Purification of Fibrinogen and the Separation of its Degradation Products in the Presence of Calcium Ions

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A desire to investigate the influence of Ca²⁺ on the structure and properties of fibrinogen and its degradation products has necessitated the development of novel purification and separation procedures. The purification scheme of Mosesson & Finlayson (1963) yields a fibrinogen free from factor XIII and plasminogen, but involves the use of Tris/phosphate buffers and is therefore incompatible with the presence of Ca²⁺. Separation of the plasmin degradation products, fragments D and E, can be achieved by using CM-cellulose (Kemp et al., 1973) or DEAE-cellulose (Nussenzweig et al., 1961). We wished to avoid the use of acidic buffers, since Marguerie et al. (1977) have reported that the binding of at least one Ca²⁺ ion is abolished at pH values below 6.5. Published DEAE-cellulose methods are again incompatible with Ca²⁺, because they use phosphate-buffer systems.

The starting material was human fibrinogen (grade L) from KABI Pharmaceuticals (Stockholm, Sweden). Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate was carried out as previously described (Lawrie & Kemp, 1979). Fractions were tested for the presence of plasminogen as follows. Streptokinase was added to a final concentration of 200 i.u./ml and the test sample incubated at 37°C for 2 h. Samples were examined for proteolytic degradation by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. In order to detect factor XIII, samples were incubated with a final concentration of 5 National Institute of Health units of thrombin/ml and 20 mm⁻¹ CaCl₂ for 2 h at 37°C. The presence of cross-linked γ chains after electrophoresis of the reduced sample indicated the presence of factor XIII.

Fibrinogen purification

Fibrinogen at a concentration of 5 mg/ml was dialysed at 4°C against 0.05 M-Tris/HCl buffer, pH 8.6, made 0.05 M with respect to NaCl and 2 mm⁻¹ with respect to CaCl₂. At 4°C there was an extensive precipitate, which almost completely dissolved on warming to 37°C for 5 min. Any remaining precipitate was removed by centrifugation and discarded. The supernatant was applied to a column (1.5 cm x 25 cm) of DEAE-cellulose equilibrated with the Tris/NaCl/CaCl₂ buffer. The column was eluted first with the equilibration buffer, followed by this buffer made 0.1 M and then 1.0 M in NaCl. The elution profile shown in Fig. 1 resulted.

As judged by gel electrophoresis, peak 1 was fibrinogen with very little degradation apparent. There was a faint band with a mobility between that of the (A)α and (B)β chains, but no other indication of (A)α-chain degradation. This peak contained no detectable plasminogen or factor XIII.

Peak 2 was a degraded fibrinogen showing little intact (A)α chain, but significant amounts of plasminogen and factor XIII.

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Fig. 1. DEAE-cellulose chromatography of fibrinogen

Stepwise elution was carried out with increases in NaCl concentration as indicated by the arrows.

Peak 3 was composed almost entirely of a high-molecular-weight component with a lower mobility than fibrinogen on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. After reduction, the mobility increased, but there was still one band. This material could be fibronectin, which is known to associate with fibrinogen (Yamada & Olden, 1978).

Separation of fragments D and E

Fibrinogen was digested in the presence of 2 mM CaCl₂ as previously described (Lawrie & Kemp, 1979). The digest was dialysed against 0.05 M Tris/HCl buffer, pH 7.5, 2 mM in CaCl₂, and applied to a column (20 cm × 2.5 cm) of DEAE-cellulose equilibrated with the same buffer. Fragment D passed straight through, but fragment E was eluted with the equilibration buffer, 0.3 M in NaCl. Fragment D was sometimes contaminated with fragment Y. In this case, gel filtration on Sephadex G-200 was necessary to separate them.

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