EPC) because the patent provided only one method for producing crosslinked avidin and did not disclose how a crosslinked avidin could be produced which consisted of either a crosslinked avidin monomer or a crosslinked avidin polymer.

Additionally, the claimed subject-matter lacked novelty (Article 54 EPC) *inter alia* in view of documents D2 and D9. Page 1009, column 2 of document D2 disclosed that three different kinds of covalently crosslinked streptavidin were mixed with excess D-[carbonyl-^{14}C]biotin, resulting in a complex according to claim 1. Example 37 of document D9 disclosed that the cross-linked streptavidins of Example 31 were mixed with end-biotinylated, single-stranded 18-base DNA, resulting in complexes according to claim 1, with two, three and four biotinylated DNA molecules bound to a crosslinked streptavidin molecule. These complexes as disclosed in Example 37 of document D9 were also disclosed in document D2, page 1009, column 1, paragraph 3.

XII. The submissions made by the respondents, as far as they are relevant to this decision, may be summarized as follows:

Documents D8, D9 and D10 were late-filed since they had neither been submitted within the nine months opposition period, nor was there any reason why they could not have been filed earlier. Moreover, it was not highly likely that any of these documents prejudiced the maintenance of the patent as held allowable by the opposition division. Hence the documents should not be admitted into the proceedings.

Main request

The term "consisting of" as used in claim 1 had a basis on page 12, paragraph 2 of the application as filed, which taught that nothing else apart from the crosslinked avidin and the biotinylated substances was

present in the complex of the invention. Hence the requirements of Article 123(2) EPC were fulfilled.

The claimed complex was sufficiently disclosed (Article 83 EPC) since paragraph [0029] of the patent in suit disclosed how crosslinked avidin could be prepared using the specified crosslinking agents, and the skilled person would know to either adjust the concentration of avidin during crosslinking, or to separate monomeric products from polymeric products, for instance by size exclusion chromatography.

The claimed subject-matter was novel over the cited prior art. In particular, neither document D2, nor document D9 disclosed that a complex was obtained which comprised at least two biotinylated substances. The molar ratio of D-[carbonyl-^{14}C]biotin and streptavidin specified in document D2, page 1011, column 1, last four lines did not allow any conclusions with respect to the absolute concentrations, and at low concentrations, it was very unlikely that two biotinylated substances were bound to a streptavidin molecule. In document D9, Example 37, the sentence referring to monomeric, dimeric, trimeric and tetrameric biotinylated targets DNA bound via single streptavidin molecules was not clear, and could refer to multibiotinylated, double-stranded DNA. Example 27 referring to dimeric and trimeric biotinylated DNA did not provide a general definition and could thus not be used to interpret Example 37. Finally, none of the prior art documents on file provided a direct and unambiguous disclosure of the claimed subject-matter.

XIII. The requests of the parties were as follows:

The appellant (opponent) requests that the decision under appeal be set aside and that the patent be revoked in its entirety. Furthermore, the appellant requests that documents D8 to D10, filed with the statement of grounds of appeal, be admitted into the proceedings. Moreover, the appellant requests that the first to seventh auxiliary requests filed by the respondents with the letter of 6 April 2011 not be admitted into the proceedings.

The respondents (patent proprietors) request that the appeal be dismissed (main request) or, alternatively, that the decision under appeal be set aside and the patent be maintained in amended form on the basis of the claims of one of the first to seventh auxiliary requests submitted with the letter of 6 April 2011. Furthermore, the respondents request that documents D8 to D10 not be admitted into the proceedings.

Reasons for the Decision

1. The appeal is admissible.

2. Admission of documents D8, D9 and D10 into the proceedings

2.1 Documents D8, D9 and D10 were submitted by the appellant with its grounds of appeal.

2.2 Document D8 relates to engineered chimeric streptavidin tetramers as novel tools for bioseparation and drug delivery (see title). The document discloses streptavidin mutants which form intramolecular disulfide bridges (see for instance Figure 1B), as well as biotin-avidin-biotin complexes (see for instance

Figures 3 and 6). The board therefore considered that document D8 is *prima facie* highly relevant for the claimed subject-matter and decided to admit the document into the proceedings.

2.3 Document D9 discloses the preparation of crosslinked streptavidin mutants (Examples 29 to 34) and the mixing of the crosslinked streptavidins with excess D-[carbonyl-^{14}C]biotin (Example 35) and with end-biotinylated oligonucleotides (Example 37). Document D9 is thus *prima facie* highly relevant for the claimed subject-matter, and the board decided to admit the document into the proceedings.

2.4 Document D10 relates to the synthesis and *in vitro* evaluation of biotin derivatives for cross-linking of streptavidin (see abstract). Figures 4 E, G and H of the document show crosslinked avidin complexed with at least two biotinylated substances. Document D10 is thus *prima facie* highly relevant for the claimed subject-matter, and the board decided to admit the document into the proceedings.

Main request

3. Amendments (Article 123(2) EPC)

3.1 Claim 1 refers to a biotin-avidin-biotin complex **consisting of** at least two biotinylated substances which are the same or different, and a crosslinked avidin sandwiched there between, whereas claim 1 of the application as filed refers to a biotin-avidin-biotin complex **comprising** such components.

3.2 The meaning of the word "comprising" is generally understood as encompassing all the specifically

mentioned features as well as optional, additional unspecified ones, whereas the term "consisting of" only includes those features as specified in the claim.

In the present case, page 12, lines 9-13 of the application as filed describes the preparation of the claimed biotin-avidin-biotin complex, in conformity with Example 1 (see pages 19 to 21), by first preparing the crosslinked avidin and the biotinylated substances separately in advance, and then coupling them to form the biotin-avidin-biotin complex. According to this teaching, in conjunction with original claim 1 and page 7, lines 9-15, a complex thus prepared does not comprise components other than those specified in claim 1. Consequently, the skilled reader would directly and unambiguously derive from the application as filed the disclosure of a complex consisting of the components specified in claim 1.

On this basis, the requirements of Article 123(2) EPC are met.

4. Sufficiency of disclosure (Article 83 EPC)

4.1 The appellant submitted that the claimed invention lacked sufficiency of disclosure under Article 83 EPC, because the patent provided only one method for producing crosslinked avidin and did not disclose how a crosslinked avidin could be produced that consisted of either a crosslinked avidin monomer or a crosslinked avidin polymer.

4.2 Sufficiency of disclosure under Article 83 EPC requires that the claimed invention is disclosed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art, taking into account

the common general knowledge of the skilled person. In the present case, the board is convinced that a skilled person would know from common general knowledge that crosslinked monomeric avidin can be separated from crosslinked polymeric avidin by chromatographic methods such as gel filtration. Therefore, the board is satisfied that the requirements of Article 83 EPC are fulfilled.

5. Novelty (Article 54 EPC)

Claim 1 is directed to a biotin-avidin-biotin complex consisting of at least two biotinylated substances which are the same or different, and a crosslinked avidin sandwiched therebetween, wherein the crosslinked avidin is a crosslinked avidin monomer composed of an avidin molecule or a crosslinked avidin polymer composed of plural avidin molecules which are bound to each other by intermolecular crosslinkages, wherein the crosslinked avidin possesses crosslinkages at least between its subunits.

According to paragraph [0026] of the patent in suit, the avidin to be used can be any avidin which binds to biotin, for instance egg-white avidin, a streptavidin, or recombinant avidin.

5.1 Document D2

5.1.1 Natural avidin molecules ("monomers" in the sense of claim 1 of the patent in suit) are composed of four subunits which are not crosslinked. In the prior art, avidin is therefore referred to as a tetrameric protein. It is known that in the tetrameric protein, two respective subunits are associated very tightly to form a so-called stable dimer. A tetramer is formed by

two of these stable dimers that are associated relatively weakly (see the introductory part of document D2, page 1007, column 1, lines 1-9). Each of the subunits binds D-biotin with an exceptionally high affinity.

Document D2 is a scientific publication which relates to streptavidins with intersubunit crosslinks having enhanced stability. The document describes introducing specific crosslinking sites into streptavidin by site-directed mutations of His-127 residues. Disclosed are *inter* alia a first and a second streptavidin construct having disulfide bonds and irreversible covalent bonds, respectively, between Cys-127 residues of different subunits, and a third streptavidin which is a hybrid tetramer consisting of two different streptavidin species, one having lysine and the other aspartic acid at position 127, which are covalently crosslinked (see abstract).

It is undisputed between the parties that these streptavidin constructs with intersubunit crosslinks are crosslinked avidin monomers as defined in claim 1.

5.1.2 The questiton is whether or not document D2 discloses a "biotin-avidin-biotin complex consisting of at least two biotinylated substances which are the same or different, and a crosslinked avidin sandwiched therebetween".

5.1.3 In its experimental protocol on page 1011, column 1, last four lines, document D2 states that "To determine the biotin-binding ability of each streptavidin construct after heat treatment, D-[carbonyl-^{14}C]biotin was added to each streptavidin sample at a ratio of biotin:biotin-binding site of 1.4 in 150 mM NaCl, 50 mM Tris-Cl (pH 7.5). After the mixtures were incubated at

room temperature for 30 min, unbound biotin was separated from streptavidin-biotin complexes (...) and quantitated by liquid scintillation counting." On page 1009, column 2, first full paragraph, in the "Results and discussion" section of the document, it is reported that the experiments with excess D-[carbonyl-^{14}C]biotin indicated that the introduction of covalent bonds across the dimer-dimer interface enhanced the thermal stability of streptavidin, making it more resistant to both subunit dissociation and loss of biotin-binding ability (see the paragraph bridging pages 1009 and 1010). According to Figure 6, at room temperature, the crosslinked streptavidins have the same biotin-binding ability as natural core streptavidin.

5.1.4 D-[carbonyl-^{14}C]biotin is to be considered as a "biotinylated substance" in the sense of claim 1, especially in view of claim 5 which defines the biotinylated substance as a biotinylated radioactive isotope.

5.1.5 However, the respondents have argued that it had not been convincingly shown that in the experiments of document D2, a complex with at least two biotinylated substances bound to a crosslinked avidin was actually obtained; the specified molar ratio in document D2 said nothing about the absolute concentration, and at low concentrations, it would be very unlikely to have at least two biotins per avidin.

5.1.6 Document D2 describes that D-[carbonyl-^{14}C]biotin was used in excess, at a ratio of 1.4 biotin:biotin-binding site of the crosslinked streptavidin molecule. This means that the samples contained 5.6 biotinylated substances per molecule of crosslinked streptavidin, and the board sees no reason to doubt that at least

two, if not essentially all four biotin-binding sites of each of the crosslinked avidin molecules bound D-[carbonyl- ^{14}C]biotin after 30 minutes of incubation at room temperature, all the more so in view of the commonly known exceptionally high affinity between biotin and (strept)avidin, which is essentially irreversible. Additionally, it would not have made any sense if the authors of document D2 had used concentrations of D-[carbonyl-^{14}C]biotin and crosslinked streptavidin so low that the binding of these binding partners would be impaired, because the experiments in question were carried out with the explicit aim of determining the biotin-binding ability of the crosslinked streptavidins; the document reports that the introduced covalent bonds did in fact result in enhanced biotin-binding ability. The board is thus convinced that the experiments were carried out with concentrations of the two binding partners that did not prevent their binding. Therefore, the board judges that document D2 discloses a biotin-avidin-biotin complex with at least two biotinylated substances and a crosslinked avidin sandwiched therebetween.

5.1.7 Consequently, the subject-matter of claim 1 lacks novelty over document D2.

5.2 Document D9

5.2.1 In Example 37 (see page 54 ff. of document D9) "two-chain tetramers" of streptavidin were used for a test of their ability to bind biotinylated macromolecules. Example 31 (see page 47 ff. of document D9) describes the preparation of reversible and irreversible two-chain tetrameric streptavidins using streptavidin with cystein at position 127. Consequently, these "two-chain tetramers" are crosslinked avidins wherein the

crosslinked avidin is a crosslinked avidin monomer composed of an avidin molecule, wherein the crosslinked avidin possesses crosslinkages at least between its subunits as set out in current claim 1.

Example 37 reports that all of the two-chain tetramers were able to bind biotin, and that it was tested whether the introduction of covalent bonds across the dimer-dimer interface would affect the binding to biotinylated macromolecules. This was done by mixing each two-chain tetrameric streptavidin with an end-biotinylated 18-base oligonucleotide and analysis by non-denaturing polyacrylamid gel electrophoresis (PAGE). It is stated that "Each **mixture** contained monomeric, **dimeric, trimeric and tetrameric biotinylated targets DNA, bound via single streptavidin molecules**. There was no significant difference in the amounts of these bound biotinylated DNA molecules among two-chain tetrameric streptavidins and natural core streptavidin" (page 54, lines 23-26; emphasis added by the board).

Consequently, Example 37 describes the mixing of a crosslinked avidin as defined in claim 1 with a biotinylated substance.

5.2.2 The question is whether or not said mixing resulted in a biotin-avidin-biotin complex in which at least two biotinylated DNA molecules were bound to a single streptavidin molecule.

5.2.3 Example 27 (see page 43 ff. of document D9) relates to the binding of streptavidin to biotinylated DNA, as does Example 37, and describes experiments comparing the binding ability of biotinylated DNA for natural core streptavidin with that for a partially truncated

streptavidin "Stv-13". Page 44, lines 1-5 states that "an end-biotinylated double-stranded DNA target was mixed with core streptavidins at various ratios and the mixtures were separated by agarose gel electrophoresis, followed by staining the DNA targets with ethidium bromide", whereby the "DNA used was a 3179-bp linear double-stranded DNA target, in which one of the 3-termini contains a biotin moiety." Electrophoretic analysis showed "that larger amounts of **dimeric** (approximately 6.4 kilobasepair) and **trimeric** (9.5 kilobasepair) **biotinylated DNA targets, which are connected via single streptavidin molecules**, were formed with Stv-13 than with natural core streptavidin at any molar ratio of streptavidin subunits to biotin used" (page 44, lines 17-21; emphasis added by the board).

5.2.4 In Example 27, the passage referring to dimeric and trimeric biotinylated DNA targets can only mean that two or three biotinylated DNAs were bound to single streptavidin molecules, because the DNA is said to be double stranded and to contain a biotin moiety only at one of the 3'-termini (see page 44, lines 4-5), and because the approximate sizes of the dimeric and trimeric biotinylated DNA targets as determined by electrophoretic analysis were 6.4 and 9.5 kilobase-pairs, respectively (see page 44, lines 17-21), i.e. the two- or three-fold of the size of the 3179-bp DNA used.

The board is thus convinced that, accordingly, the expression "monomeric, dimeric, trimeric and tetrameric biotinylated targets DNA, bound via single streptavidin molecules", as used in Example 37, must be understood to mean that one, two, three or four biotinylated DNAs were bound to single streptavidin molecules.

For this reason, Example 37 of document D9 discloses a biotin-avidin-biotin complex in which at least two biotinylated DNA molecules are bound to a single streptavidin molecule.

5.2.5 Additionally, the respondents' argumentation that Example 37 referred to the binding of only a single double-stranded DNA carrying either one, two, three or four biotins to a streptavidin molecule, does not convince the board for the following reasons: The aim of the experiments described in Example 37 was to test whether the crosslinked streptavidins would bind biotinylated macromolecules (page 54, lines 19-21). It would therefore not make any sense to determine the number of biotin residues bound to the 18-base oligonucleotide. It is true that neither Figure 18, nor any other figure in document D9 shows the non-denaturing PAGE referred to in Example 37. However, the conclusions drawn by the authors of document D9 from the described experiments that there was no significant difference in the amounts of biotinylated DNA molecules bound by crosslinked streptavidin and natural core streptavidin (page 54, lines 24-26) can only mean that the expression "monomeric, dimeric, trimeric and tetrameric biotinylated targets DNA" refers to the binding of one, two, three and four biotinylated DNA molecules to a single streptavidin molecule.

5.2.6 In view of the above, also document D9 is prejudicial to the novelty of the subject-matter of claim 1.

6. Remittal to the opposition division (Article 111 EPC)

In view of the board's finding of lack of novelty (Article 54 EPC) of the subject-matter of claim 1 of

the main request, which the opposition division had considered to be allowable, and in view of the auxiliary requests and documents on file not yet discussed in the first instance, the board decides to exercise its discretion under Article 111(1) EPC to remit the case to the opposition division for further prosecution.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance for further prosecution.

The Registrar: The Chairman:

N. Maslin H. Kellner

Decision electronically authenticated