Journal of Chromatography, 280 (1983) 165–166
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,156

Note

The reproducibility of fast protein liquid chromatography of pyridoxalated haemoglobin copolymerized with serum albumin

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(Received July 20th, 1983)

Fast protein liquid chromatography (FPLC)[1] is a new sensitive technique which has been found also very useful in our laboratory for rapid fractionation and detailed characterization of modified human stroma-free haemoglobin (SFH)[2]. The present communication presents recent results achieved with repeated FPLC runs of a variant of modified SFH. The aim of our experiments was to check the reproducibility of the FPLC method under intentionally strict conditions using a very complex protein mixture and a relatively high sensitivity during registration of the elution curves.

MATERIALS AND METHODS

A mixture of human stroma-free haemoglobin and serum albumin was modified by means of pyridoxal-5-phosphate, sodium borohydride and glutaraldehyde under standard conditions[1,3] suitable for the preparation of a variant of an oxygen-transporting blood substitute[4]. FPLC was done on a HR5 column pre-packed with Mono Q anion exchanger (Pharmacia FPLC system)[5]. A 1.6-mg sample of protein in 0.13 ml of 0.02 *M* Tris buffer (pH 8.0) was eluted with a linear sodium chloride gradient up to 0.3 *M* at 22–25°C as in our previous work[2]. Samples were run repeatedly and standard deviations from the mean were calculated at the most significant deflection points (maxima and minima) of the elution curves.

RESULTS AND DISCUSSION

Elution curves registered at sensitivities 2 and 1 were relatively smooth while at higher sensitivity a number of smaller peaks appeared. To ascertain their reproducibility under routine analytical conditions a randomly selected sample was run five times repeatedly at identical conditions at sensitivity 0.5. Curve 1 in Fig. 1 is the mean of those five elution curves. The dotted area covers the standard deviation (S.D.) from the mean. The S.D. did not exceed 8% which is a very good result in favour of FPLC. Curve 2 belongs to another batch of modified haemoglobin pre-

0021-9673/83/$03.00

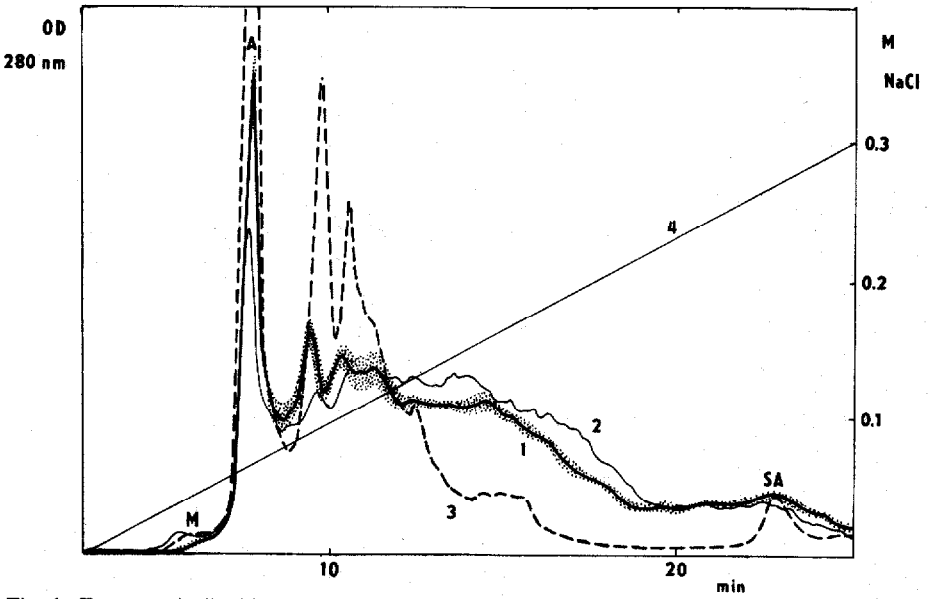

Fig. 1. Fast protein liquid chromatography of pyridoxalated (P) stroma-free haemoglobin (SFH) and serumalbumin (SA) copolymerized by means of glutaraldehyde (G). Column: HR5 pre-packed with Mono Q anion exchanger. Eluent: 0.02 *M* Tris pH 8.0 with pH gradient gradient up to 0.5 *M*. Curves: 1 = SFH–SA–P–G; dotted area covers the standard deviation from the mean of five repeated runs; 2 = another batch of SFH–SA–P–G; 3 = SFH–SA–P; 4 = gradient of sodium chloride; M = methaemoglobin; A = oxyhaemoglobin.

pared in a similar way but from a different haemolysate. Although the general shapes of both curves were mutually similar, there were certain deviations between them surpassing the S.D. which were due to slight differences in the material and in the preparative process. Curve 3 corresponds to the pyridoxalated inter-product taken during the preparation of sample 1 just before the addition of glutaraldehyde. Marked differences between the curves allows the logical deduction that glutaraldehyde reacts with a part of the intact or slightly pyridoxalated haemoglobin (the three or four peaks on the left) with formation of more acidic copolymerized subfractions. The identification of the peaks of haemoglobin A, methaemoglobin (M) and serum albumin (SA) was done from separate FPLC experiments. There is a good general accordance between the conclusions concerning the heterogeneity of modified SFH derived from FPLC under the above conditions and from flat-bed isoelectric focusing[2,6] and chromatofocusing[7]. FPLC has the advantage of direct quantitation of the results with good reproducibility, even in routine work.

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