ISOELECTRIC FOCUSING OF FIBRINOGENS ON POLYACRYLAMIDE GELS

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1. Introduction

Previous investigations demonstrated that cobalt ions change the conformation of rabbit fibrinogen as shown by increased electrophoretic mobility and heterogeneity on agarose gel electrophoresis [1–3]. The present study extends this work by further analysis of normal and cobalt modified fibrinogen. Normal rabbit fibrinogen was separated into 3 fractions (isoelectric points 5.45–5.5) by means of isoelectric focusing in polyacrylamide gels. Cobalt modified fibrinogen (fibrinogen prepared from plasma of rabbits injected with cobalteous ions) separated into 7 fractions (isoelectric points 5.0 to 5.15). All these fractions showed antigenic identity with the unfocused protein when tested by gel diffusion against sheep anti-rabbit fibrinogen.

2. Material and methods

Normal and cobalt modified fibrinogen (prepared from plasma of rabbits given 5 mg Co/kg b.w. as cobalt (II)-L-glutamate or cobalt (II) chloride) were prepared from pooled plasma as described [2]. Both proteins were rechromatographed on a Sephadex G-200 column (2.5 × 100 cm). Normal fibrinogen was 99 to 100% clottable as estimated according to Blombäck [4]. No impurities could be detected on immuno- and disc-electrophoresis of normal and cobalt fibrinogens.

Gel solutions (10 ml) containing 0.5 g acrylamide, 55 mg *N,N'*-methylene bisacrylamide, 5 µl *N,N,N',N'*-tetramethyl ethylene diamine, 0.05 mg riboflavin, 0.25 ml of 40% Ampholine pH range 3–6 (LKB Producter AB, Bromma) and 0.2 to 0.4 mg of protein were photopolymerized in glass tubes 7 cm × 0.5 cm inner diameter. The amount of protein was doubled in experiments on immunological characterization. The anodal solution was 0.12% H_3PO_4 and the cathodal was 0.1% ethylene diamine solution. Electrofocusing was carried out for 2 min at 1 mA per gel, and the voltage then increased to 300 V during 60 min at a maximum of 5 mA per gel. Electrophoresis was continued for 24 hr, during which time the current decreased below 0.3 mA per gel. Gels were stained according to the method described by Awdeh [5] and the pH gradients in gels determined as

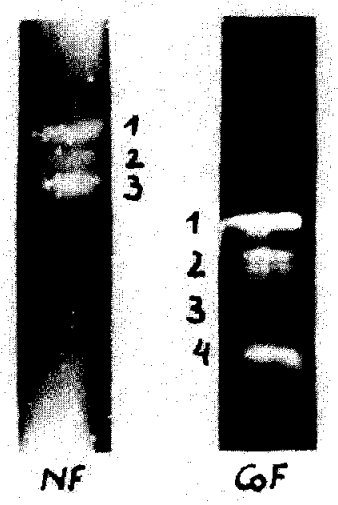

Fig. 1. Isoelectric fractionation of normal (NF) and cobalt (CoF) fibrinogens.

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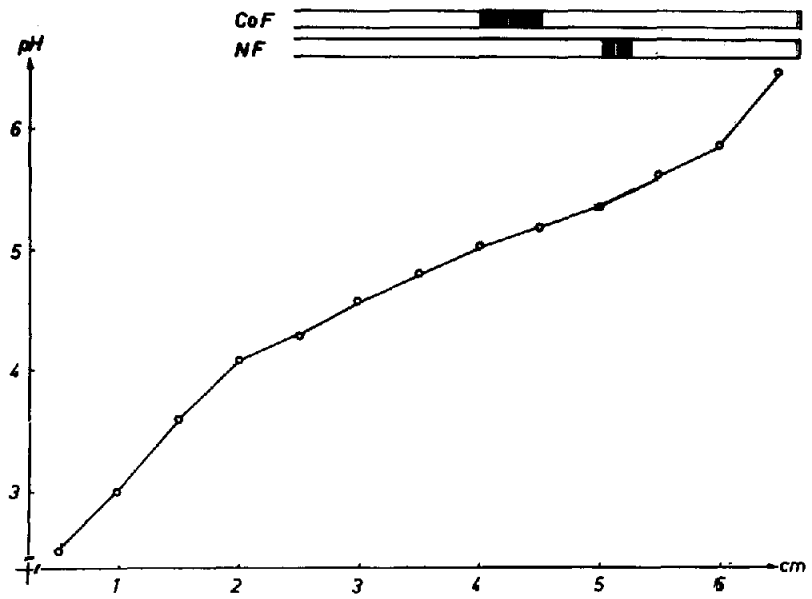

Fig. 2. pH gradients after electrofocusing (1% carrier ampholyte, pH range 3–6), showing positions of normal and cobalt modified fibrinogen bands drawn from stained gels.

described [8, 9]. Immunological experiments employed Catsimpoolas' sectional immuno-electrofocusing technique [6] using Ouchterlony plates (1% agarose, 0.1 M tris pH 7.4) and sheep antisera against rabbit fibrinogen and rabbit serum proteins [7].

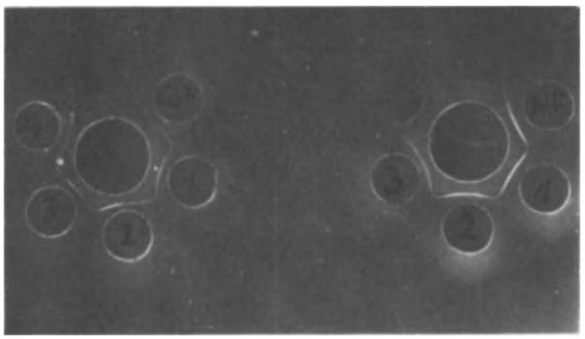

Fig. 3. Gel diffusion of normal (NF) and cobalt (CoF) fibrinogen and their focused fractions reacted with anti-fibrinogen serum. Numbers of the sample wells correspond to number pictured in fig. 1.

3. Results and discussion

Normal fibrinogen separated into 3 closely adjacent zones in the pH region 5.45 to 5.5. Cobalt modified fibrinogen resolved into 7 fractions in the pH range 5.0 to 5.15 (figs. 1 and 2). The focused bands of normal fibrinogen precipitated with anti-fibrinogen serum (fig. 3) but not with an antiserum to rabbit serum proteins. The four fractions (fig. 1) of cobalt modified fibrinogen also reacted with anti-fibrinogen but not antiserum proteins and gave reactions of identity with the unfocused fibrinogen (fig. 3).

The heterogeneity of normal fibrinogen is not surprising, because microheterogeneity of fibrinogen chains has been shown (*S*-sulfoderivatives of the α(A), β(B), γ chains) [10–12] and fibrinogen derivatives with distinct electrophoretic mobility, molecular weight and solubility have been described [13]. Furthermore, aggregates or precipitates may be formed at high protein concentration near the isoelectric points giving a false impression of heterogeneity. The isoelectric point of normal fibrinogen was estimated to be within the pH range of 5.45 to 5.5 confirming the value observed on agarose gel electropho-

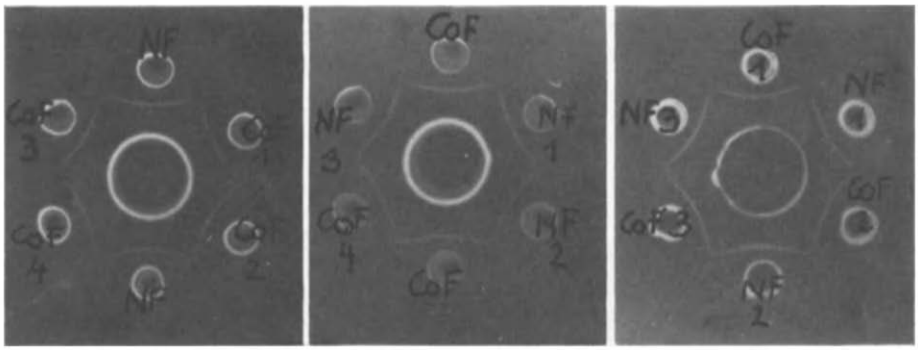

Fig. 4. Gel diffusion showing reactions of identity between normal (NF) fibrinogen and cobalt fibrinogen (CoF) and their electrophoretic components. Centre wells contain sheep antifibrinogen. Numbers refer to fractions shown in fig. 1.

resis where fibrinogen moves as a single fraction [14]. The isoelectric points of cobalt fibrinogen components are lower as indicated by earlier results [1,2]. No immunochemical difference between the normal and cobalt modified fibrinogen [1] and their focused bands have been detected (fig. 4).

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