Purification and Characterization of the Protein Component of Tissue Factor*

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ABSTRACT: Tissue factor is a particle-bound lipoprotein which initiates coagulation *via* the extrinsic system. The present study presents a modification and extension of previous techniques by which 40 mg of soluble tissue factor can be prepared from 100 g of lung acetone powders with 800- to 1500-fold purification. Although the purified protein contains 7% residual phospholipid, recombination with 7.5 mg of phospholipid per mg of protein is required for maximal coagulant activity; this lipid to protein ratio stimulates activity 950-fold. The protein has been characterized by gel filtration, sucrose density gradient ultracentrifugation, and disc gel electrophoresis. By all of these techniques, the protein appears as two major species. The apparent molecular weights of these species are 330,000 and 220,000; the smaller component has a higher specific activity. Application of histochemical staining techniques to disc gels suggests that each species contains acidic lipid and acid mucopolysaccharide. One species, however, appears to contain more total lipid. Since the two species are not readily interconvertible, the relationship of one to the other is considered in terms of the residual lipid with resultant effects on coagulant activity and nonspecific protein binding.

The predominant mechanism of blood coagulation involves the sequential conversion of proenzymes into active, proteolytic enzymes. Tissue factor is a particle-bound lipoprotein that can initiate blood coagulation by combining stoichiometrically with factor VII, a plasma protein (Nemerson, 1966; Williams and Norris, 1966). The complex formed by these factors enzymatically converts factor X into its activated form which generates thrombin from prothrombin. The mechanisms of complex formation and factor X activation are not yet understood in detail.

The coagulant activity of tissue factor is a function of its lipid content (Nemerson, 1968; Deutsch *et al.*, 1964; Hvatum and Prydz, 1966). Removal of phospholipids from tissue factor results in loss of coagulant activity which, however, can be restored by recombining the protein with appropriate phospholipids (Nemerson, 1968). Removal of lipids from particulate tissue factor solubilizes the protein component. The biological activity of the soluble protein is also restored by phospholipids (Nemerson, 1969).

Tissue factor activity is the property of only certain proteins found in brain and lung (Nemerson, 1969). The tissue factor apoproteins derived from these organs are similar with respect to lipid requirements, biological activity, and behavior on gel filtration. On the basis of slight differences in elution from DEAE-cellulose, however, it appears as if tissue factor may exist in more than one molecular form. Investigation of this point was limited by the instability of tissue factor.

In this study, we present a modification of our previous method for the purification of tissue factor. The product is stable and has been prepared in high yield. The lipid-depleted soluble protein has been partially characterized, and is shown to exist as at least two major species in bovine lung.

Materials

Frozen bovine lungs were obtained from Pel-Freeze Biologicals, Rogers, Ark. Sodium deoxycholate was a product of Mann Research Laboratories, New York, N. Y. DEAE-Sephadex and Sepharose 6B were purchased from Pharmacia, Piscataway, N. J. 2,5-Diphenyloxazole and *p*-bis[2-(5-phenyloxazolyl)]benzene were products of Pilot Chemicals, Watertown, Mass. [^{32}P]Phosphorylase *a* was a gift from Dr. Edmond H. Fischer and was kindly prepared by Miss Susan D. Elsom; 4S RNA was a gift from Dr. Paul Leibowitz. γ-Globulin was a product of E. R. Squibb & Sons, New York, N. Y. Other reagents were purchased from the usual sources of supply.

Methods

Preparation of Apoprotein. Bovine lungs were processed immediately after slaughter, or else were rinsed in tap water and frozen. On occasion, commercial frozen lungs were used as starting material. To prepare acetone powders, the lungs were minced and ground in a Waring Blendor with an equal volume of 0.15 M NaCl. The homogenate was allowed to settle and the turbid supernatant was discarded. The remaining tissue was dehydrated by repeated washing with large volumes of acetone, air-dried, and stored at −20°.

To remove phospholipids, the acetone powders were extracted with freshly distilled heptane–butanol (2:1, 20 ml/g) for 30 min with vigorous stirring. The tissue was recovered by filtration through Whatman No. 40 paper prewashed with the solvents. The procedure was repeated for a total of five extractions. The tissue was air-dried and stored at −20°.

Some extraneous proteins were removed by extracting the delipidated powders with 0.5 M NaCl (40 ml/g in a Waring

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Blendor for 15 sec). The tissue was recovered by vacuum filtration through Whatman No. 40 paper covered with a cake of HyFlo and the filtrate was discarded. The apoprotein was solubilized by extracting the tissue (and the HyFlo) with 0.25% sodium deoxycholate (40 ml/g of starting material) for 30 min with vigorous stirring. The soluble material was collected by filtration as above. Solid ammonium sulfate was added to the clear deoxycholate solution to 30% saturation at room temperature (176 g/l.). The material was then filtered as before and the precipitate discarded. The filtrate was brought to 60% saturation with solid ammonium sulfate (198 g/l.) and the precipitate was collected by centrifugation (37,000*g* for 15 min at 4°), dissolved in a minimal amount of distilled water and dialyzed overnight against large volumes of 0.05 M imidazole·HCl–0.1 NaCl, pH 7.2 at 4°. The apoprotein derived from this preparation was combined with similar material prepared the following day and used for further purification.

DEAE-Sephadex was converted into the chloride form by washing with dilute NaOH, dilute HCl, water, and then with several changes of 0.05 M imidazole·HCl–0.1 M NaCl, pH 7.0. Excess buffer was removed from the slurry by aspiration with a filter candle. The apoprotein prepared by ammonium sulfate fractionation was added to the DEAE-Sephadex (1 g of moist DEAE-Sephadex per 15 mg of protein) and stirred at room temperature for 30 min. The slurry was centrifuged at 2000*g* for 5 min and the supernatant aspirated and discarded. The DEAE-Sephadex was washed with a volume of starting buffer equal to the volume of the starting material and again centrifuged. The slurry was then eluted with 0.05 M imidazole·HCl–0.5 M NaCl, pH 7.0 (equal in volume to the starting material) and again centrifuged. The elution was repeated with 0.5 volume of the same buffer and the eluates combined.

The apoprotein was concentrated by filtration through a Diaflo XM-50 filter to a volume of about 20 ml. It was further concentrated (to 6–9 ml) by placing it in dialysis tubing packed in dry sucrose, and then gel filtered at room temperature on 6% agarose (Sepharose 6B) in a 2.5 × 100 cm column fitted with upward-flow adapters. The column was pumped with a peristaltic pump at a rate of about 10 ml/hr. Fractions (5 ml) were collected.

Biological Activity. For assay of coagulant activity, the apoprotein was recombined with lipid (at the indicated concentrations) as previously described (Nemerson, 1969) by adding one volume of protein (in 0.05 M imidazole·HCl–0.375 M NaCl, pH 7.2 at 4°) to one volume of lipid (Nemerson, 1968; Method 1) and two volumes of 0.25% sodium deoxycholate. The material was dialyzed against the same imidazole buffer for 18 hr, then for at least 2 hr against imidazole buffer containing 0.1 M NaCl. Coagulant activity was determined according to the assay previously described (Nemerson, 1968). The results of the assay are presented as arbitrary units which refer to the activity of a standard tissue factor preparation that has been in use in this laboratory for several years (Nemerson, 1968).

In those cases where the amount of protein was insufficient for relipidation, the coagulant activity of the apoprotein was determined. For this assay, the incubation with the factor VII–X mixture was continued for 4 min (instead of the usual 1 min), and the appropriate correction was made for the prolonged incubation. The dilution curve for the apoprotein was parallel to that of the lipoprotein, so that the activities could be directly compared.

Electrophoresis. Disc gel electrophoresis was performed in 5% polyacrylamide gels at pH 9.5 using the discontinuous buffer system of Davis (1964). The gels were photopolymerized using riboflavin as a catalyst. Prior to electrophoresis, the samples were dialyzed against 0.5 strength reservoir buffer, and approximately 100 μg of protein (in a maximum volume of 100 μl) were layered on the gels. The samples were stabilized against convection disturbances by the addition of dry Sephadex G-25 or dry sucrose. At the completion of electrophoresis the gels were either fixed in 7% acetic acid for staining or eluted for activity assay. Aniline blue black in 7% acetic acid was used as the protein stain; carbohydrate was located with the periodic acid–Schiff stain suggested by Canalco Instruments (Model 12 System instructions). The stains for acid mucopolysaccharide (alcian blue) and acidic lipids (nile blue sulfate) were adapted from histochemical techniques described by Bancroft (1967). The gels were stained for the same period of time as recommended for tissues, then destained in the suggested solutions overnight. The method of Chiffelle and Putt (1951) using sudan black B in propylene glycol was used as a lipid stain; 85% propylene glycol was used for the initial destaining steps, then water. The gels were stained for nucleic acid with acridine orange according to the method of Richards *et al.* (1965). For elution, the gels were cut into 3-mm sections; each slice was placed in a tube and triturated with 0.5 ml of 0.05 M imidazole·HCl–0.1 M NaCl, pH 7.2. The tubes were centrifuged and the supernatants assayed directly.

Ultracentrifugation. Linear sucrose gradients were prepared from solutions containing 5 and 30% (w/v) sucrose in 0.05 M imidazole·HCl–0.375 M NaCl–0.001 M EDTA, pH 7.2 at 4°. The volume of the gradient was 4.6 ml. Following the application of the sample (0.2 or 0.3 ml) the gradients were centrifuged in a Spinco SW-39 rotor at 36,000 rpm at 4°. At the termination of a run, the tubes were pierced and ≈0.2-ml fractions collected.

Chemical Methods. Phospholipid content was calculated from the total phosphorus estimated by the method of Chen *et al.* (1956). Protein concentrations were determined by the biuret technique (Gornall *et al.*, 1949) or by the method of Lowry *et al.* (1951). Radioactivity was determined in a scintillant containing 200 g of naphthalene, 10 g of 2,5-diphenyloxazole, and 250 mg of *p*-bis[2-(5-phenyloxazolyl)]benzene in 1 l. of dixoane using a Nuclear-Chicago Spectrometer Model 6850. No correction was made for quenching.

Results

Preparation of the apoprotein on a large scale as well as substantial purification has been accomplished (Table I). Extraction of the delipidated powder with 0.5 M NaCl resulted in a much higher specific activity of the ammonium sulfate fraction than previously obtained. The NaCl wash removed much extraneous protein, but little tissue factor. The resultant ammonium sulfate fraction has been increased in purity by a factor of 3–7 over similar fractions prepared from nonextracted powders (Nemerson, 1969).

Our previous attempts to purify the apoprotein by DEAE-cellulose chromatography resulted in little purification and excessive losses (about 85%). Column chromatography on

TABLE I: Purification of Lung Tissue Factor.

Preparation	Vol (ml)	Protein (mg/ml)	Total Protein (mg)	Lipid:Protein[a] (mg/mg)	Units ($\times 10^6$)	Yield (%)	Units/mg of Protein	Purification
Acetone powder			90,000		2.9	100	32	1
$(NH_4)_2SO_4$ 60% ppt	55	8.0	440	2.5:1	2.10	72	4,771	149
DEAE-eluate	117	1.75	205	2.5:1	2.17	74	10,640	333
Agarose chromatography	114	0.63	72	7.5–10.0:1	1.85	64	25,690	803
Agarose rechromatography	88	0.52	46	7.5–10.0:1	1.40	48	30,430	951

[a] Preliminary experiments were done with each preparation at lipid:protein ratios of 1.5:1; 2.5:1; 5.0:1; 7.5:1, and 10.0:1. The results obtained with the optimal ratio are indicated. These experiments were performed with mixed brain lipids.

DEAE-Sephadex resulted in somewhat better purification (about 1.5-fold) but the losses were still high (50%). Best results were obtained from batch elution of the apoprotein from DEAE-Sephadex: 2.0- to 2.5-fold purifications and about 100% recovery.

Gel filtration on columns of 6% agarose (which has about the same elution characteristics as Sephadex G-200, but gives sharper separations with this protein) resulted in additional purification (Table I). Rechromatography using the same column further increased the specific activity. The overall recovery from chromatography on agarose was 65%. In the experiment illustrated (Figure 1) coagulant activity was determined after relipidation and appeared as a peak with a distinct shoulder (the material used for this experiment is of lower specific activity than the preparation shown in Table I, although prepared identically). The activity profile contained two regions of constant specific activity; the later emerging shoulder (lower molecular weight component) had a specific activity of 25,000 units/mg, about 65% greater than that of the earlier component (17,000 units/mg). It is unlikely that this was due to contaminating proteins in the ascending limb as the specific activities of several fractions under this peak were approximately constant (Figure 1).

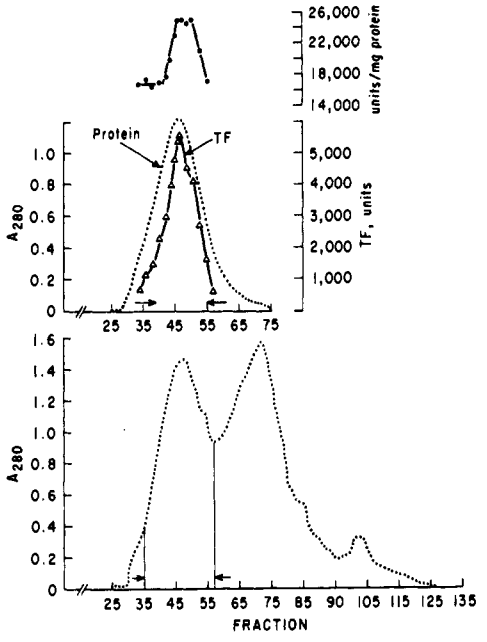

FIGURE 1: Gel filtration on 6% agarose (see Methods). The lower chromatogram depicts the first filtration. The fractions between the arrows were pooled and rechromatographed (upper chromatogram). Activity was located after relipidation of individual fractions with 7.5 mg of mixed brain lipid per mg of protein; the specific activity is presented in the same figure. Arrows on the upper chromatogram depict fractions which were pooled for further characterization.

As it was possible that the differences in specific activity reflected changing lipid requirements across the peak, every third fraction was recombined with lipid at ratios of 2.5, 5.0, 7.5, and 10.0 mg of lipid per mg of protein. In each fraction, optimal activity was obtained with 7.5 and 10.0 mg of lipid per mg of protein. Thus, the observed increase in specific activity reflects some intrinsic property of the protein, and not changing requirements for lipids.

It is of interest that the amount of lipid required for optimal activity appeared to rise with the purity of the protein. This is noted in Table I where the ratio is seen to rise from 2.5 mg of lipid per mg of protein at 4771 units/mg of protein to 7.5–10.0 mg of lipid per mg of protein at 30,400 units/mg of protein. As previous studies using a purification technique that did not include extraction with 0.5 M NaCl revealed an optimal ratio of 1.5 mg of lipid per mg of protein (Nemerson, 1969), a similar preparation was made and relipidated with the batch of mixed lipids currently in use. The material was carried through the ammonium sulfate fractionation, and had a specific activity of 775 units/mg of protein. The optimal ratio was again found to be 1.5 mg of lipid per mg of protein. It is clear, therefore, that the lipid requirement of tissue factor is a function of its purity.

The protein obtained from the second agarose column was pooled and further characterized. It contained 7% phospholipid by weight and was stimulated 950-fold by the addition of optimal amounts of mixed lipids. The procedure detailed in Table I yielded 46 mg of protein which was 950-fold purified over the acetone powders. Routinely, purifications of 800- to 1500-fold were obtained. This material is stable at room temperature for several days, at 4° for weeks, and for at least several months at −20° in 50% glycerol.

Ultracentrifugation of the purified protein in 5–30% sucrose gradients revealed the presence of two distinct activity peaks (Figure 2), with molecular weights of 220,000 and 330,000 (mean of three determinations). When protein from the 60%

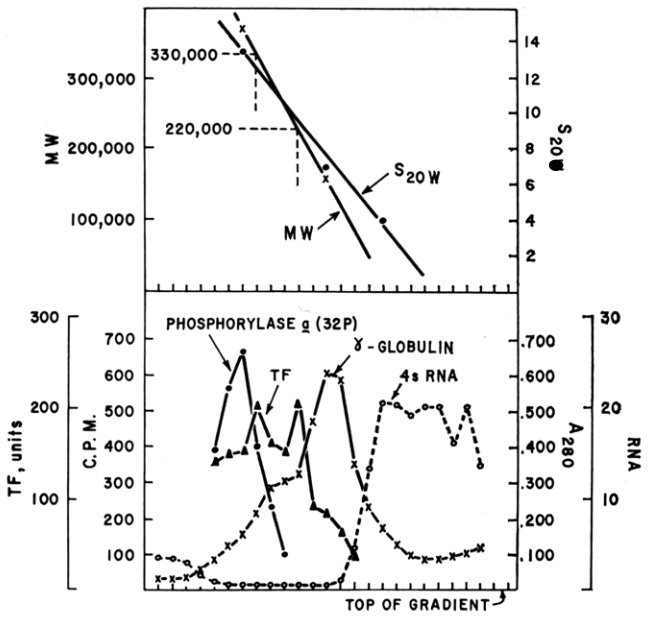

FIGURE 2: Sucrose density gradient centrifugation (18 hr at 36,000 rpm) of tissue factor (0.052 mg) and the molecular size markers 4S RNA (0.1 mg), γ-globulin (3.2 mg), and [^{32}P]phosphorylase *a* (0.548 mg, 12,088 cpm). Tissue factor activity was located after relipidation of each fraction with 0.05 mg of mixed brain lipids. The markers, centrifuged in a separate tube, were located after addition of 1.0 ml of H_2O to each fraction. Protein was determined as A_{280}; 4S RNA was located from A_{260}/A_{280} using the values of Chaykin (1966); 0.5 ml of each diluted fraction was added to 10 ml of scintillant and counted for 4 min. The values used for the markers were: phosphorylase *a*, 13.5 S, molecular weight, 370,000 (Seery *et al.*, 1967); γ-globulin, 6.6 S, molecular weight, 160,000; RNA, 4 S.

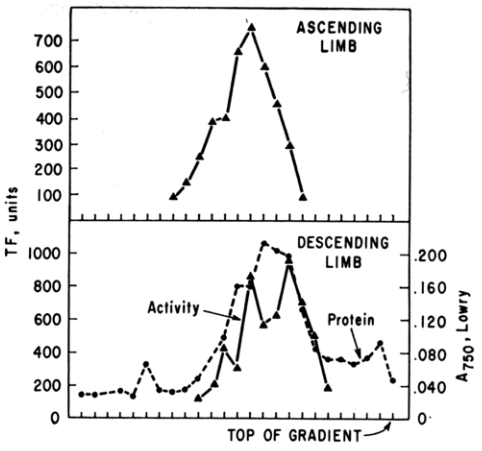

FIGURE 3: Sucrose density gradient centrifugation (6 hr at 36,000 rpm) of pools from the ascending and descending limb of the agarose column. Ascending limb, 70.5 μg of protein; descending limb, 141 μg of protein. Activity was located after relipidation of each fraction with mixed brain lipids (ascending limb, 50 μg of lipid per fraction; descending limb, 100 μg of lipid per fraction). Protein was located by the method of Lowry *et al.* (1951), using a complete fraction from a separate centrifuge tube for each determination.

ammonium sulfate precipitation was centrifuged, the same results were obtained.

The two activity peaks could not be resolved by gel filtration and therefore, the possibility of a dynamic equilibrium between the two species was considered. Accordingly, the ascending and descending limbs obtained from gel filtration were separately pooled and centrifuged as above (Figure 3). The activity derived from the ascending limb sedimented more rapidly than that from the descending limb, suggesting that the two species are indeed stable and distinct.

The tissue factor protein was electrophoresed in 5% polyacrylamide gels (Figure 4). Again two species were detected. Protein stains revealed two major bands (plus a minor contaminant running with the front). In addition, the region between the bands and for about 1 cm in front of the leading band was diffusely stained. An unstained gel was sectioned immediately after electrophoresis, eluted and assayed for coagulant activity. The coagulant activity was detected in two peaks in the region of the intensely stained protein bands. Interestingly, activity was also found between the bands and in front of the leading major band in areas corresponding to diffusely stained portions of the gel. This suggests that forms of tissue factor with lower molecular weights than the major components exist in an active state. It should be stressed that the disc gels yield only semiquantitative data with respect to clotting activity. Since measurement of the protein concentration of the gel eluates was not feasible, relipidation was not performed and the clotting activity of the apoprotein was determined.

In order to determine if one species of tissue factor contained more nonprotein material (which could account for the heterogeneity of tissue factor), disc gels were stained for lipid, acidic lipids, polysaccharide, acid mucopolysaccharide, and nucleic acid. As a control, chymotrypsin was electrophoresed under the same conditions and the gels were stained in the same manner. Chymotrypsin stained intensely with the protein stain, but not with the others. The tissue factor preparation did not contain nucleic acid. However, the areas which stained positively with aniline blue black, including the areas between the bands and in front of the anionic band, also reacted positively with the other stains. Only with sudan black B (lipid stain) was a difference noted between the two

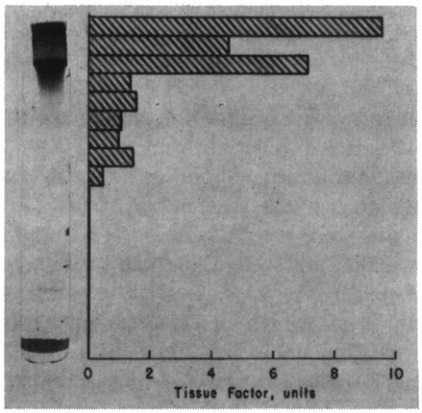

FIGURE 4: Disc gel electrophoresis at pH 9.5 in 5% acrylamide (100 μg of protein). One gel was stained with aniline blue black; the other gel was sliced in 3-mm sections and eluted for direct determination of coagulant activity. The direction of electrophoresis was from the top (cathode) to the bottom (anode).

bands: the upper (less mobile) band stained more intensely than the lower, and therefore, probably contained more lipid.

Discussion

Tissue factor is a particle-bound lipoprotein that initiates coagulation via the extrinsic system. The lipid component of tissue factor apparently is responsible for its insolubility since removal of most of its lipid renders the protein water soluble. The present study describes a technique for the preparation of about 40 mg of tissue factor protein from 100 g of lung acetone powder; this material is purified 800- to 1500-fold over the acetone powders (Table I).

The present technique of purification modifies and extends our previous methods. Although lung is a more difficult tissue to work with than brain, its tissue factor content is greater (Nemerson, 1969). Extraction of the delipidated acetone powder with 0.5 M NaCl prior to the solubilization of tissue factor with deoxycholate apparently increased the stability of the product. Previously, tissue factor decayed at a rate that made substantial purification impossible. Now, the product is stable.

We previously demonstrated that the coagulant activity of the solubilized apoprotein was increased by a factor of 500–1000 by the addition of phospholipids (Nemerson, 1969). The impure preparation used for these earlier experiments (775 units/mg of protein) contained less than 1% phospholipid and required 1.5 mg of lipid/mg of protein for maximal activity. Tissue factor prepared by the present method, however, requires at least 7.5 mg of lipid/mg of protein for maximal activity. This suggests that nonlipophilic contaminants are removed during purification. This is further supported by the parallel increase in residual lipid observed during purification: less than 1% at the lower specific activity to 7% at about 20,000 units/mg of protein. Phospholipid, presumably bound to tissue factor, is purified *parri passu* with the apoprotein. It is noteworthy, however, that 7% phospholipid confers only minimal coagulant activity on tissue factor; phospholipid must be increased 100-fold for maximal activity.

Disc gel electrophoresis clearly demonstrates the heterogeneity of tissue factor. Two major bands of protein were detected which correspond to the major portion of coagulant activity (Figure 4). The gels, however, also contained regions which stained diffusely for protein and had coagulant activity. This phenomenon is not due to overloading the gels as chymotrypsin banded sharply at the same concentration, and tissue factor was diffuse at even lower concentrations. It is not clear whether the recovery of protein is similar for these species, and, therefore, it is not certain that the relative amounts of activity detected following elution of the gels reflects the true distribution of the various tissue factor species.

The disc gels were treated with several specific strains to determine whether nonprotein material could account for the difference between the species. Both major bands stained equally with stains which detect acid mucopolysaccharide, polysaccharide, and acidic lipid. Nucleic acid was not present in either. Sudan black, however, which stains all lipids was significantly more intense in the upper band (cathodal) than in the lower. It therefore appears as if one species contains greater amounts of a neutral lipid than the other, although other hydrophobic material could account for the staining.

Two peaks of tissue factor activity were also detected by sucrose density gradient ultracentrifugation (Figure 2). The molecular weight estimates of these peaks (220,000 and 330,000) assume a partial specific volume of 0.73 ml/g for tissue factor and the protein markers. However, a lipoprotein complex containing 7% phospholipid would have a partial specific volume of 0.75 ml/g (assuming 1.07 ml/g for the lipid), which for any given sedimentation velocity would yield a higher molecular weight. Since it is not known how the lipid is distributed between the species seen in the gradients, suitable corrections could not be made. If the species in the gradient correspond to those detected in the disc gels, one component would presumably contain more lipid and have a correspondingly higher molecular weight.

When the apoprotein was gel filtered on 6% agarose, two species were also detected, the later emerging one appearing as a shoulder on the activity profile (Figure 1). Two regions of constant specific activity were found; the ratio of these specific activities, 1.47, closely approximates the ratio of the molecular weights estimated by ultracentrifugation, 1.50. This suggests that each tissue factor molecule may contain a single biologically active subunit irrespective of its molecular weight.

As the two species were regularly observed in approximately the same proportions, we investigated the possibility that they were in rapid equilibrium. The ascending and descending limbs obtained by gel filtration were centrifuged in a sucrose gradient. The ascending limb sedimented more rapidly than did the descending limb (Figure 3). Thus, the ultracentrifugal patterns were consistent with the gel filtration data; the earlier emerging species was of greater molecular weight than the later emerging species. We obtained no evidence, therefore, that the two species were in rapid equilibrium.

There are several possible explanations for the occurrence of the different species of tissue factor. First, tissue factor may exist as two different, but related proteins. Second, and perhaps more likely, is that the heterogeneity is a function of residual lipid. Lipid, for example, could serve as a nidus for the aggregation of tissue factor molecules or, perhaps of subunits. The differing specific activities of the species, then, could be due to aggregates in which some molecules are inactive because their access to factor VII is restricted. Alternatively, the presence of excess lipid may hold the protein units in a conformation which limits their ability to interact with phospholipids during the recombination procedure. Finally, the lipid may bind contaminating proteins which are not resolved by the conditions used in these experiments. Until the two species can be isolated in quantities sufficient for analysis, or all the lipid can be removed while retaining potential biological activity the explanation for the heterogeneity of tissue factor must remain speculative.

Acknowledgment

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Binding of the Protein Component of Tissue Factor to Phospholipids*

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ABSTRACT: The coagulant activity of tissue factor, a particle-bound lipoprotien, is lipid dependent. Phosphatidylethanolamine and phosphatidylcholine restore activity to delipidated tissue factor protein if lipid and protein are combined in the presence of deoxycholate, then dialyzed. After this recombination procedure, tissue factor activity, protein and phospholipid band isopynically as a complex in a 5–30% sucrose gradient. If deoxycholate is omitted during the recombination, lipid does not bind to tissue factor nor is activity restored. The complex incorporates increasing amounts of phosphatidylethanolamine from 2.5 to at least 7.4 mg of lipid per mg of protein, although the optimal ratio for restoration of activity for these preparations is 5.0 mg of lipid per mg of protein. Phosphatidylcholine, which does not restore activity as effectively as phosphatidylethanolamine, nevertheless, is bound as well. If reduced phosphatidylcholine or lysophosphatidylcholine is substituted for phosphatidylethanolamine, activity is not restored. These phospholipids do interact with the protein, however, since the sedimentation characteristics of each are altered after the recombination procedure. Reduced phosphatidylcholine complexes with apoprotein only at very high lipid to protein ratios. Since a lipoprotein complex containing lysophosphatidylcholine cannot be isolated, it is concluded that these complexes are unstable to centrifugation in a sucrose gradient. Thus, restoration of tissue factor activity requires a specific interaction of lipid and protein which results in a stable lipoprotein complex. Since tissue factor also binds ineffective phospholipids, restoration of activity is considered in terms of phospholipid structure. Although binding to phospholipid is required for restoration of coagulant activity to tissue factor, binding alone does not restore activity.

The role of tissue factor in the initiation of blood coagulation has been studied in detail by Nemerson (1966) and in Williams' laboratory (Williams, 1966; Williams and Norris, 1966). Experiments with purified bovine lung microsomes and crude bovine brain particles have established that tissue factor, in the presence of calcium, forms a complex with factor VII; this complex then enzymatically converts factor X into its activated, enzymatic form.

Tissue factor is a lipoprotein that requires the presence of its lipid component for coagulant activity. The specificity of the lipid requirement has been reported previously from this laboratory (Nemerson, 1968, 1969). Crude tissue factor preparations are 38–45% phospholipid by weight (Nemerson, 1968). The solubilization and partial purification of the protein moiety of tissue factor has been reported earlier (Nemerson, 1969). An accompanying article (Nemerson and Pitlick, 1970), describes in detail the further purification of the apoprotein. Although the apoprotein possesses residual tissue factor activity, recombination of tissue factor with certain phospholipids enhances this activity 500- to 1000-fold.

Crude delipidated brain particles have been demonstrated to bind phospholipid; however, PS[1], which did not restore biological activity, was bound as effectively as PE and PC

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[1] Abbreviations used are: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidyl-L-serine; lysoPC, lysophosphatidylcholine; reduced PC, reduced phosphatidylcholine.