used in the separation process. The molecular weight of the enzyme attacking Pro-Val-Gly was greater than 150000 and that of the enzyme attacking Pro-Gly-Gly was 55000 (Fig. 2).

It thus appears that there may be at least two different enzymes in bovine kidney involved in the catabolism of tripeptides containing N-terminal proline. Any relationships between them must await further purification and study.


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Infrared Studies on Pig Brain Thromboplastin

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Thromboplastin or factor III is a membrane-bound lipoprotein that can initiate the extrinsic system of blood coagulation by combining with factor VII, a plasma protein. The complex formed by these factors enzymically converts factor X into its activated form, which generates thrombin from prothrombin. The coagulant activity of tissue factor III is a function of its lipid content. Separation of phospholipids from the protein component results in loss of coagulant activity, which can be restored by recombining the protein with phospholipids of appropriate charge (Nemerson, 1969).

In the present study the protein component of thromboplastin was isolated from pig brain by the procedure of Nemerson & Pillick (1970) and its activity was restored by relipidation with a mixture of soya-bean phospholipids. It was found that optimum activity was restored when 1 mg of the apoprotein was recombined with 10 mg of phospholipid dispersed in 0.25% (w/v) sodium deoxycholate. A circular-dichroism (c.d.) technique has been used by Howell & Rezvan (1977) to determine whether or not the function of thromboplastin is related to a particular protein configuration in the presence of those lipids and detergents which restore its activity. The c.d. studies in the far-u.v. region showed that relipidation induced no significant conformational change in the α-helical part of the protein. This contrasts with the increased α-helical content which occurs when plasma lipoproteins are reconstituted (Andrews et al., 1976) and emphasizes the membranous source of thromboplastin. However, there were indications that a conformational change had occurred in the β-structure of the apoprotein.

In the work reported here we have therefore attempted to gain further information on any lipid-dependent conformational changes of thromboplastin as a membrane-derived factor by using i.r. spectroscopy. I.r. analysis complements c.d. because it can distinguish the β-structure (parallel and anti-parallel) as a distinctive band at 1630 cm⁻¹ (Timasheff & Gorbunov, 1967), whereas the maximum ellipticities due to α- and β-configurations merge in the c.d. spectrum. However, the amide I band in the i.r. spectrum, which arises mostly from carbonyl stretchings, is difficult to interpret, since it occurs at about 1650 cm⁻¹ for both the α-helical and random-coil conformations; the position of the amide II band (N–H bending) is more variable than the amide I band.

Spectroscopic samples were prepared by drying either apoprotein or relipidated thromboplastin on AgCl discs. Alternatively freeze-dried material reconstituted in ²H₂O was sandwiched between two AgCl discs. Spectra were then recorded in each case on a Perkin–Elmer 325 double-beam grating i.r. spectrophotometer at room temperature.

The spectrum of a dry film of the apoprotein is shown in Fig. 1(a). This shows a sharp peak at 1650 cm⁻¹ indicating the presence of α-helix with random coil. There also exists a distinct shoulder at 1630 cm⁻¹ typical of β-structure, which is in contrast with
the findings of Chapman et al. (1968) for erythrocyte membranes. Fig. 1(b) shows the spectrum of the same protein recombined with an optimum concentration of soya-bean phospholipids dispersed in 0.25% (w/v) sodium deoxycholate. An important feature is the marked splitting of the amide I band with peaks at 1630cm⁻¹ and 1650cm⁻¹, a finding in accordance with those of Cherry et al. (1971) for recombinant lipids and proteins of erythrocyte membrane. These observations confirm to a large extent the results of c.d. in that there appears to be a definite conformational change in the limited amount of α-structure present in the apoprotein.

A spectrum of a heat-denatured sample of the apoprotein shows a sharp broadening of the shoulder at 1630cm⁻¹. When another portion of the apoprotein was heated similarly at 70°C for 30 min 80% of clotting activity was destroyed. This close association between loss of clotting activity and β-structure suggests an important role for a limited amount of the latter for the apoprotein to function in clotting.

When the apoprotein, in the presence and absence of phospholipids and detergent, was transferred to ²H₂O a change was noted in both of the amide absorptions (Fig. 2). Compared with the dry-film preparations in Fig. 1, the amide I band at 1650cm⁻¹ is now diminished relative to that at 1630cm⁻¹. In Fig. 2(a) it can be seen that the intensity of the amide II band at 1540cm⁻¹ has now decreased, and it is replaced by a much sharper band at 1450cm⁻¹ indicating that most protons are accessible for exchange with ²H from ²H₂O. However, in Fig. 2(b) a greatly decreased amide II band at 1540cm⁻¹ has reappeared, due apparently to some of the protons not being exchangeable because they are buried in the hydrophobic lipid phase.

When the above findings are considered together with the earlier results from c.d. experiments we concluded that the presence of both α-helical and β-configurations of the apoprotein are necessary for optimal biological activity of thromboplastin. Furthermore, the rearrangement of the β-region of the protein on addition of specific phospholipids, such as phosphatidylethanolamine, might indicate an optimum orientation.
Fig. 2. *I.r. spectra of thromboplastin apoprotein in $^2\text{H}_2\text{O}$

Spectra were recorded of (a) 1 mg of apoprotein/ml and (b) 1 mg of apoprotein/ml in the presence of soya-bean phospholipids (10 mg/ml) dispersed in 0.25 % (w/v) sodium deoxycholate. Measurements were carried out after dissolving freeze-dried apoprotein in 0.25 M-NaCl in $^2\text{H}_2\text{O}$, buffered with 0.025 M-Tris/HCl, pH 7.0.

of thromboplastin protein for its subsequent interaction with factor VII in the next step of blood coagulation. It is noteworthy that immunological studies (Zeldis et al., 1971) have shown that the protein component is exposed on the external face of plasma membranes, whereas the procoagulant lipids, such as phosphatidylethanolamine, are normally unavailable, being located on the cytoplasmic side. Tissue damage could be expected to promote clotting by turning the membrane inside out.

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