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ADSORPTION AND DESORPTION OF PROTEINS IN METAL CHELATE AFFINITY CHROMATOGRAPHY

PURIFICATION OF ALBUMIN

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SUMMARY

Adsorption and desorption of protein in metal chelate affinity chromatography depend to a great extent on the type of the buffer ions used. Acetate and phosphate ions with a low affinity for the chelated metal facilitate adsorption while amines prevent adsorption or cause desorption of adsorbed protein. These findings were used to purify albumin from a very crude and strongly discoloured Cohn IV extract in 65% yield. Coloured contaminants were removed by washing protein adsorbed to the metal affinity column with an ethanol–water mixture.

INTRODUCTION

In 1975 Porath *et al.*[1] published a new method for protein fractionation termed "metal chelate affinity chromatography". They showed that proteins vary in their affinity to metal ions bound by Sepharose-fixed chelating groups. Since then a number of papers have shown that the method can be used to purify polypeptides and proteins; *i.e.*, human plasma α_2-SH glycoprotein[2], interferon[3–5], lactoferrin[6], gastrointestinal polypeptides[7], pepsin and bovine serum albumin[8], granule proteins from granulocytes[9], α_2-macroblobulin and α_1-antitrypsin[10], and nucleoside diphosphatase[11]. A Japanese patent claims that endotoxins can be removed from useful proteins by the use of chelating gels[12].

We have studied the conditions for adsorbing and desorbing proteins on chelating gels and used the results to design a protocol for the purification of albumin from a Cohn fraction IV extract.

EXPERIMENTAL

Synthesis of chelating gels

Iminodiacetic acid was coupled to Sepharose activated with 1,4-bis-(2,3-epoxypropoxy)butane (a commercial product from Pharmacia, Uppsala, Sweden, or a product activated in the laboratory)[1]. Variations in the amount of iminodiacetic acid gave different degrees of substitution as shown in Table I. The capacity for Zn^{2+}

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TABLE I

IMINODIACETIC ACID DERIVATIVES OF SEPHAROSE USED AS CHELATING GELS

Gel No.	*Sepharose*	*Capacity for Zn^{2+} (μmole/ml)*
I	6B	21
II	4B	18
III	4B	12

ions was determined by complexiometric titration of excess Zn^{2+} with EDTA after passing a solution of $ZnCl_2$ through the gel [indicator tablets (article No. 8430 from E. Merck, Darmstadt, G.F.R.) were used].

Chromatography

All experiments were carried out at room temperature. Columns were charged with metal ions by elution with excess of $ZnCl_2$, 3 mg/ml, pH adjusted to 6.4 or $CuSO_4 \cdot 5H_2O$, 5 mg/ml, and were equilibrated with the starting buffer before introduction of the sample. Between each experiment columns were stripped of Cu^{2+} or Zn^{2+} ions with 0.05 *M* EDTA, pH 7.0, containing 0.5 *M* NaCl. Samples of albumin were prepared by dilution of the original salt-free albumin solution with the starting buffer, 1:2. In experiments with Cohn IV extracts, solid salts were added to give concentrations and pH as in the starting buffer. For further details, see figure legends.

The Cohn fraction IV extract was kindly supplied by the Finnish Red Cross Blood Transfusion Service. It had been prepared according to published procedures[13].

In the chromatographic setup we used a peristaltic pump (P 3) and a UV detector (UV-2) from Pharmacia.

Analytical procecures

Electrophoresis in polyacrylamide gradient gels (4/30) was done according to the manufacturer's instructions (Pharmacia) after concentration of samples in Minicon™ concentrators (Amicon, Lexington, MA, U.S.A.). Cellulose acetate electrophoresis was carried out with an DCD-16 apparatus (Gelman). Immunodiffusion in agarose was used to detect α_2-HS glycoprotein and α_1-lipoprotein (antisera from Behringwerke, Marburg, G.F.R.).

RESULTS AND DISCUSSION

Conditions for adsorption and desorption of albumin

When albumin in 0.05 *M* Tris–HCl, 0.15 *M* NaCl, pH 8.0, was introduced on a Zn^{2+}-loaded chelating gel, the major part of the protein came off only slightly retarded (Fig. 1a). Some tailing of the main peak was observed. A sharp peak which was eluted with 0.1 *M* acetate, 0.8 *M* NaCl, pH 4.5, contained only a minor part of the protein. Electrophoresis in a polyacrylamide gradient gel showed that dimers and oligomers had been enriched in this peak, which also contained α_2-HS glycoprotein. Stronger retardation and more pronounced tailing was noticed when the column was carrying Cu^{2+} instead of Zn^{2+} (Fig. 1b). Changing to phosphate

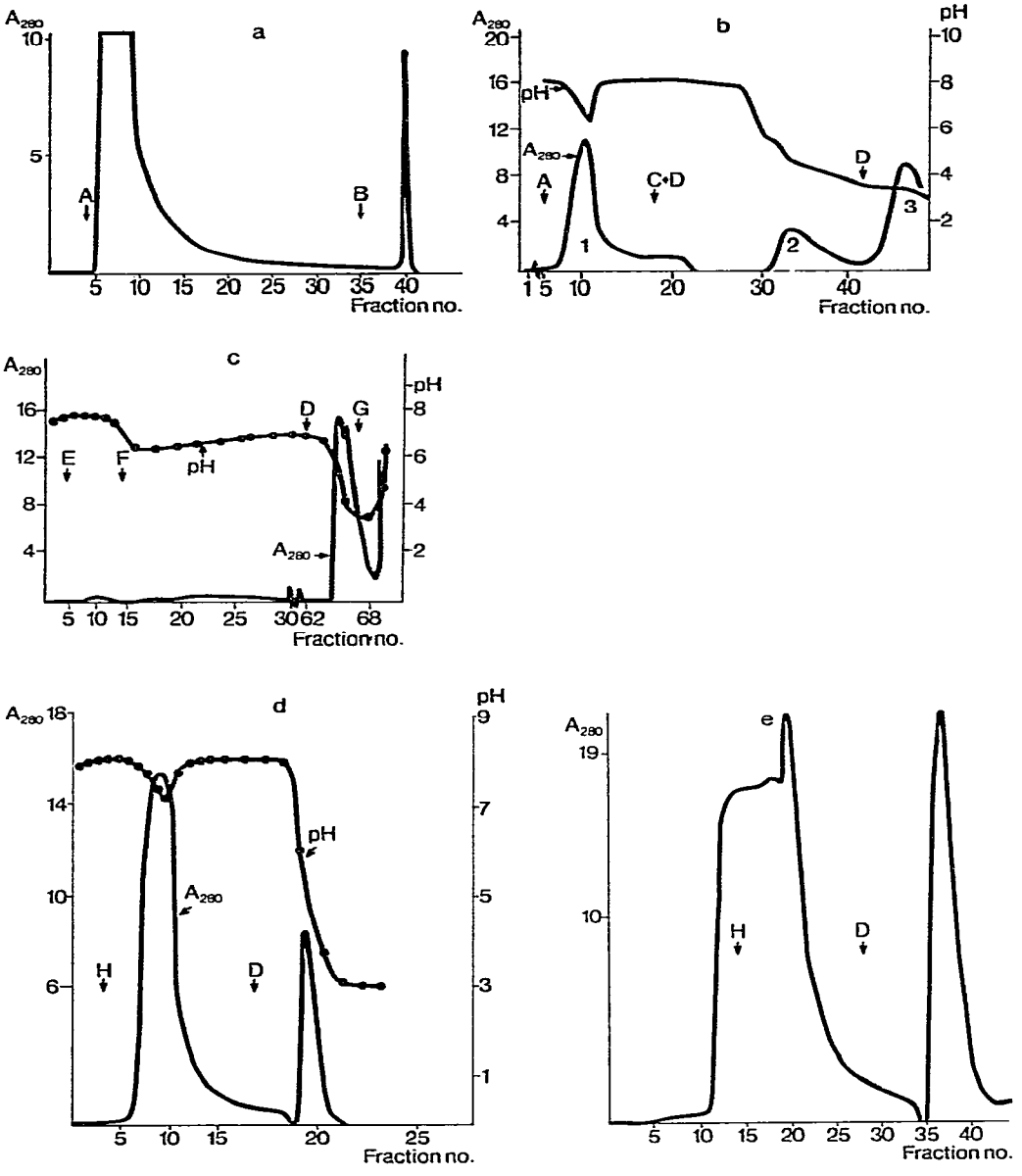

Fig. 1. Metal affinity chromatography of albumin on Zn^{2+}- and Cu^{2+}-loaded columns of chelating Sepharose. Eluents: (A) 0.05 *M* Tris–HCl, 0.15 *M* NaCl, pH 8.0; (B) 0.1 *M* sodium acetate, 0.8 *M* NaCl, pH 4.5; (C) 0.02 *M* sodium phosphate, 0.5 *M* NaCl, pH 7.7; (D) 0.1 *M* acetic acid, 0.5 *M* NaCl, pH 2.8; (E) 0.1 *M* sodium acetate, 0.5 *M* NaCl, pH 7.7; (F) 0.1 *M* ammonium acetate, 0.5 *M* NaCl, pH 6.5; (G) 0.05 *M* EDTA, 0.5 *M* NaCl, pH 7.0; (H) 0.05 *M* Tris-HCl, 0.15 *M* NH_4Cl, pH 8.0. Metal ion, column, gel volume, flow-rate, sample size, fraction size: (a) Zn^{2+}, K 16/20, 18 ml, 0.39 ml/min, 18 ml (1,4 g protein), 3.9 ml; (b) Cu^{2+}, K 16/20, 18 ml, 0.40 ml/min, 14 ml, (1.1 g protein), 4.0 ml; (c) Cu^{2+}, K 16/20, 18 ml, 0.40 ml, 14 ml (1.1 g protein), 4.0 and 8.0 ml; (d) Cu^{2+}, K 16/20, 18 ml, 0.42 ml/min, 14 ml (1.1 g protein), 4.2 and 8.4 ml; (e) Cu^{2+}, K 26/40, 58 ml, 1.1 ml/min, 154 ml (3.6 g protein), 11.0 ml. Gel 1 (Table I) was used in all experiments. In (b) a pH gradient was applied using eluents C and D, 50 ml of each.

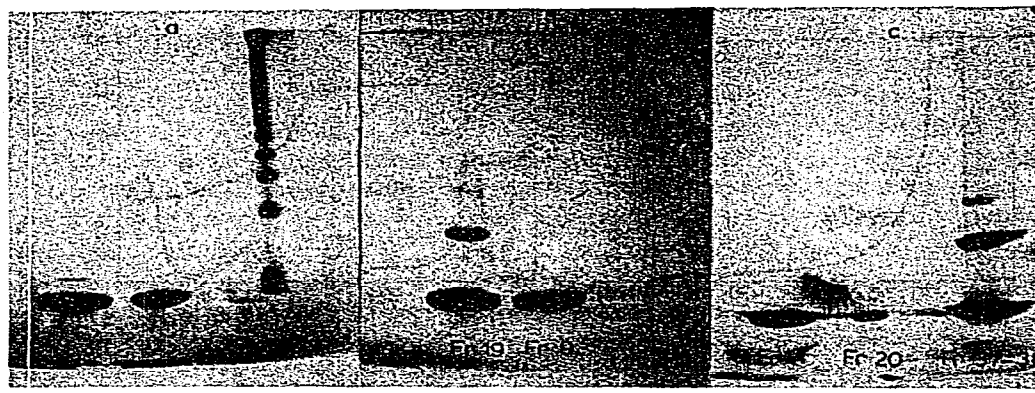

Fig. 2. Polyacrylamide gradient gel electrophoresis of fractions obtained in experiments described in Fig. 1: (a) Fig. 1b, (b) Fig. 1d, (c) Fig. 1e.

buffer, pH 7.7, caused even stronger adsorption as indicated by the abrupt cessation of the tailing. The albumin which did not elute in peak 1 started to leave the column only when the the applied pH gradient reached below pH 5. Both peak 1 and 2 contained quite pure albumin as shown by gradient gel electrophoresis (Fig. 2a).

Fig. 1c shows that albumin was almost completely adsorbed by a Cu^{2+} chelating gel when applied in sodium acetate–sodium chloride, pH 7.7. The change to ammonium acetate–sodium chloride, pH 6.5, caused some protein to leave the column but the major part remained adsorbed. A strong peak appeared on elution with acetic acid, pH 2.8.

Fig. 1d presents the results obtained with 0.15 *M* NH_4Cl instead of NaCl in the starting 0.05 *M* Tris buffer, pH 8.0. Most of the albumin was eluted directly and there were no dimers or oligomers at all in the main peak as judged from gradient gel electrophoresis. Immunodiffusion in agarose showed the presence of some α_2-HS glycoprotein in fraction 19, while none could be detected in fraction 9.

The removal of α_2-HS glycoprotein from a less pure albumin sample was also demonstrated on a larger scale using similar conditions (Fig. 1e). Only monomeric albumin was present in the main peak. α_2-HS glycoprotein could be detected by immunodiffusion at a protein concentration of 0.6% in the sample, while fractions 14 and 20 concentrated to 20% failed to give any precipitate lines.

The above series of experiments show that conditions can be chosen to allow a protein such as albumin to pass directly through the metal-loaded chelating gel whilst contaminants such as albumin oligomers and α_2-HS glycoprotein are adsorbed.

By choosing other conditions albumin can be made to bind to the gel. Fig. 1 demonstrates that this can be done by using buffer ions such as acetate and sodium ions, which do not compete effectively with the protein for the coordination sites of the chelated metal ions.

Fig. 1b demonstrates that Tris to some extent expels the protein from the gel. Ammonia (NH_3 from NH_4Cl) is more efficient as reflected in Fig. 1d and e, where the ammonia concentration can be assessed to be only *ca.* 6 n*M* and yet the major part of the albumin elutes from the column directly.

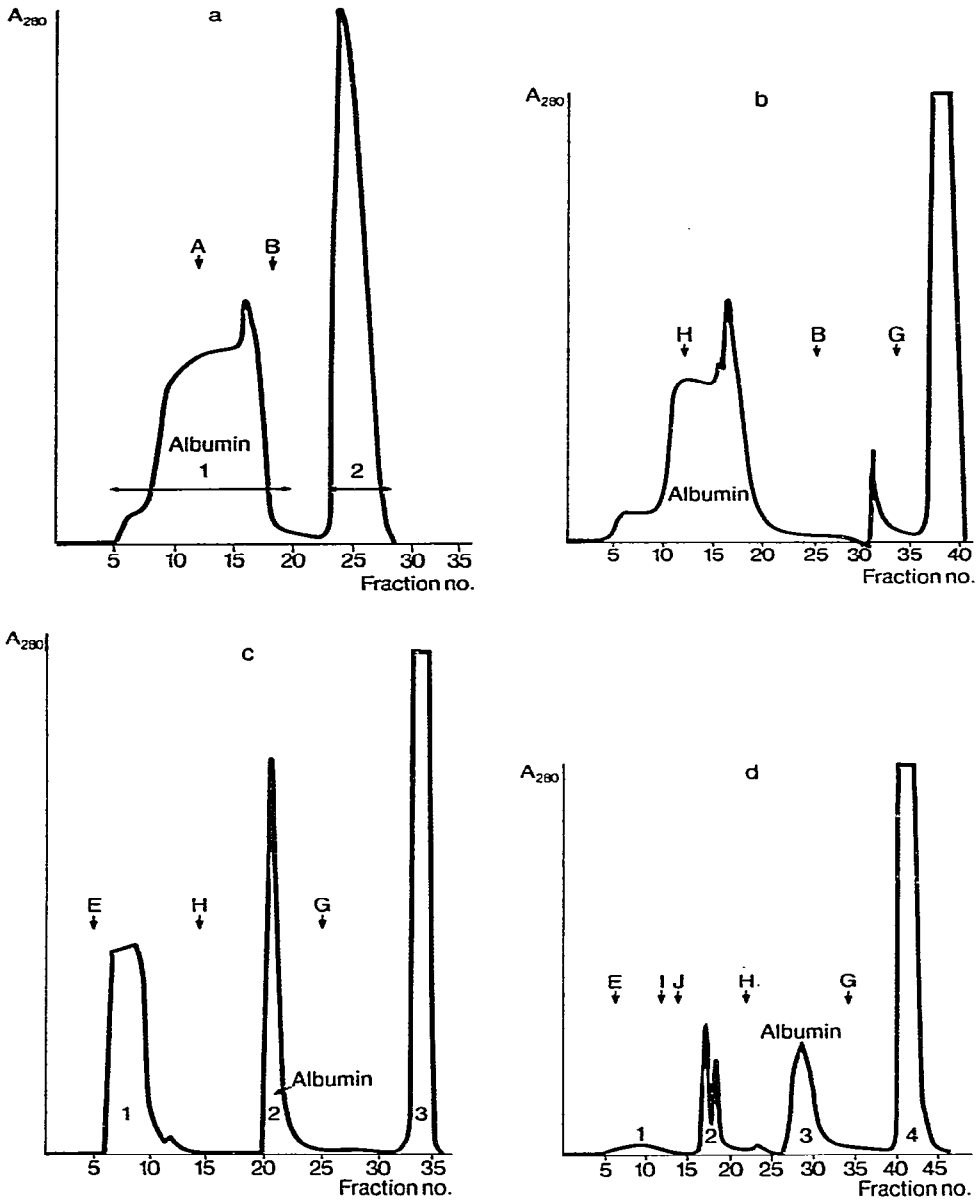

Fig. 3. Isolation of albumin from Cohn fraction IV extracts by metal affinity chromatography. Eluents: (A)–(H) see legend to Fig. 1; (I) ethanol–water (1:9); (J) ethanol–water (1:1). Metal ion: (a) Zn^{2+}, (b)–(d) Cu^{2+}. Gel volume: 55–58 ml. Flow-rate: 1.1–1.2 ml/min. Fraction size: 11–12 ml. Sample size: (a) 130 ml, (b) 130 ml, (c) 42 ml, (d) 67 ml. Gel (see Table I): (a) II, (b)–(d) III.

Albumin from Cohn fraction IV extracts

The Cohn fraction IV extract was a dark coloured solution containing 9.5 g albumin/l. The total protein content was 21.5 g/l. Transferrin was the major protein contaminant.

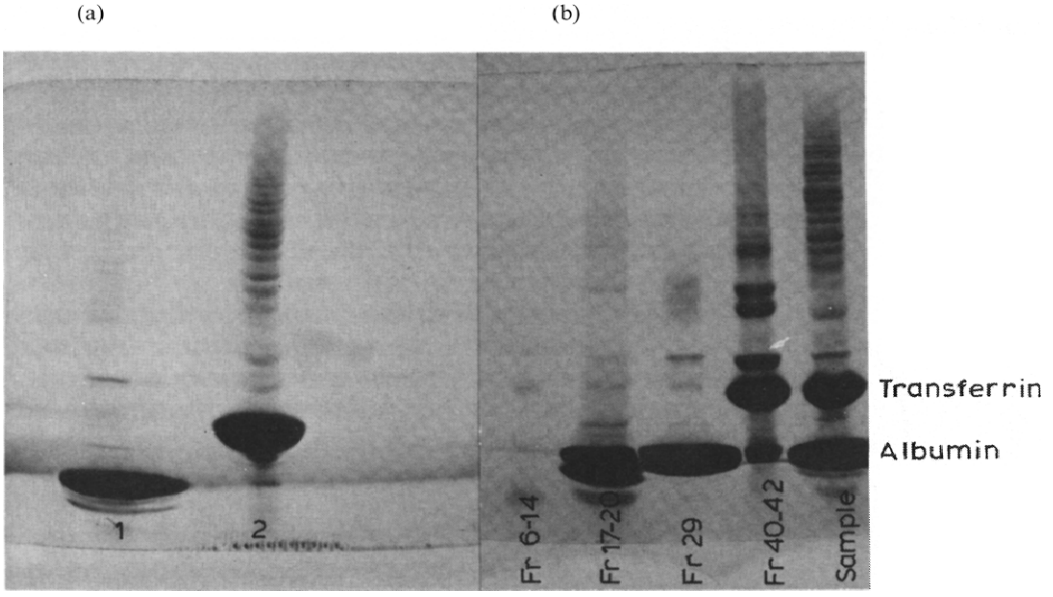

Fig. 4. Polyacrylamide gradient gel electrophoresis of fractions obtained in experiments described in Fig. 3: (a) Fig. 3a, (b) Fig. 3d.

When the Cohn IV extract was charged onto the Zn^{2+}-loaded column under conditions given in Fig. 3a, the albumin passed through without being adsorbed. The upper part of the column became strongly coloured. According to gradient gel electrophoresis (Fig. 4a), all the transferrin and most of the other high-molecular-weight proteins were eluted in peak 2. Peak 1 contained fairly pure albumin as judged from gradient gel and and cellulose acetate electrophoresis (the latter not shown). Neither α_2-HS glycoprotein nor α_1-lipoprotein could be detected in peaks 1 or 2 by immunodiffusion with protein concentrations of 14 and 22%, respectively.

A_{403} was 0.6 in a 1% solution of albumin from peak 1 (Fig. 3a), indicating that removal of haemoglobin was not complete. (The U.S. Pharmacopeia requires $A_{403} < 0.25$ for a 1% solution.)

As expected, when the Cohn IV extract was applied to a Cu^{2+}-saturated column with 0.15 *M* NH_4Cl in 0.5 *M* Tris–HCl, pH 8.0, the albumin passed through only slightly retarded (Fig. 3b). The albumin was, however, somewhat coloured ($A_{403} > 0.7$ at 1% concentration).

After introduction of the sample under conditions which caused the albumin to be adsorbed (Fig. 3c), it could be eluted as a sharp peak with 0.05 *M* Tris–HCl containing 0.15 *M* NH_4Cl. However, there still was some contamination with haemoglobin ($A_{403} = 0.32$). The major part of the discolouration could be removed by elution with ethanol–water (1:1) before desorbing the albumin (Fig. 3d). In this experiment a small column of chelating Sepharose, not loaded with chelated ions, was placed after the main column to adsorb eluted Cu^{2+}. This procedure is recommended to trap Cu^{2+} ions which to some extent escape from the column.

Peak 2 (Fig. 3d) was strongly coloured while the albumin peak was almost colourless, $A^{1\%}_{403} = 0.04$. The yield of albumin isolated in peak 3 was 65% and its

purity was 99.9% according to cellulose acetate electrophoresis. By immunodiffusion α_2-HS glycoprotein could be detected at an albumin concentration around 0.5%, while α_1-lipoprotein gave no precipitate line up to 10% albumin concentration.

CONCLUSIONS

By choosing a suitable buffer a protein such as albumin can be made to pass directly through a metal affinity column or to be completely adsorbed. The relevant property of the buffer is its ability to compete with the solutes to be fractionated for the coordination sites of the chelated metal ions. Albumin has a comparatively low affinity for chelated metals. After adsorption on chelated Cu^{2+} it can be desorbed with NH_4Cl in Tris–HCl, pH 8.0, while proteins such as albumin oligomers, haptoglobin, transferrin and others require stronger eluents or a lower pH to be eluted. Also the possibility of removing contaminants from adsorbed proteins by washing the column with alcohol–water mixtures might be useful in some cases.

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