THE USE OF IMMOBILIZED CIBACRON BLUE IN PLASMA FRACTIONATION

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1. Introduction

Human serum albumin is a major contaminant in the isolation of many plasma proteins. It constitutes about 55% of the total plasma protein and is responsible for non-specific binding to many affinity columns. The use of Blue Dextran to abstract albumin from plasma was first reported by Travis and Pannell in 1974 [1]. The efficiency of the system was improved [2] by the use of the chromophore of Blue Dextran, viz. Cibacron Blue, fig.1, attached directly to agarose.

We have already reported the influence of the matrix on the interaction of immobilized Cibacron Blue with albumin [3]. In this paper we describe the effect of pH on the adsorption of plasma by Cibacron Blue-Sepharose.

2. Materials and methods

Sepharose 6B was obtained from Pharmacia (G.B.) Ltd., London W.5. Cibacron Blue F3G-A was a gift from Ciba-Geigy, Manchester. Late pregnancy plasma was kindly provided by the North West Regional Blood Transfusion Centre, Liverpool.

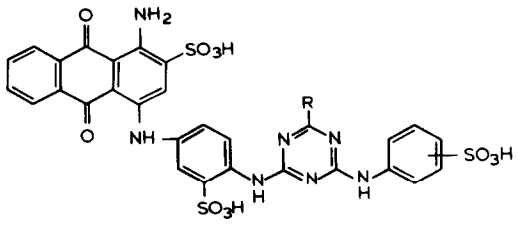

Fig.1. The structure of Cibacron blue F3G-A.

Anti-prealbumin and anti-retinol binding protein were kindly given by Professor Glover, Department of Biochemistry, Liverpool. Antiserum to a pregnancy specific protein, SP_1 was a gift from DAKO-Immunoglobulins, Denmark. All other antisera were generously donated by Dr P. Johnson, Department of Immunology, Liverpool University. All other chemicals were obtained from BDH Chemicals, Poole, Dorset.

Cibacron Blue-Sepharose (CBS) was prepared by the method of Heyns and De Moor [4]. One ml quantities were packed in columns, diameter 6 mm and equilibrated with 10 mM buffers at various pHs. The buffers used were as follows:

Acetic acid/sodium acetate; pH 4.0, 4.4, 4.8, 5.2
Potassium dihydrogen phosphate/sodium hydroxide; pH 4.9, 5.2, 5.5, 5.8
Potassium dihydrogen phthalate/sodium hydroxide; pH 5.8, 6.3, 6.8, 7.2, 7.6, 8.0
Tris/hydrochloride; pH 7.85, 8.2, 8.6, 8.9
Glycine/hydrochloride; pH 8.6, 9.0, 9.4, 9.8, 10.3

All chromatographic operations were carried out at 4°C with a flow rate 4 ml/h.

Late pregnancy plasma was dialysed against the appropriate buffer and 100 μl of the clarified dialysate were applied to each column. The columns were washed with their respective buffers and unadsorbed protein was estimated by the method of Lowry [5]. Final desorption was carried out with 0.5 M sodium thiocyanate.

In the second part of the experiment a similarly treated column at pH 5.85 was eluted with a pH gradient (10 mM KH_2PO_4/NaOH pH 5.85 (10 ml) against 20 mM K_2HPO_4/NaOH, pH 11.0 (10 ml)). 0.9 ml Fractions were collected, the pH and A_{280} determined and concentrated to 100 μl using an Amicon Minicon B15 concentrator. 5 μl of each

Table 1
Immunodiffusion analysis of proteins present in fractions of the pH gradient from fig.3

	pI	Unadsorbed protein	Fraction number 2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Orosomucoid	2.7	+++																						
Prealbumin	4.7	+++																						
Transferrin	5.9	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+	+	+	+	+	+	+	+	+	–
Retinol-binding protein	4.7	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	+	+	+							
Haptoglobin	4.1	–	–	–	–	–	+	+	++	+++	+++	+++	++	+	–	–	–	–	–	–	–	–	–	–
Ceruloplasmin	4.4	–	–	–	–	–	–	–	–	+	++	+++	+++	+	–	–	–	–	–	–	–	–	–	–
α_2-Macroglobulin	5.4	–	–	–	–	–	–	–	–	–	+	++	++	+	+	–	–	–	–	–	–	–	–	–
Immunoglobin G	5.8–7.3	–	–	–	–	–	+	+	+	+	++	+	+++	+++	+++	+++	+++	+++	+++	++	+	–	–	–
C_3 Factor of complement		–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	++	++	+	–	–	–	–	–
SP_1	~4.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	++	+++	++	–	–	–	–	–
Albumin	4.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+

The eluted fractions from the pH gradient were reduced to 100 μl and tested against the available antisera by double immunodiffusion on microscope slides. (–) No precipitate observed. (+, ++, +++) Increasing density of precipitin lines

sample were tested against the available antibodies by the double diffusion method of Ouchterlöny [6].

3. Results and discussion

As expected from the negative charge on the sulphonate groups of Cibacron Blue increasing amounts of protein were found to bind to CBS as the pH was lowered. At pH 4 about 96% of the total protein was bound to the adsorbent. Fig.2 shows the percentage of bound protein at various pH values, as estimated from the unbound protein content.

The results of the double diffusion tests were recorded qualitatively, depending on the density of the precipitates (see table 1). Fig.3 illustrates the elution profile obtained when a pH gradient was applied to a column at pH 5.85.

It is clear from table 1 and fig.3 that the appearance of a protein on the pH gradient is not necessarily related to its pI, especially in the cases of prealbumin, transferrin and SP_1. With respect to the known physicochemical properties of the proteins concerned the reason for the elution pattern obtained is not understood. It is, however, obvious that as well as abstracting albumin this adsorbent is capable of fractionating plasma proteins in a manner different to that of the usual ion exchange celluloses. It may be used in conjunction with the latter as a cheap and simple general method for plasma fractionation.

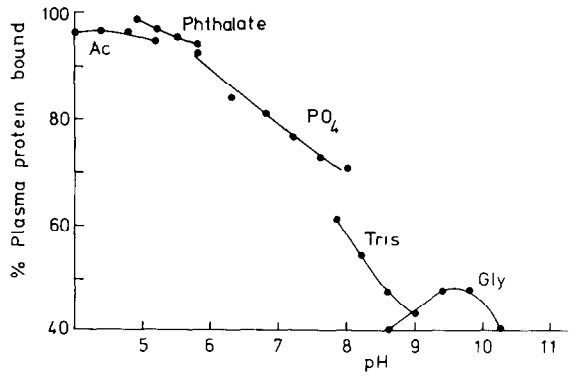

Fig.2. The effect of pH on the adsorption of plasma proteins to Cibacron Blue–Sepharose. Columns, 1 ml, of CBS were equilibrated with 10 mM buffers at various pH values. 4°C, 4 ml/hr. 100 μl of late pregnancy plasma was applied to each column. The protein adsorbed was calculated from the percentage of protein remaining in the washing buffers.

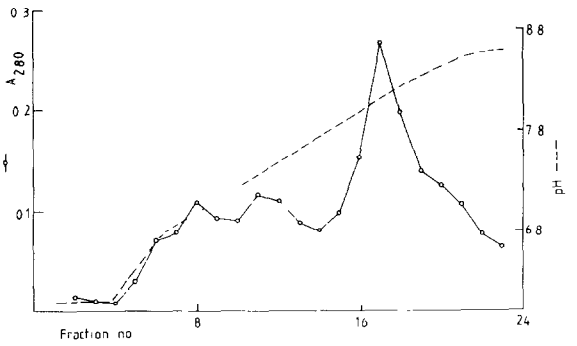

Fig.3. pH gradient elution of protein from Cibacron Blue–Sepharose 100 μl of pregnancy plasma was passed through a 1 ml column of CBS at pH 5.85. After adequate washing a pH gradient was applied (10 mM KH_2PO_4/NaOH (10 ml) against 20 mM K_2HPO_4/NaOH, pH 11.0 (10 ml)). Protein and pH of the 0.9 ml eluted fractions were determined.

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