PURIFICATION OF THE HUMAN PLASMA ALPHA$_2$-SH GLYCOPROTEIN BY ZINC CHELATE AFFINITY CHROMATOGRAPHY

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1. Introduction

Plasma alpha$_2$-SH glycoprotein was first obtained and named alpha$_2$-Z by Heremans [1]. This protein was soluble together with IgA globulin at concentrations of zinc ions higher than 0.05 M. In 1961, Schmid and Bürgi [2] showed that the former, as well as another glycoprotein named alpha$_2$-Zn [3], exhibited minimum solubility between 0.01 M and 0.03 M zinc acetate in the ethanol procedure. These two proteins were separated to a great extent by using low concentration of Ba^{2+} ions [2]. Schultze et al. [4] demonstrated that the protein obtained by Heremans [1] and the one discovered and named alpha$_2$-Ba by Schmid and Bürgi [2] were identical, and this protein was finally called alpha$_2$-SH.

Recently, Porath et al. [5] showed that chelate-forming ligands for metal ions (Cu, Zn, Cd, Hg, Co, Ni) could be attached to agarose derivatives. These authors demonstrated that the affinity of proteins for such adsorbents is pH dependent.

As little is known about the structure of alpha$_2$-SH, metal chelate affinity chromatography was used to obtain a better resolution of the human plasma alpha$_2$ globulin fraction. The present paper shows that, by this technique, alpha$_2$-SH was obtained free from alpha$_2$-Zn with a similar pI and molecular weight.

2. Materials and methods

CM–Sepharose CL-6B, Sephadex G-200 and epoxy-activated Sepharose 6B were purchased from Pharmacia Fine Chemicals Co., Uppsala, Sweden. Immunoelectrophoresis and double immunodiffusion were performed on 1.3% agar in 0.05 M barbital buffer, pH 8.2, by using antibody to human serum (Organon Teknika S.A.) and anti-alpha$_2$ monospecific immune sera (Behringwerke A. G., Marburg, FRG). The alpha$_2$-SH content was measured by single radial diffusion (Partigen plate, Behringwerke A.G.). Protein determinations were performed by the Biuret method. SDS–Polyacrylamide gel electrophoresis was carried out in 10% or 7.5% acrylamide. Gel-filtration (Sephadex G-200 column (2.5 × 100 cm)) was carried out with 0.1 M. Tris–HCl buffer, pH 8.0, containing 0.1 M NaCl. The column was standardized with blue dextran 2000 (Pharmacia Fine Chemicals Co.). Fractions of 5 ml were collected.

A human serum alpha$_2$ globulin fraction rich in alpha$_2$-SH, obtained from pooled normal human sera, was prepared by precipitation at 1.8 M $(NH_4)_2SO_4$, followed by ion-exchange chromatography on a CM–Sepharose CL-6B column equilibrated with a 0.025 M acetate buffer, pH 5.0, containing 2 mM $CaCl_2$, 2 mM $MgCl_2$, 2 mM $MnCl_2$ and 50 mM ϵ-aminocaproic acid. The proteins were eluted with the same buffer and then step-wise with increasing concentrations of 0.02–0.2 M NaCl. Alpha$_2$-SH was present in the first fractions eluted (up to 0.06 M NaCl). All the protein fractions which contained alpha$_2$-SH and alpha$_2$-Zn were pooled and two preparations were retained for the zinc-chelate affinity chromatography: the first one contained apparently intact alpha$_2$-SH as shown by immunoelectrophoresis and immunodiffusion in comparison to fresh human serum against a monospecific anti-alpha$_2$-SH; the second one showed apparently intact alpha$_2$-SH

together with a spontaneous fragment of this protein, as shown by immunodiffusion and by immunoelectrophoresis.

Preparation of the chelate gel was carried out essentially as described by Porath et al. [5]: 30 ml of swollen epoxy-activated Sepharose 6B (bis-oxirane activated agarose containing approx. 15–20 μmol epoxy-groups/ml) were added to 20 ml 2 M Na_2CO_3 containing 4 g iminodiacetic acid, disodium salt. The gel suspension was heated to 65°C and continuously stirred for 24 h in a water-bath. After coupling, at pH 12.4, the gel was thoroughly washed with distilled water. A water solution of $ZnCl_2$ (1 $g.l^{-1}$) was passed through a column containing the bis-carboxymethylamino–Sepharose 6B: 1.5 × 12 cm (15 ml) gel bed, flow rate 40 ml/h. Fractions of 2 ml were collected. The pH was determined in each tube and the presence of Zn^{2+} was assessed by the formation of an insoluble $ZnCO_3$ precipitate (0.2 ml 2 M Na_2CO_3).

3. Results and discussion

The gel-bound chelate of zinc was obtained by passing a $ZnCl_2$ solution through bis-carboxymethylamino–Sepharose 6B. In a typical experiment, the formation of the metal chelate was shown by a pH drop (pH 2.4) which remained constant from 42–85 ml elution vol. Furthermore, Zn^{2+} appeared at the same time as the pH dropped. When the pH returned to neutrality, the column was equilibrated with 0.05 M Tris–HCl buffer, pH 8.0, containing 0.15 M NaCl, an $alpha_2$ fraction (26 mg in 2 ml) rich in apparently intact $alpha_2$-SH applied (see fig.1) and eluted with the same buffer. When the $A_{280\,nm}$ was less than 0.02, elution was continued with 0.1 M Na phosphate buffer, pH 6.5, containing 0.8 M NaCl. In the same way, 0.1 M Na acetate buffer, pH 4.5, containing 0.8 M NaCl was passed through the column but no appreciable absorbance was noticed. Finally,

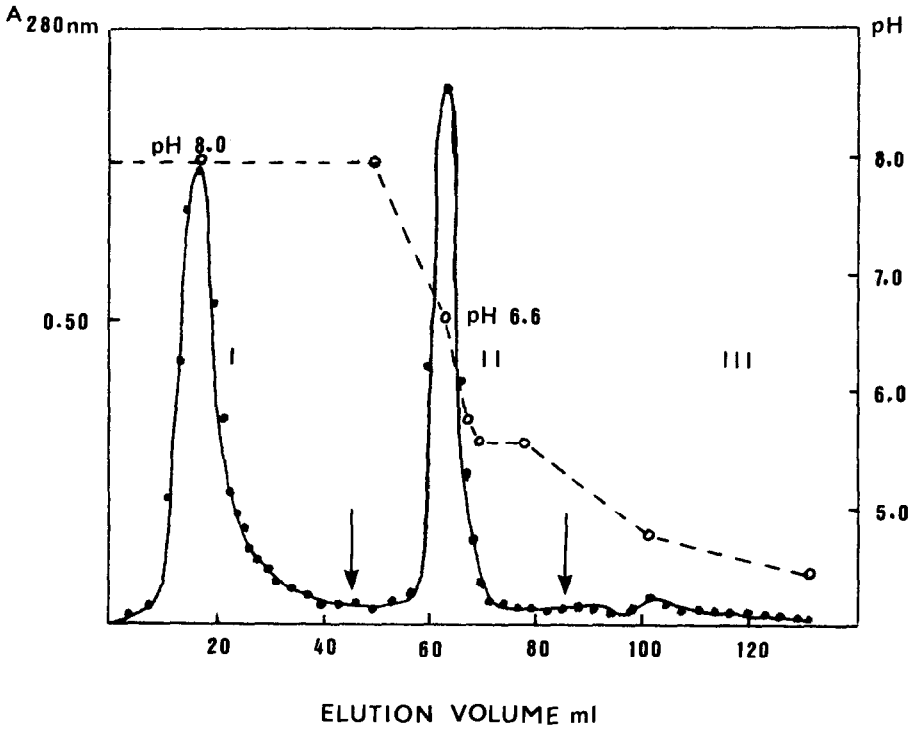

Fig.1. Zinc chelate affinity chromatography (ZCAC) of a human serum $alpha_2$ globulin fraction rich in $alpha_2$-SH. Conditions were: 1.5 × 12 cm (15 ml) gel bed, sample vol. 2 ml (26 mg $alpha_2$ fraction). Elution system: (I) 0.05 M Tris–HCl buffer, pH 8.0, containing 0.15 M NaCl; (II) 0.1 M Na phosphate buffer, pH 6.5, containing 0.8 M NaCl; (III) 0.1 M acetate Na buffer, pH 4.5, containing 0.8 M NaCl, flow rate 20 ml/h.

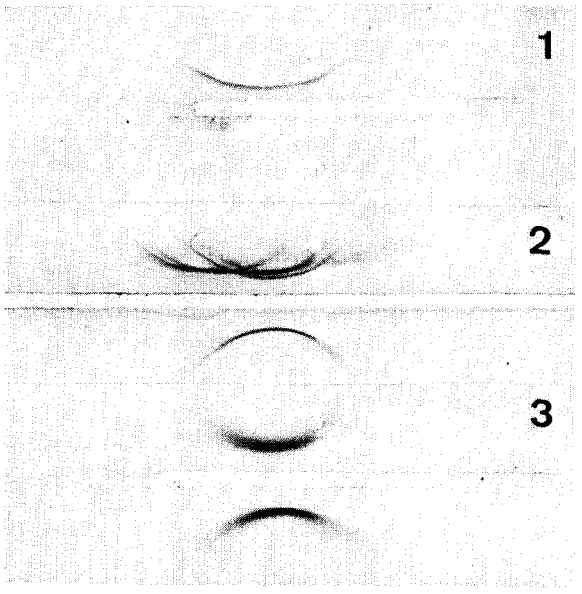

Fig.2(a)

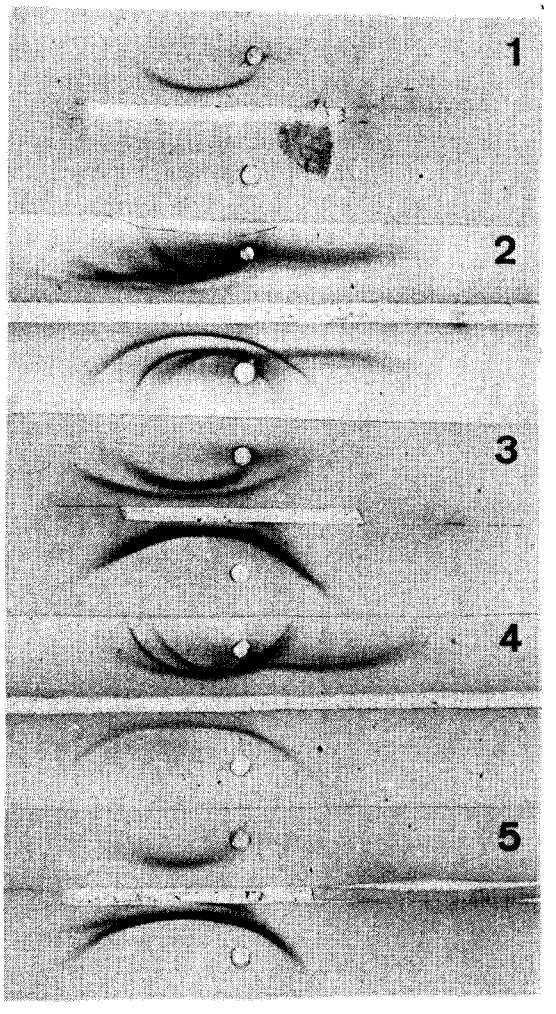

Fig.2(b)

Fig.2(a). Immunoelectrophoretic patterns of the proteins eluted after zinc-chelate affinity chromatography (ZCAC: see fig.1). In 1: upper well, peak I; lower well, peak II (developed with anti-alpha$_2$-Zn immuneserum). In 2: upper well, peak I; lower well, peak II (developed with anti-human total immuneserum). In 3: upper well, peak I; lower well, peak II (developed with anti-alpha$_2$-SH immuneserum). Fig.2(b). Controls after ZCAC (second experiment: same elution profile as that in fig.1; see text for details). 1,2,3: same disposition as that in fig.2a. In 4,5: controls after Sephadex G-200 gel filtration of peak II of ZCAC. In 4: upper well, first protein fraction eluting in the void volume of the G-200 column; lower well, second protein fraction, (developed with anti-human immuneserum). In 5: same disposition that in 4 (developed with anti-alpha$_2$-SH immuneserum). Anode is at the left.

the column was eluted with 0.05 M EDTA, pH 7.0, 0.5 M in NaCl, then washed with distilled water. The zinc chelate was regenerated by passing zinc chloride through the column as indicated above.

Material from peaks I and II were concentrated and analyzed. The unique protein identified by immunoelectrophoresis in peak II (fig.2a) was alpha$_2$-SH glycoprotein, and after concentration of the volume, unidentified traces of a beta$_1$ and an alpha$_1$ appeared. In peak I, alpha$_2$-Zn glycoprotein, a prealbumin, two alpha$_2$ glycoproteins and traces of alpha$_2$-SH were identified. By radial immunodiffusion of the two protein fractions (peak I and II) the recovery of alpha$_2$-SH was shown to be about 85% in peak II. By PAGE–SDS of the latter, a prominent band of app. mol. wt 50 000–55 000 was obtained. In a second experiment, a protein fraction which contained partially denatured alpha$_2$-SH, alpha$_2$-Zn, some unidentified alpha$_2$ and beta globulins, and alpha$_2$ macroglobulin, was applied to the column. An alpha$_2$-SH fragment, as shown by immunodiffusion and a small proportion of native alpha$_2$-SH, were not retained, at pH 8.0 (fig.2b, 3). Alpha$_2$ macroglubulin co-eluted, at pH 6.6, with apparently intact alpha$_2$-SH (fig.2b, 2). Sephadex G-200 filtration of this fraction showed two peaks: in the first one, alpha$_2$ macroglobulin was

eluted in the void volume of the column. Alpha$_2$-SH was obtained into the second peak (fig.2b, 4,5) with PAGE–SDS of this fraction showing a major band of app. mol. wt 50 000–55 000.

The chelate column was reactivated and used eight times. Under the following conditions (column 23 × 1.5 cm), more than 60 mg protein were chromatographed with identical elution profile and when the column was used in the absence of zinc ions, all proteins eluted, at pH 8.0, in the starting buffer.

This chromatographic procedure was highly reproducible. Thus, under the present experimental conditions, alpha$_2$-SH was the most strongly absorbed protein, at pH 8.0, along with some alpha$_2$-M, alpha$_1$-B, and traces of unidentified alpha$_1$ and beta glycoproteins. On the other hand, alpha$_2$-Zn and some other alpha$_2$ glycoproteins were never absorbed onto the column. As noticed by Porath et al. [5], imidazole and thiol groups may interact with Zn^{2+} ions fixed on the column but it is speculative to draw a parallel between the histidine and cysteine (or other amino acids) content of these proteins and their binding capacity, before their quaternary structure is elucidated.

The use of zinc-chelate chromatography may make easier the purification of alpha$_2$-SH which is frequently present in alpha$_2$-kininogen preparations [6]. So far, the primary structure of alpha$_2$-SH is unknown, and further information is needed about its susceptibility to cleavage. Furthermore, its biological function is not established although Van Oss et al. [7] observed that alpha$_2$-SH possessed opsonic properties. Dickson et al. [8] showed that this protein was a constituent of both adult and foetal human bone matrix. Also, the concentration of alpha$_2$-SH is decreased in the serum of trauma patients [9]. This observation, added to my preliminary results, that the concentration of the protein is significantly lowered in sera of patients suffering from inflammatory processes (in inverse ratio to the increase of 'acute phase' proteins) emphasizes the need of further investigation of this plasma protein.

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