

THIS OPINION WAS NOT WRITTEN FOR PUBLICATION

The opinion in support of the decision being entered today (1) was not written for publication in a law journal and (2) is not binding precedent of the Board.

Paper No. 33

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte BRUCE E. FURIE, BARBARA C. FURIE,
HOWARD A. LIEBMAN and
RICHARD M. LEWIS

Appeal No. 1996-0223
Application No. 07/931,563

ON BRIEF

Before WILLIAM F. SMITH, GRON, and SPIEGEL, *Administrative Patent Judges*.
SPIEGEL, *Administrative Patent Judge*.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 2, 3, 5 through 8, 10, 11, 13 through 15, 17, 23 through 28 and 32. Claims 1, 18 through 22 and 29

through 31, the only other claims pending in the application, have been withdrawn from further consideration under 37 CFR § 1.142(b) as not readable on the elected invention.¹

Claims 2, 10 and 17 are representative of the subject matter on appeal and read as follows:

2. A method for isolating a vitamin K-dependent protein, which is complexed with a metal cation and exists in a stabilized conformational state, from a mixture containing said protein, said method comprising

(a) providing an antibody immobilized on a solid support, said antibody being reactive with said protein complexed with a divalent or trivalent metal cation and substantially unreactive with said protein not complexed with said metal cation,

(b) contacting said mixture, in the presence of said metal cation, with said immobilized antibody to bind said protein, complexed with said metal cation, to said immobilized antibody to form an immune complex, and

(c) contacting said immune complex with a compound having a binding affinity for said metal cation higher than the binding affinity of said protein for said metal cation, to remove said metal cation from said protein to induce a non-stabilized conformational state of said protein and thereby release said protein from said immobilized antibody.

10. A method for isolating a vitamin K-dependent protein, which is uncomplexed with any metal cations and exists in a non-stabilized conformational state, from a mixture containing said protein, said method comprising

(a) providing a conformation-specific antibody immobilized on a solid support, said antibody being reactive with said uncomplexed protein in a non-stabilized conformational state and substantially unreactive with said protein complexed with a metal cation in a stabilized conformational state,

¹ Appellants have requested official cancellation of non-elected claims 1, 18-22 and 29-31 in the amendment filed November 14, 1994 (Paper No. 27), which was **not acknowledged** by the examiner in the communication mailed February 23, 1995 (Paper No. 28). This oversight should be corrected upon return of the above identified application to the jurisdiction of the examiner.

(b) contacting said mixture, under conditions under which no metal cation forms a complex with said protein, with said immobilized antibody to bind said protein to said immobilized antibody to form an immune complex, and

(c) contacting said immune complex with a metal cation under conditions under which said metal cation can complex with said protein to induce a stabilized conformational state of said protein, and thereby release said protein from said immobilized antibody.

17. The method of claim 2 wherein said protein is capable of forming three conformational states in the presence of said metal cation, and said antibody is capable of forming an immune complex with one of said conformational states and is substantially unreactive with the other two of said conformational states.

The references relied on by the examiner are:

Furie et al. (Furie '320)	4,769,320	Sep. 6, 1988 ²
Zimmermann	5,055,557	Oct. 8, 1991 ³

Falb et al. (Falb), "Immobilized Proteins and Peptides" in POLYMERS GRAFTS IN BIOCHEMISTRY 197-221 (Hixson, Jr. and Goldberg, eds., Marcel Dekker, Inc., New York, 1976).

Furie et al. (Furie 1979), "Conformation-specific Antibodies as Probes of the (- Carboxyglutamic Acid-rich Region of Bovine Prothrombin," 254 *The Journal of Biological Chemistry* 19, 9766-71 (October 10, 1979).

Swanson et al. (Swanson), "Vitamin K Dependent in Vitro Production of Prothrombin," 21 *Biochemistry* 23, 6011-18 (November 9, 1982).

ISSUES

² Furie '320 issued from application no. 661,187, filed October 15, 1984, which was a continuation-in-part of application no. 402,318, filed July 27, 1982, abandoned, which was a continuation-in-part of application 198,444, filed October 20, 1980, abandoned.

³ Zimmerman issued from application no. 275,466, filed November 23, 1988, which was a continuation of application no. 910,315, filed September 2, 1986, which was a continuation of application no. 800,902, filed November 22, 1985, abandoned, which was a continuation of application no. 707,179, filed March 1, 1985, abandoned, which was a continuation of application no. 472,413, filed March 4, 1983, abandoned.

Appeal No. 1996-0223
Application No. 07/931,563

I. Claims 10, 11 and 13-15 stand rejected under 35 U.S.C. § 102 as anticipated by Zimmerman.

II. Claims 2, 3, 5-8 and 17 stand rejected under 35 U.S.C. § 103 as being unpatentable over Swanson in view of Falb. Claim 32 stands rejected under 35 U.S.C. § 103 as being unpatentable over Swanson in view of Falb as applied to claims 2, 3, 5-8 and 17, and further in view of Zimmerman. Claims 23-28 stand rejected under 35 U.S.C. § 103 as being unpatentable over Swanson in view of Falb as applied to claims 2, 3, 5-8 and 17, and further in view of Furie 1979.

III. Claims 2, 3, 5-8, 17, 23-28 and 32 stand rejected under 35 U.S.C. § 103 as being unpatentable over Furie '320 in view of Falb, Zimmerman and Furie 1979.

We REVERSE all of the examiner's above rejections.

In reaching our decision in this appeal, we have given careful consideration to the appellants' specification and claims and to the respective positions articulated by the appellants and the examiner. We make reference to the examiner's answer (Paper No. 24, mailed September 9, 1994) for the examiner's reasoning in support of the rejections and to the appellants' brief (Paper No. 23, filed May 19, 1994) and to the appellants' reply brief (Paper No. 26, filed November 14, 1994) for the appellants' arguments thereagainst.

BACKGROUND

Vitamin-K dependent proteins, i.e., Factor IX, Factor X, Factor VII, prothrombin, protein S and protein C, all contain metal binding amino acid (-carboxyglutamic acid. When these proteins complex with metal ions, e.g., calcium, magnesium, manganese and gadolinium ions, they undergo a structural conformational change that includes changes in the three-dimensional structure of the (-carboxyglutamic acid-rich regions of the proteins. (specification, pages 2, 5-6)

THE INVENTION

As succinctly stated by appellants, the claimed invention is directed to

... methods for purifying vitamin-K dependent proteins with conformation-specific antibodies that react only with (i) specific protein-cation complexes (claims 2, 3, 5, 6-8, 17, 23-28, and 32) or (ii) specific cation-free uncomplexed proteins (claims 10, 11, and 13-15). In these methods, the protein to be purified forms an immune complex with a conformation-specific antibody, and is then released from the immune complex by induction of a conformational change of the protein so that it is no longer recognized by the antibody.

When the antibody is specific for the protein-cation complex, the protein is removed from the immune complex by exposing the complex to a compound, e.g., a chelating-agent, that has a higher binding affinity for the cation than does the protein. This compound removes the cation from the protein to induce a non-stabilized conformational state that is not recognized by the antibody, which disrupts the protein-antibody complex.

When the antibody binds exclusively to a cation-free protein, the protein is removed from the immune complex by exposing the complex to a metal cation that

Appeal No. 1996-0223
Application No. 07/931,563

induces a stabilized conformational state of the protein that is not recognized by the antibody, which again disrupts the protein-antibody complex. [Brief, pages 1-2.]

According to the specification, this releasing step is specific and mild in contrast to the non-specific, harsh conditions under which proteins are conventionally eluted from immunoaffinity columns, thereby providing a high degree of protein purification without the risk of denaturation and loss of biological activity (pages 6-7).

OPINION

I. Rejection of claims 10, 11 and 13-15 under 35 U.S.C. § 102 as anticipated by Zimmerman.

Anticipation requires that all elements of the claimed invention be described, either expressly or under the principles of inherency, in a single prior art reference. *In re Paulsen*, 30 F.3d 1475, 1478-79, 31 USPQ2d 1671, 1673 (Fed. Cir. 1994); *In re Spada*, 911 F.2d 705, 708, 15 USPQ2d 1655, 1657 (Fed. Cir. 1990).

Claims 10, 11 and 13-15 all require use of an antibody which binds to a vitamin K-dependent protein in its non-metal complexed or “non-stabilized conformational” state but does not bind to the protein in its metal complexed or “stabilized conformational” state. Zimmerman describes separation

and purification, i.e., isolation, of Factor IX, a vitamin K-dependent protein, by immunoaffinity chromatography using an immobilized antibody which binds to Factor IX in its “non-stabilized conformational” state (i.e., binds to Factor IX in *citrated* plasma) (col. 6, lines 9-66). However, as argued by appellants to no avail, the Zimmerman antibody also binds to Factor IX in its active “stabilized conformational” state because Zimmerman only identified “positive clones” as those in which “the [hybridoma] supernatant substantially neutralized Factor IX clotting activity” (Zimmerman col. 3, lines 45-46; brief, pages 6-7). In other words, the Zimmerman antibody binds to Factor IX irrespective of whether or not the Factor IX is itself bound to a metal ion. The Zimmerman antibody is *not* a conformation-specific antibody.

Secondly, while Zimmerman suggests that a calcium chloride containing buffer may be used to elute purified Factor IX from the immunoaffinity column, it is stated to be “preferably at a concentration of about 0.1 to 1.0 M” (col. 6, lines 61-65). As argued by appellants, this is elution by normal ionic disruption using a strong salt, i.e., a high ionic concentration of calcium and chloride ions, not elution by inducing any change in conformational state as recited in the claims (brief, pages 7-8). The skilled artisan would have expected changes in conformational states of blood proteins to occur under normal physiological conditions. For example, human adult blood serum normally contains 2.10 - 2.55 mM

Appeal No. 1996-0223
Application No. 07/931,563

total calcium.⁴ Thus, Zimmerman does not disclose or suggest the elution or “releasing step” of the claimed invention.

Based on the foregoing, we REVERSE the examiner’s rejection of claims 10, 11 and 13-15 as anticipated by Zimmerman.

II. Rejection of claims 2, 3, 5-8, 17, 23-28 and 32 under 35 U.S.C. § 103 as being unpatentable over Swanson in view of any one or more of Falb, Zimmerman and Furie 1979.

The examiner bears the initial burden of establishing a *prima facie* case of obviousness. To establish a *prima facie* case of obviousness, there must be both some suggestion or motivation to modify the reference or combine reference teachings and a reasonable expectation of success.

In re Vaeck, 947 F.2d 488, 493, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

Swanson describes calcium-dependent and calcium independent antibodies, i.e., conformationally specific antibodies, and their use to study prothrombin precursors (see e.g., pages 6011 and 6017-18). Figure 2 illustrates the binding of plasma prothrombin and microsomal precursors to the Ca⁺⁺-independent antibody, which occurs in the presence of either 2 mM EDTA or 1 mM CaCl₂ (2A) and to the Ca⁺⁺-dependent antibody, which occurs in the presence of CaCl₂ but not in the presence of EDTA (2B) (page 6013, col. 2 - page 6014, col. 1).

Falb discloses that immobilized antibodies can be used to purify antigens (page 202).

⁴ According to Tietz’s TEXTBOOK OF CLINICAL CHEMISTRY (W.B. Saunders Company, Philadelphia, 1986) (copy attached).

According to the examiner, it would have been obvious to immobilize the calcium-dependent antibodies of Swanson in order to purify (-carboxyglutamyl modified prothrombin which is an *active* protein, while prothrombin without (-carboxyglutamyl residues is inactive, (answer, page 5) because “[t]he teachings of Swanson et al. on the characterization of calcium-dependent antibodies and the release of prothrombin from these antibodies by EDTA and the teachings of Falb et al. on the use of immobilized antibodies to purify protein antigens provide the requisite motivation and expectation of success for purifying prothrombin by the use of calcium-dependent antibodies” (answer, page 11).

Appellants do not dispute that using immobilized antibodies to purify antigens is old in the art (brief, page 11). The argument is that “normal human plasma contains only fully carboxylated, fully active protein, and non-conformation specific antibodies can be used to isolate the active form of prothrombin” (brief, page 13, footnote omitted). In other words, the examiner has not explained why the skilled artisan would have chosen Swanson’s calcium-dependent antibody as the immobilized antibody for the purification of prothrombin, as opposed to either a non-conformation specific antibody or a calcium-independent antibody of Swanson (N.B. Figure 2A clearly shows that Swanson’s calcium-dependent antibody binds plasma prothrombin).

The examiner opines that EDTA would be of no use in releasing prothrombin from a calcium-independent antibody (answer, page 10) and that the “harsh treatments reported by Swanson et al. in their in vitro experiments would not be of value in purifying bioactive prothrombin” (answer, page 11).

The former comment is nonresponsive to appellants' arguments and the latter comment is not understood. The use of "strong salts or acids" to elute proteins from immunoaffinity columns was discussed by appellants on page 8 of their brief in reference to Zimmerman, not Swanson. Moreover, Zimmerman indicates that these "strong salts or acids" are useful in eluting *active* vitamin K-dependent proteins from immunoaffinity columns (abstract and col. 6, lines 61-66), from plasma or recombinant DNA protein sources (col. 2, lines 36-46). The examiner has not pointed out and we do not find where Swanson or Falb disclose or suggest contacting a protein mixture containing Ca^{++} •prothrombin with an immobilized calcium-dependent antibody to form an immobilized antibody• Ca^{++} •prothrombin complex and then releasing prothrombin therefrom by contacting with EDTA which removes (chelates) the Ca^{++} from the immobilized antibody• Ca^{++} •prothrombin complex. Neither Zimmerman⁵ nor Furie 1979⁶ cure this deficiency. Rather, the only place we find such suggestion is in the appellants' specification. Thus, we find that the examiner has relied on impermissible hindsight in making his determination of obviousness. *W.L. Gore & Assocs. v. Garlock, Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984) ("To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of

⁵ The examiner relies on Zimmerman for its disclosure of using prothrombin derived from recombinant cells as a protein source material for purification (answer, page 6).

⁶ The examiner relies on Furie 1979 to show that Mg^{+2} , Mn^{+2} or Gd^{+3} can replace Ca^{+2} for binding to (-carboxyglutamic acid residues in prothrombin (answer, page 7).

Appeal No. 1996-0223
Application No. 07/931,563

record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher”).

As to claims 17 and 32, it is the examiner’s opinion that “formation of three conformational states in the presence of calcium, only one of which binds the calcium-dependent antibody, is considered to be an intrinsic property of prothrombin” (answer, page 6). Assuming *arguendo* that prothrombin is inherently capable of forming three conformational states, the examiner has not established that binding calcium to metal-free prothrombin induces not one but two different conformational changes in the structure of prothrombin or that Swanson’s calcium-dependent antibody will differentially bind to only one of the two calcium-induced conformational states to the substantial exclusion of the other. As noted by in appellants’ reply brief (pages 5-6), the answer never addressed this issue.

Based on the foregoing, we conclude that the examiner has not established a *prima facie* case of obviousness as to claims 2, 3, 5-8, 17, 23-28 and 32 over Swanson in view of any one or more of Falb, Zimmerman and Furie 1979. Having concluded that the examiner has not established a *prima facie* case of obviousness, we do not reach appellants’ discussion of rebuttal evidence on pages 14-19 of the brief and on pages 3-5 of the reply brief.

III. Rejection of claims 2, 3, 5-8, 17, 23-28 and 32 under 35 U.S.C. § 103 as being unpatentable over Furie ‘320 in view of Falb, Zimmerman and Furie 1979.

Appeal No. 1996-0223
Application No. 07/931,563

Furie '320 discloses antibodies which specifically form complexes with human native prothrombin only in the presence of Ca^{+2} and are nonreactive with human abnormal prothrombin (col. 3, lines 60-63) as well as antibodies which specifically form complexes with human abnormal prothrombin in the presence of human normal prothrombin and in the presence or absence of Ca^{+2} (col. 3, lines 63-67).

According to the examiner,

It would have been obvious to one of ordinary skill in the art at the time the invention was made to purify prothrombin using immobilized calcium-dependent antibodies, as per Furie et al. (US Pat. 4,769,320) and Falb et al., and to use Mg^{2+} , Mn^{2+} , or Gd^{3+} in place of calcium when forming the prothrombin/metal/antibody complex since Furie et al. (1979) teach that any of these metals can replace calcium for binding to gla residues in the formation of prothrombin/metal/antibody complex. One would be motivated to replace calcium with these other metals in order to reduce the activation of calcium-dependent proteases and to optimize the conditions for protein/antibody complex formation. [Answer, page 8.]

We reverse this rejection for the same reasons set forth in rejection II. above, i.e., none of the applied prior art references disclose or suggest use of conformation-specific antibodies for protein purification.

OTHER MATTERS

Claims 7 and 14 in appellants' "Appendix - Claims on Appeal" incorrectly use the Greek letter tau (τ) instead of gamma (γ).

Appeal No. 1996-0223
Application No. 07/931,563

CONCLUSION

To summarize, the decision of the examiner (I) to reject claims 10, 11 and 13-15 under 35 U.S.C. § 102 as anticipated by Zimmerman, (II) to reject claims 2, 3, 5-8, 17, 23-28 and 32 under 35 U.S.C. § 103 as being unpatentable over Swanson in view of any one or more of Falb,

Zimmerman and Furie 1979, and (III) to reject claims 2, 3, 5-8, 17, 23-28 and 32 stand rejected under 35 U.S.C. § 103 as being unpatentable over Furie '320 in view of Falb, Zimmerman and Furie 1979 is **reversed**.

REVERSED

Appeal No. 1996-0223
Application No. 07/931,563

WILLIAM F. SMITH
Administrative Patent Judge

TEDDY S. GRON
Administrative Patent Judge

CAROL A. SPIEGEL
Administrative Patent Judge

)
)
)
)
)
) BOARD OF PATENT
) APPEALS
) AND
) INTERFERENCES
)
)
)
)
)

CAS/kis

Appeal No. 1996-0223
Application No. 07/931,563

PAUL T. CLARK
FISH & RICHARDSON
225 FRANKLIN ST.
BOSTON, MA 02110-2804