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Purification of C-reactive protein, an acute phase protein of human serum

An alternative method of isolation of C-reactive protein which would avoid the troublesome preliminary preparation of pneumococcal C-polysaccharide[1] employed in the classical isolation procedure of this protein devised by MCCARTY[2] would facilitate study of the biochemistry of CRP. In addition, the production of clinically useful CRP antiserum would be simplified.

We have utilized chromatographic technics employing DEAE-cellulose and have devised a simple procedure which gives good yields of CRP.

Within the typical protocol, given below, figures for total protein concentration were estimated from the absorbancy of samples at 280 mμ. CRP was assayed on dilutions of samples utilizing the capillary precipitin test[3].

A 1.75-l sample of pleural exudate having a CRP titer of 1:64, obtained from a patient with carcinoma of the tongue with pulmonary metastasis, was brought to 0.5 saturation with $(NH_4)_2SO_4$, filtered and the filtrate raised to 0.75 saturation with the same salt. The precipitate, recovered by filtration, was slurried in sufficient distilled water to permit transfer to dialysis tubing, dialyzed against running tap water for approx. 4 h and then dialyzed at 4° against several changes of Buffer A (0.05 M sodium citrate, pH 7.0, containing 0.1 M NaCl). This fraction, containing approx. 17.4 g of protein in 700 ml of solution, was passed at a rate of approx. 1.5 ml/min through a 2.2 × 20-cm column of DEAE-cellulose which had been previ-

Abbreviation: CRP, C-reactive protein.

ously washed and equilibrated with Buffer A. Under these conditions CRP binds strongly to the DEAE-cellulose, while nearly all other proteins in serous fluids elute readily[4]. The column was then washed with 250 ml of Buffer A to wash out most of the residual weakly absorbed or unabsorbed protein, and CRP was eluted at higher electrolyte concentration by Buffer B (0.05 M sodium citrate, pH 7.0, containing 1.5 M NaCl). Three times the retention volume (3 × 25 ml in this instance) was required to elute approx. 90% of the CRP added to the column initially. Eluate containing CRP was concentrated by pervaporation to 0.1 vol. and dialyzed against buffer A. It contained about 1% of the total protein originally passed through the chromatograph column or about 0.5% of the protein in the exudate. "Disc" electrophoresis on polyacrylamide revealed proteins in the γ-globulin, albumin and prealbumin regions of the gel column. Immunological test indicated it to be essentially free of β-lipoprotein. This batch eluate was subjected to a second chromatographic fractionation on DEAE-cellulose utilizing a variable gradient device (Varigrad) to

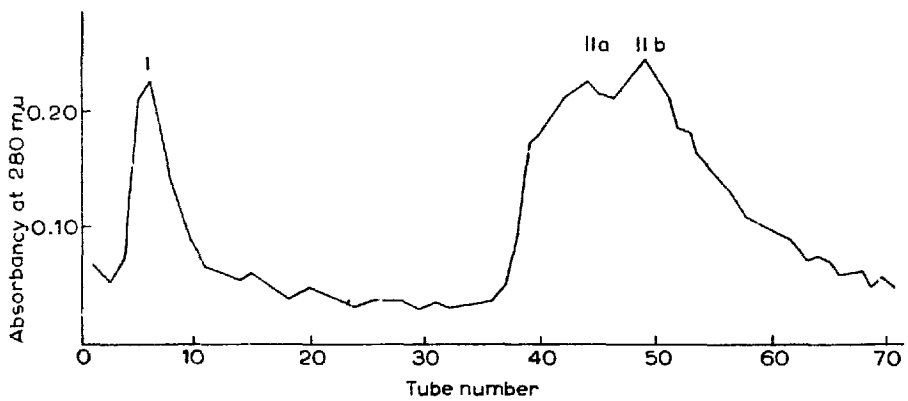

Fig. 1. Gradient-elution chromatogram of partially purified CRP on DEAE-cellulose. Buffer gradient from 0.05 M sodium citrate (pH 7.0), containing 0.1 M NaCl to 0.05 M sodium citrate (pH 4.5) containing 0.5 M NaCl.

ensure a smooth salt and pH gradient during elution. A 5-ml sample (2/3) of the concentrated CRP obtained from the first chromatogram was introduced into a 1.0 × 27-cm column of DEAE-cellulose which had been equilibrated with Buffer A, and the column then connected in tandem through a micro-pump to a Varigrad employing 4 chambers. A volume of 75 ml of Buffer A was added to each of Chambers 1 and 2, and 75 ml of limit Buffer C (0.05 M sodium citrate, pH 4.5, containing 0.5 M NaCl) was added to each of Chambers 3 and 4 to provide a non-linear decrease in pH from 7.0 to 4.5 and an increase in NaCl concentration from 0.1 to 0.5 M NaCl in the course of the delivery of 300 ml of the mixture to the column. The column was developed at room temperature with the Varigrad in operation and the micro-pump set to provide a flow rate of 0.75 ml/min. Outflow was monitored at 280 mμ and fractions of 4 ml were collected.

The result of the second chromatogram is shown in Fig. 1. This chromatogram showed two major groups of components. The first, designated Fraction I, Tubes 4–14, contained about 1/4 of the protein added to the column. This combined fraction gave no precipitation when tested with CRP antiserum in the capillary precipitin test. The second and larger group of components designated IIa and IIb, respectively, appeared to be heterogeneous in that 2 peaks were noted in Tubes 44 and 49. Materials

in Tubes 37–70 contained detectable CRP on testing with CRP antiserum. Approx. 80 % of the CRP added to the column was recovered in Tubes 37–70.

Fraction I, Tubes 4–14, concentrated by pervaporation to 1/20 vol. of the pooled eluate, and then equilibrated against 0.05 M NaCl by dialysis, gave positive precipitin reactions in the capillary tube test with antisera to human crystalline albumin, Fraction $IV_{5,6}$, plasminogen, and a somewhat weaker reaction with anti-human complement endpiece. No precipitation was noted with CRP antiserum or with anti-human β-lipoprotein. Ring tests with pneumococcal C-polysaccharide dissolved in 1 mM $CaCl_2$ in physiological saline were also negative.

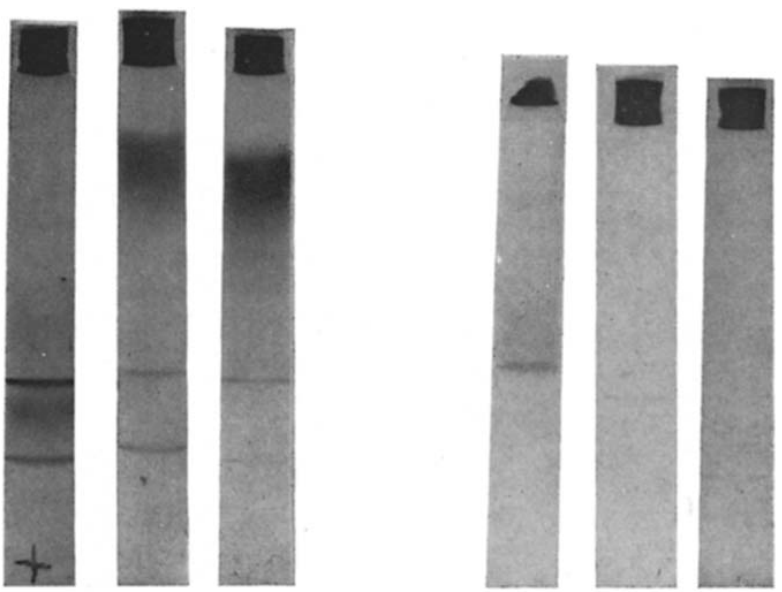

Fig. 2. The photographs of the disc polyacrylamide electrophoresis gel columns of Fractions I, IIa and IIb, respectively, stained with amidoblack (left) and periodic acid-Schiff's stain (right). The solid-black section at the top (cathodic end) is opaque spacer gel.

Fractions IIa, Tubes 37–46, and IIb, Tubes 47–70, concentrated and tested in the same manner, gave no visible precipitates in the micro-capillary tube test with the antisera listed above, with the exception of CRP antiserum which reacted strongly. Similarly, strong interfacial ring tests were obtained when Fractions IIa and IIb were tested with pneumococcal C-polysaccharide.

Results of examination of these fractions by electrophoresis on polyacrylamide are illustrated in Fig. 2. Fraction I showed 2 distinct discs and 2 somewhat diffuse discs in the prealbumin, albumin and postalbumin regions when stained with amido-black. When Fraction I was stained with periodic acid-Schiff's stain, 5 discs were noted. Four of these can be seen in the photo shown in Fig. 2, while 1 disc closest to the anodic end (bottom) was not intense enough to reproduce clearly. The periodic acid-Schiff's stain showed a component in the γ-globulin region not detected by the amidoblack stain. Fractions IIa and IIb were analyzed similarly. Both fractions were very similar. The majority of the material staining with amidoblack occurred in the γ-globulin region as a diffuse disc, while weakly staining discs were noted in the prealbumin and albumin areas. The latter discs were more pronounced in Fraction IIa. Periodic acid-Schiff's staining revealed no components in the γ-globulin region, while weakly staining discs were noted in the prealbumin and albumin areas, though these

are not adequately illustrated by the photographs. The diffuse disc in the γ-globulin region of Fractions IIa and IIb was identified as CRP. This was demonstrated by embedding unstained polyacrylamide columns in agar and diffusing against CRP antiserum[5] and by precipitin tests on eluates of segments of unstained columns.

A number of other individual samples of CRP positive exudates, fractionated as above, showed essentially the same 2 major groups of components as noted in Fig. 1, but the ratio of Fraction I to Fraction II varied with the individual sample. Nonetheless, CRP was invariably found in Fraction II, though usually Fraction II did not show evidence of splitting as shown here. Similar separations have also been effected using buffers in which citrate has been replaced by phosphate and buffers containing sodium EDTA, though these systems have not been studied thoroughly.

Antiserum obtained following multiple injections of rabbits with CRP isolated in the manner described above was capable of detecting CRP in concentrations down to 1 μg/ml. In addition, the antiserum reacted strongly with a solution of crystalline CRP courteously provided by Dr. H. F. WOOD. It showed no reaction with normal human serum and no reaction in capillary precipitin test with 1 % solutions of Cohn Fractions IV_1, IV_4, $IV_{5,6}$, V and VI, or with β-lipoprotein.

The specificity of the antiserum obtained is somewhat surprising in view of the presence of contaminants in the antigen shown in Fig. 2. This could be due to their poor antigenicity. Preliminary evidence indicates, however, that one if not two of them arise from CRP by dissociation.

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