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Note

Fast protein liquid chromatography of pyridoxalated and glutaraldehyde-treated human haemoglobin

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Pyridoxalated and polymerized haemoglobin derivatives have been investigated as prospective oxygen-transporting blood volume expanders[1–6]. Sensitive analytical methods are necessary to characterize and check the quality and distribution of protein subfractions of these complex products. Electrophoresis[2], isoelectric focusing (IEF)[1] and chromatofocusing[7] have been found suitable for this purpose.

In the present paper we report on the feasibility of fast protein liquid chromatography (FPLC) of the above haemoglobin derivatives using an anion-exchange column, a salt concentration gradient and a new FPLC apparatus[8].

MATERIALS AND METHODS

Stroma-free human haemoglobin (SFH) was prepared from outdated human erythrocytes by a standard technique[9]. Pyridoxalated and borohydride-treated SFH was subsequently modified with 25 mg glutaraldehyde per 1 g of haemoglobin (SFH-P-G/25) as described previously[1] and stored for 6 days at −5°C before fractionation by FPLC. The haemoglobin (Hb) concentration was 60 g/l, and the methaemoglobin content was 15% of the total Hb. P_{50} (37°C, pH 7.40) was 1.86 kPa for SFH and 2.30 kPa for SFH-P-G/25. Before application to the chromatographic column HR5 (the Pharmacia FPLC system), the samples were equilibrated with 0.020 *M* Tris buffer, pH 8.0, by using gel filtration on Sephadex G-25. The two samples were applied to a Mono Q prepacked column HR5 and eluted with a linear gradient of NaCl in the same buffer.

RESULTS AND DISCUSSION

A typical FPLC result is shown in Fig. 1. Modified haemoglobin was fractionated into ten or eleven peaks. The elution pattern of SFH-P-G/25 was markedly different from that of native haemoglobin, showing only four peaks, eluted at concentrations of up to about 0.1 *M* NaCl. The fractions eluted at NaCl concentrations above 0.1 *M* correspond to chemically modified haemoglobin molecules possessing a higher negative charge, predominantly due to the induction of phosphate groups. The

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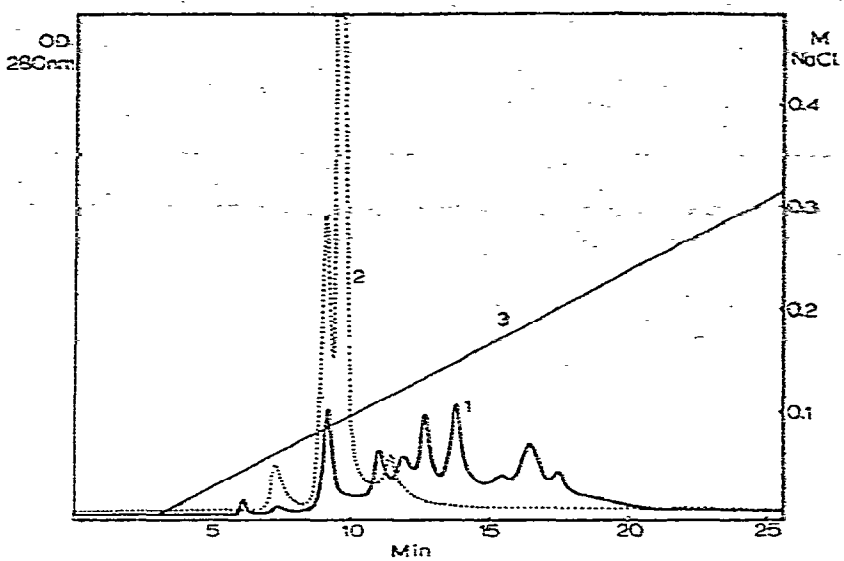

Fig. 1. Fast protein liquid chromatography of SFH and SFH treated with pyridoxal-5-phosphate and glutaraldehyde (SFH-P-G/25). Column: HR5 pre-packed with Mono Q anion exchanger. Eluent: 0.02 *M* Tris pH 8.0 with NaCl gradient up to 0.5 *M*. Curves: 1 = SFH; 2 = SFH-P-G; 3 = gradient of NaCl.

portion of modified SFH is about 75% of the total, in good agreement with previous results[1,2].

In general, there is a similarity between the fractionation of SFH-P-G by IEF in a pH gradient (*cf.*, ref. 1) and by FPLC in a salt gradient, since both separation techniques are based on the charge differences between individual molecular subpopulations. The number of fractions was ten to twelve in both cases. Moreover, the relative positions of the first six peaks in Fig. 1, curve 1, were comparable to those observed in IEF of SFH-P-G. Faster chromatography at steeper salt gradients also led to a fair fractionation within about 5 min, however, the conditions used in Fig. 1 seemed to be preferable. The present results do not enable a detailed identification of protein fractions present in a very complex and dynamically equilibrated system of different variants of haemoglobin molecules (*cf.*, refs. 3, 7). Nevertheless, FPLC has proved to be a very sensitive and useful method for rapid analysis of the heterogeneity of pyridoxalated haemoglobin derivatives.

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