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Purification of Bovine and Human Fibrinogen

Birger Blombäck and Margareta Blombäck

Chemistry Department II, Karolinska Institutet, Stockholm, Sweden

A prerequisite for the study of different coagulation systems is a supply of purified and stable substrates. When studying the inhibition of thrombin by heparin + cofactor we found fibrinogen prepared by salt precipitation or by freezing out [1] to be too impure and unstable. During the course of our work we succeeded in getting a stable and pure fibrinogen by treating fraction I of Cohn's [2] method 6 with glycine.

The effect of glycine on the solubility of proteins has been thoroughly studied by a number of authors [3-6]. As has been pointed out by Cohn *et al.* a polar substance such as glycine strongly reduces the protein-protein interaction. This principle was adopted by Cohn *et al.*[7] in their method 10 of 1950 for the isolation of γ-globulin and β_1-lipoprotein from a human plasma precipitate.

We have found that the salting-in effect on fibrinogen increases sharply in rising the glycine concentration to 0.5 M (at an ionic strength of 0.3) and that at higher glycine concentrations there is a salting-out effect. Thus if Cohn's fraction I is extracted during one hour at —3° C with a solution of 1 M glycine in a citrate buffer of pH 6 and ionic strength 0.3 containing 6.5 % ethanol the fibrinogen and the cold insoluble globulins are almost insoluble. On the other hand the other proteins contaminating fibrinogen in fraction I, including prothrombin, are dissolved and thus removed.

The insoluble fraction is then dissolved at +25° C in a citrate buffer, pH 6.35 and ionic strength 0.3, containing 0.12 M glycine. When this solution is cooled to 0° C and ethanol added to a concentration of 2 % the cold insoluble globulins, described by Edsall [8,9] are precipitated. They are removed and the fibrinogen is precipitated by increasing the ethanol concentration to 6.5 % at —3° C. The final product has a clottable / non clottable ratio of 96—97 %. It is now free of prothrombin, plasmin, heparin-cofactor and the antihemofilic globulin. It is also practically free of plasminogen and of the proactivator of plasminogen. Electrophoresis in the Tiselius apparatus at pH 6.6 showed only one single boundary. The yield was 70 % calculated on the fibrinogen in fraction I, thus considerably higher than in the procedure of Kekwick *et al.*[10]. The technique works equally well on human as on bovine plasma.

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