Europäisches Patentamt
Beschwerdekammern

European Patent Office
Boards of Appeal

Office européen des brevets
Chambres de recours

Aktenzeichen / Case Number / N° du recours : T 293/85 - 3.3.2

17

Anmeldenummer / Filing No / N° de la demande : 78 300 597.8

Veröffentlichungs-Nr. / Publication No / N° de la publication : 1930

Bezeichnung der Erfindung: Method for polypeptide production involving expression of a heterologous gene, recombinant microbial vehicle, containing said gene and bacterial culture transformed by said cloning vehicle
Title of invention:
Titre de l'invention :

Klassifikation / Classification / Classement : C12K 1/02

ENTSCHEIDUNG / DECISION

vom / of / du 27 January 1988

Anmelder / Applicant / Demandeur : Genentech, Inc.

Patentinhaber / Proprietor of the patent / Titulaire du brevet :

Einsprechender / Opponent / Opposant :

Stichwort / Headword / Référence : Hybrid polypeptides/GENENTECH II

EPÜ / EPC / CBE Articles 56, 83, 84, 123(2) and Rule 27(1)(f) EPC.

Kennwort / Keyword / Mot clé : "Sufficiency (affirmed)"
"Inventive step (affirmed)"

Leitsatz / Headnote / Sommaire

Europäisches Patentamt
Beschwerdekammern

European Patent Office
Boards of Appeal

Office européen des brevets
Chambres de recours

Case Number : T 293/85 - 3.3.2

D E C I S I O N
of the Technical Board of Appeal 3.3.2
of 27 January 1988

Appellant : Genentech, Inc.
460 Point San Bruno Boulevard
So. San Francisco
California
USA

Representative : Goldin, Douglas Michael et al
J. A. KEMP & CO.
14, South Square
Gray's Inn
London WC1R 5EU
GB

Decision under appeal : Decision of Examining Division 023 of the European Patent Office dated 15 May 1985 and notified on 15 July 1985 refusing European patent application No. 78 300 597.8 pursuant to Article 97(1) EPC

Composition of the Board :

Chairman : P. Lançon
Members : G. Szabo
F. Antony
G. Paterson
R. Schulte

Summary of Facts and Submissions

I. European patent application 78 300 597.8 filed on 6 November 1978 and published on 16 May 1979 with publication number 1930, was refused by the decision of the Examining Division 023 of the European Patent Office dated on 15 May 1985 and notified on 15 July 1985. The decision was based on claims 1-51 submitted on 15 May 1985. Claims 1, 12, 16 to 19 and 29 were worded as follows:

1. A process which comprises producing a heterologous polypeptide in a bacterium transformed with a recombinant plasmid comprising a DNA sequence encoding, in proper reading frame, both said heterologous polypeptide and additional protein, the additional protein having a selective cleavage site adjacent the heterologous polypeptide, wherein the DNA sequence is expressed, and the resulting conjugate polypeptide is specifically cleaved to give said heterologous polypeptide.

12. A recombinant plasmid comprising a regulon, a DNA sequence encoding both a desired specific heterologous polypeptide and additional protein, and one or more termination codon(s), wherein the said DNA sequence is interposed in reading frame between said regulon and termination codon(s) such that a conjugate polypeptide comprising both the desired heterologous polypeptide and additional protein results from expression in a bacterium transformed with the plasmid, there being a selective cleavage site between the desired heterologous polypeptide and the additional protein.

16. A plasmid according to any one of Claims 12 to 15, wherein the heterologous polypeptide is somatostatin.

17. A plasmid according to any one of Claims 12 to 15, wherein the heterologous polypeptide is the A chain of insulin or the B chain of insulin.

18. A bacterium transformed with a plasmid according to any one of Claims 12 to 17.

19. A bacterium transformed with a plamid selected from the group consisting of pSOM1, pSOM11, pSOM11-3, pIA1 and pIB-1.

29. A process which comprises producing a heterologous polypeptide, selected from the group consisting of somatostatin, the A chain of human insulin and the B chain of human insulin, in a bacterium transformed with a recombinant plasmid comprising a DNA sequence encoding, in a proper reading frame, both said heterologous polypeptide and additonal protein, the additional protein having a selective cleavage site adjacent the heterologous polypeptide, wherein the DNA sequence is expressed, and the resulting conjugate polypeptide is specifically cleaved to give said heterologous polypeptide.

II. The stated grounds for the refusal were that the disclosure was not sufficient under Article 83 EPC, including requirements arising from Rule 27(1)(f) EPC. There was lack of clarity for certain claims under Article 84 EPC, and an inventive step could not be acknowledged as long as sufficiency of disclosure was questionable. No specific objections were raised against independent Claim 29 involving the preparation of somatostatin or the A or B chain of human insulin, or dependent Claims 16, 17, and 30-51.

III. The Examining Division raised the same arguments against the sufficiency of the disclosure as in the related case T 292/85 ("Polypeptide expression / GENENTECH I, 27 January 1988, to be reported in OJ) which involved an application relying for support on the same specific disclosure and partly on the same features in the claims. In particular, the Division insisted that all embodiments within the claims must have been capable of being carried out by the skilled person at the priority date and in a repeatable manner without practicing inventive skill. No claims should rely on constituents which represent further inventions. In addition to the impossibility of providing such embodiments at the present, the later patentability of such constituent variants might be adversely affected. Claims should, in effect, at least be limited to what is available at the priority date, i.e. known bacteria, plasmids and DNA relating to known polypeptides. A process for the preparation of a human hormone could not be identically repeated since the source of the DNA in humans varied with the individual. In general, no component should be defined in functional terms in this field of technology.

Although earlier communications from the Examining Division expressed doubts about the inventive step, no particular objections were raised in the decision itself against any of the claims on this ground, in reply to the submissions by the Appellants.

IV. The Appellant submitted a Notice of Appeal against the decision, together with a payment of the fee on 13 September 1985, and filed a Statement of Grounds on 22 November 1985. A Communication was issued by the Board on 2 June 1987, raising in particular the role and provision of the regulon as one of the critical features, and the Appellant filed a reply on 28 August 1987.

V. An oral hearing was held on 27 January 1988. During the course of the hearing a new request with 33 claims was submitted on behalf of the Appellant to replace all earlier requests. Independent Claims 1 and 12 have the following wording:

1. A process for producing a desired heterologous polypeptide which comprises growing a bacterium transformed with a recombinant plasmid comprising a homologous regulon, a DNA sequence in proper reading frame therewith, and one or more termination codons, wherein said DNA sequence is positioned between said regulon and the termination codon(s) and encodes said heterologous polypeptide, additonal protein, and a selective cleavage site between the desired heterologous polypeptide and additional protein, wherein the DNA sequence is expressed and the resulting conjugate polypeptide is sufficiently large not to be degraded by endogenous proteolytic enzymes and is specifically cleaved to give said desired heterologous polypeptide.

12. A recombinant plasmid comprising a homologous regulon, a DNA sequence encoding a desired specific heterologous polypeptide, a selective cleavage site and additional protein, and one or more termination codons, wherein the said DNA sequence is interposed in proper reading frame between said regulon and termination codon(s) such that a conjugate polypeptide comprising both the desired heterologous polypeptide and additional protein results from expression in a bacterium transformed with the plasmid, there being a selective cleavage site between the desired heterologous polypeptide and the additional protein.

VI. The Appellant submitted in the proceedings and at the oral hearing the same arguments as in case T 292/85. The issues were identical and related to the same example and general

explanations in the disclosure. It was emphasised that functional terms should be allowable and it was sufficient to show one way of carrying out the invention. There was no reason to assume that the invention would be unworkable and there was no evidence to the effect that failure was inevitable even under a bona fide effort. It was argued that limitation to an actual example would be unfair since the method provided had general applicability.

VII. The Appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of the description and Claims 1 to 33 as submitted during the oral proceedings with the drawings as originally filed.

Reasons for the Decision

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

Amendments and support

2. The amendments which are incorporated in the present claims are not such that the application contains subject-matter which extends beyond the content of the application as filed (Article 123(2) EPC). Furthermore, the amended claims are supported by the description (Article 84 EPC).

In particular, the feature "homologous regulon" added to the main claim was taken from page 15, line 5-8. The requirement that the expressed conjugate polypeptide "is sufficiently large not to be degraded by endogenous proteolytic enzymes" is based on the statements from page 26, lines 12 to 27, in particular the last two lines. The term "heterologous polypeptide" in Claim 12 is implied by the corresponding term "heterologous DNA" and its

function to code for such polypeptide (cf. page 4, line 4-8). Claim 12 refers to plasmids obtained, i.e. obtainable, according to the methods described on page 27, line 25 et seq. Claim 19 is now amended by deleting pSOM1 and pSOM11. The amendments therefore comply in the formal respect with Articles 84 and 123(2) EPC. The consequential amendments of the specification presented at the oral hearing, containing also corrections of obvious typing errors, are allowable.

3. Although Claim 12 to the recombinant plasmid is not formally restricted to coding which provides conjugate polypeptides "sufficiently large not to be degraded ..." as in Claim 1, this condition is implied by the requirement that a "conjugate polypeptide ... results from expression ... " (emphasis added). The claims are therefore confined to those embodiments which are capable of providing the desired results.

Sufficiency

4. The appeal is primarily concerned with the issue of sufficiency associated with certain components of the claims, such as "bacterium", "regulon", or "plasmid". These kinds of features are essentially functional terms in this particular context, in spite of structural connotations and may cover an unlimited number of possibilities. The Board has held in the decision of the above cited case T 292/85 (cf. paragraphs 3.1.5 and 3.2.1) 4, that an invention is sufficiently disclosed if at least one way is clearly indicated enabling the person skilled in the art to carry out the invention. Then the non-availability of some particular variants or unsuitability of some unspecified particular variants of a functionally defined component feature of the invention is immaterial to sufficiency as long as there are suitable variants known to the skilled person through the disclosure or common general knowledge

which provide the same effect for the invention. The disclosure need not include specific instructions as to how all possible component variants within the functional definition should be obtained.

Since the generalisations in the present case are based on the same factual background as in the Decision in T 292/85, the same conclusions must be drawn. No insufficiency with regard to the terms objected to arises therefore under Article 83 EPC.

5. The further objection by the Examining Division was that some DNA molecules, coding for instance for certain human hormones, showed allelic variations when obtained from the human donor, and the processes could not, therefore, be repeated without having guaranteed access to such sources. The same objection was rejected by the Board in the above decision (cf. paragraph 3.3.3) holding that "generally applicable biological processes are not insufficiently described for the sole reason that some starting materials or genetic precursors therefor, e.g. a particular DNA or a plasmid, are not readily available to obtain each and every variant of the expected result of the invention, e.g. the product, provided the process as such is reproducible."

No insufficiency therefore arises in this respect either, since the processes claimed in the present case are also generally applicable and are, in fact, mere extensions of the processes in the cited Decision by a further step.

Rule 27(1)(f) EPC

6. The objection under Rule 27(1)(f) EPC (page 19, last line) was not substantiated by reasoning in the decision. The examples show that somatostatin or the insulin A or B chains can be obtained according to the invention by the expression of a conjugate polypeptide incorporating such

materials as components, and by subsequent cleavage and separation (e.g. page 34, lines 9-24, and p. 41, line 2 to page 42, line 2). In view of this and the above considerations on the availability of various features of the invention, no insufficiency arises under Art. 83 EPC. No consequential lack of support or of clarity can be recognized in relation to the wording of the claims under Article 84 EPC either.

The problem and the solution

7. The claimed subject-matter relates to the expression of certain polypeptides and the recovery of a part thereof as a desired heterologous polypeptide. There was no reference cited from the state of the art where this effect had been achieved before, involving expressions in micro-organisms.

In the view of the Board the closest state of the art is nevertheless represented by Polisky et al (Proc. Natl. Acad. Sci. USA, 1976, 73, 3900-3904(1)), describing the construction of a plasmid suitable for the transformation of E. coli bacteria, which has a bacterial regulon and heterologous DNA fragment inserted into it, coding for a ribosomal RNA sequence (rRNA). The sequence did not code for a translatable polypeptide at all, but for the rRNA which was to become part of a ribosome (cf. page 3902, right column under the heading "Expression of eukaryotic DNA under lac-control"). The article, however, speculates about the possible use of the idea for producing "eukaryotic gene products in bacteria" in general (last sentence of abstract and at the end of the discussion, page 3904, left column, last paragraph).

8. The speculative statements in the reference could be construed as a tentative reference to a technical problem arising from the disclosure. This was to provide, at last, any specific heterologous polypeptide as a useful product,

involving a step of expression in bacteria, corresponding exactly to a DNA insert. The solution of the problem requires the use of recombinant plasmid according to Claim 12. This plasmid characteristically contains, inter alia, a DNA coding for "the desired specific heterologous polypeptide, selective cleavage site, and additional protein" wherein the cleavage site between the other two coding sequences. According to Claim 1, the process of using this plasmid must lead on expression to a conjugate polypeptide which "is sufficiently large not to be degraded by endogenous proteolytic enzymes and is specifically cleaved to give said heterologous polypeptide".

Novelty

9. The success of the suggested approach to obtain the desired polypeptide via a large conjugate also depends on the other features of the claim, such as the provision of the proper reading frame with the DNA sequence for the regulon and the presence of an appropriate termination codon. As already suggested above (under 6), the results show that on using the plasmids and transformed bacteria according to the claimed process, somatostatin and the insulin constituent chains A and B were duly obtained. Since there is no doubt so far that other embodiments falling under the claimed subject-matter would equally provide the corresponding polypeptides, the solution of the stated problem appears to have been credibly achieved by the claimed plasmids and process. In the absence of any reference which disclosed either the expression of such conjugate polypeptides or their cleavage at a specifically provided cleavage site, the claimed subject-matters are novel.

Inventive step

10. As to the inventive step, it is relevant that the obtaining of the conjugate polypeptides, with or without specific

cleavage sites, was as described and claimed in the copending European application No. 78 300 596.0 (Publ. No. 1929), filed on the same date by the same Applicants (cf. Decision T 292/85). In that case the Board has recognised the inventive step for the first part of the presently claimed process, i.e. without the cleavage step, and for the plasmids which embrace the now claimed specific plasmids also carrying a codon for a cleavage site in addition. That Decision was based on the two critical modifications implemented on the plasmids disclosed in the Polisky reference (1). These were the provision of the proper reading frame for the homologous regulon and the obtaining of a polypeptide which is not degraded by endogenous proteolytic enzymes. Since the present claims expressly or implicitly also carry these features and also all the other essential features of the cited other case, part of the subject-matter of the present invention is already recognized as inventive over the cited Polisky (1) reference. A combination of such subject-matter with the further independent feature of the cleavage site and appropriate cleaving step is, in the present case, also considered as non-obvious on the same basis.

11. Nevertheless, it is also apparent that the additional features associated with cleavage represent a further contribution to the inventive step. It was not expected to seek a route to small size polypeptides through a large polypeptide. Only the discovery of the problem associated with the direct expression of small polypeptides in association with the primary invention in the other application opened the door for an indirect approach to such small products. Without the solution of the first problem of obtaining large polypeptides, the way to the small polypeptides was barred. The skilled person would not have contemplated the detour into the area of more complex products without the knowledge of the difficulties, which

were to be encountered when the basic process was tested with small polypeptides.

12. Whilst the provision of a cleavage site and the indirect approach to such products may look logical ex post facto, this had not been the case before the unexpected difficulties were discovered. In addition, there is no literature reference available to the Board which relates to the post expression cleavage of polypeptides in any context to obtain a desired polypeptide which corresponds to an incorporated specific heterologous gene, representing a desirable proteinaceous product. There is nothing in Polisky (1) or in other prior art to suggest the successful new approach. Thus the subject-matter of the present claims involves a further modification which cannot be seen to be desirable from the state of the art, and in the Board's view there is no reason to believe that their methodology would have naturally ocurred to the skilled person.

13. Original Claims 29 et seq, relating to somatostatin or the insulin chains were not objected to on any grounds. Earlier arguments during prosecution were based on the Polisky (1) reference and the assumption that the known character of the technical problem itself provided the skilled person with all the features of the solution. As explained above the Board already dealt with the inventive step with regard to the in-phase position of the regulon and the unexpected necessity of first obtaining a large protein in the cited decision, and this is equally applicable to the present case.

14. However, the Examining Divisions additional contention that cleavage was also implied by the problem of obtaining desired polypeptides is also erroneous, since only the discovery that, with small polypeptides, the process of expression would bring no results, necessitates the step of

cleavage and the means for incorporation which would enable such a step in the first place. The question is not whether the skilled person could have provided certain means but whether he would have done so in expectation of some improvement or advantage (cf. T 2/83, "Simethicon Tablet/ RIDER", OJ 6/1984, 265 at page 270). There was no reason whatsoever to expect an advantage from an indirect route through a different larger entity to be provided by any method and by an additional cleavage, before the invention was made.

In view of the above the process represents a general approach to polypeptides, partly known, partly yet unexplored, which was neither available nor derivable from the state of the art.

15. The claimed plasmids act, on the other hand, as genetic precursors for the desired polypeptides by carrying the relevant information which exactly governs the expression of the desired polypeptide conjunates and cleaved products. In view of the dependency of the product on the composition of the genetic precursor, the latter is also responsible for the unexpected result of the former and derives its inventive character therefrom. All claimed processes and products are therefore associated with an inventive step and are linked to form a single general inventive concept under Art. 82 EPC (unity), in view of their close technical interconnection.

Order

For these reasons it is decided:

1. The decision under appeal is set aside.

2. The patent is granted on the basis of the description and the Claims 1-33 as submitted during the oral proceedings with the drawings as originally filed.

The Registrar

The Chairman