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Note

Two-step purification of human α_1-acid glycoprotein

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Serum α_1-acid glycoprotein (AGP) or orosomucoid concentrations are known to increase during acute inflammatory states such as cancer [1—4], tuberculosis [5], head injury [6], surgery [7] and myocardial infarction [8—10], so this protein can be classified in the group of plasma proteins known as acute phase reactants. It is characterized by a high carbohydrate content (42%), which includes a large number of sialyl residues, a very low isoelectric point (p*I* 2.7) [11] and a high solubility.

To study the variations in sialic acid content during pathological states, it is necessary to obtain the glycoprotein in a pure form and that no desialylation occurs during the purification steps. Most of the techniques for the isolation of human α_1-acid glycoprotein use as a first step an acidic precipitation in order to remove most of the plasma proteins, followed by chromatography on DEAE- and/or CM-cellulose, generally using a strongly acidic buffer. Under these conditions, some variations in the sialic acid content may be observed.

We describe here a two-step purification of α_1-acid glycoprotein from normal plasma that prevents alterations due to exposure to strongly acidic conditions.

EXPERIMENTAL

Human plasma, obtained from healthy blood donors, was kept frozen at $-40^{\circ}C$ until used.

Isolation procedure

A 10-ml volume of plasma was dialysed overnight against 20 m*M* citrate—phosphate buffer (pH 4.0). After centrifugation at 500 *g* for 10 min, the sample was applied on a column (9 × 2.1 cm) of DEAE-Trisacryl (Industrie Biologique

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Française, Villeneuve-la-Garenne, France) equilibrated with the same buffer. The column was washed with the equilibrating buffer until no absorbance at 280 nm was recorded, then with the same buffer containing 100 m*M* disodium phosphate. The flow-rate was 140 ml h^{-1} and 2-ml fractions were collected. The remaining contaminating proteins were then eluted by addition of 1 *M* sodium chloride to the buffer (Fig. 1a). The whole procedure was run at 4°C.

After detection by radial immunodiffusion, the fractions containing α_1-acid glycoprotein were pooled, dialysed overnight against distilled water and then concentrated by ultrafiltration on Schleicher & Schüll Ultragains (UH 100).

The concentrated sample was then applied on a column (12.5 × 2.1 cm) of CM-Trisacryl equilibrated with 20 m*M* citrate—phosphate buffer (pH 4.0). The column was washed with the equilibrating buffer, then with the same buffer containing 1 *M* sodium chloride. The elution was run at 4°C at a flow-rate of 140 ml h^{-1}.

The fractions eluted as a single symmetrical peak with the equilibrating buffer (Fig. 1b) contained the purified α_1-acid glycoprotein. The fractions were pooled, dialysed overnight against distilled water and freeze-dried.

Polyacrylamide gel electrophoresis

Electrophoresis was performed using 7% acrylamide plates (Cellacryl, Sebia, Issy-les-Moulineaux, France), with migration for 90 min at 20 V cm^{-1} in 25 m*M* Tris—glycine buffer (pH 8.3). Proteins were stained with Coomassie blue.

Two-dimensional immunoelectrophoresis

This technique was used to control the purity of isolated α_1-acid glycoprotein and was performed on 1% acrylamide gel plates. For the first dimension, the migration was conducted in 25 m*M* Tris—glycine buffer (pH 9.2) for 45 min at 20 V cm^{-1}. For the second dimension, a rabbit antiserum to human serum proteins (Dako, Copenhagen, Denmark) was incorporated in the gel before running for 3 h at 17 V cm^{-1} in the above buffer. The plates were then washed, dried and stained with Coomassie blue.

Quantitative determination of sialic acid and α_1-acid glycoprotein

Sialic acid was determined according to the method of Warren [12] using 2-thiobarbituric acid after mild hydrolysis with 0.05 *M* sulphuric acid for 1 h at 80°C. α_1-Acid glycoprotein was determined by radial immunodiffusion (M-Partigen plates, Behring, Marburg, F.R.G.).

RESULTS AND DISCUSSION

The proposed two-step ion-exchange chromatography leads to purified α_1-acid glycoprotein in a 50% yield. Individual recoveries and yields are summarized in Table I.

Fig. 1 shows the elution profiles obtained in the two chromatographic steps. α_1-Acid glycoprotein is eluted in the bound fraction from the DEAE-Trisacryl column together with albumin, α_1-antitrypsin, haptoglobin and α_2-macroglobulin (Fig. 2). These proteins are further retained on the CM-Trisacryl column, except α_1-acid glycoprotein, which was unbound and eluted in a pure

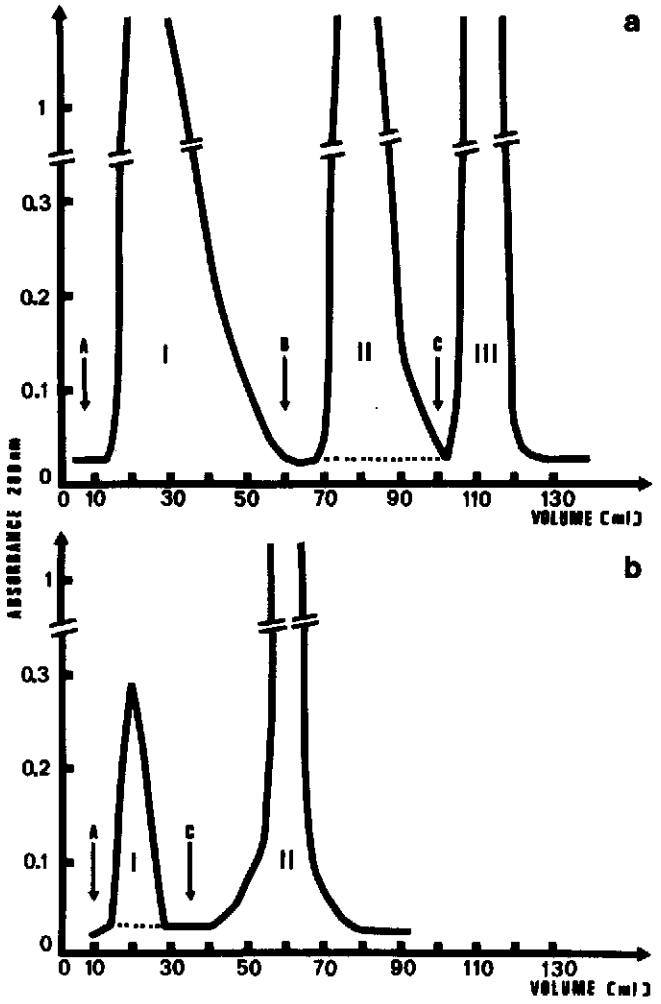

Fig. 1. Stepwise elution of human α_1-acid glycoprotein from (a) DEAE- and (b) CM-Trisacryl columns. (a) A 10-ml volume of human serum, dialysed against 20 m*M* citrate—phosphate buffer, was applied to a DEAE-Trisacryl column (9 × 2.1 cm) equilibrated with the same buffer. Elution was carried out successively with the equilibrating buffer (A), buffer A containing 100 m*M* disodium phosphate (B) and buffer A containing 1 *M* sodium chloride (C). The positions of the changes in elution buffer are indicated by the arrows. The fractions eluted with buffer B (peak II) contain mainly α_1-acid glycoprotein. (b) Fractions containing α_1-acid glycoprotein were applied to a CM-Trisacryl column (12.5 × 2.1 cm). Elution was carried out with buffers A and C. The first peak (I) eluted with buffer A contains pure α_1-acid glycoprotein.

form. No contaminants would be detected by using rabbit antiserum to human serum proteins (Fig. 3). The detection limit was 0.1 μg.

The purification procedure appears to have some advantages over previously reported two-step techniques. It does not involve denaturing steps such as preliminary acidic precipitation and/or exposure to strongly acidic buffers during chromatography. Therefore, it provides an α_1-acid glycoprotein without desialylation. When control α_1-acid glycoprotein (Sigma, St. Louis, MO, U.S.A.)

TABLE I

α_1-ACID GLYCOPROTEIN RECOVERIES AND YIELDS AFTER THE DIFFERENT PURIFICATION STEPS

Material	Volume (ml)	α_1-AGP* (mg)	Overall yield (%)
Dialysed human serum	10	9	100
Eluate from DEAE-Trisacryl column	50	5.4	60
Eluate from CM-Trisacryl column	15	4.5	50

*α_1-AGP concentrations were measured by radial immunodiffusion.

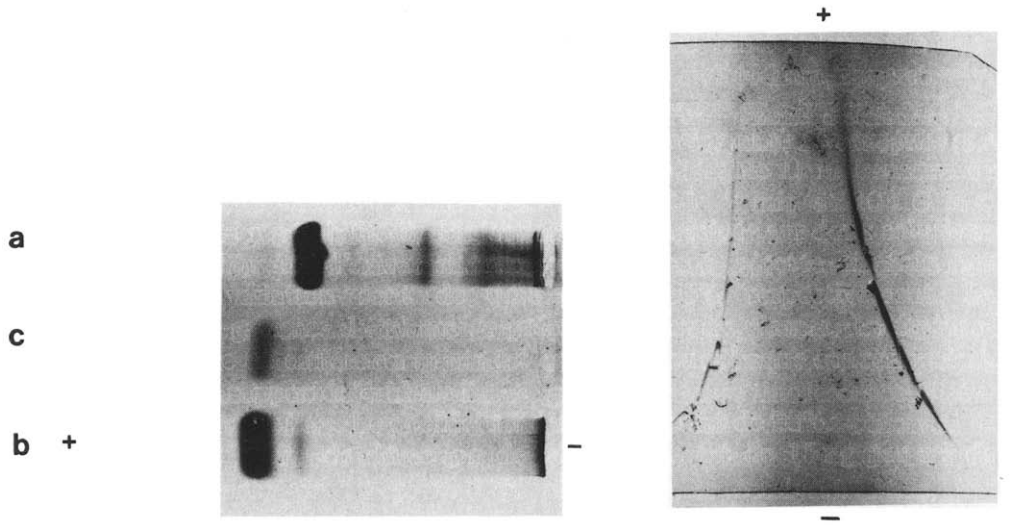

Fig. 2. Polyacrylamide gel electrophoresis at various stages of purification of α_1-acid glycoprotein. (a) Human serum; (b) peak II from the DEAE-Trisacryl column; (c) α_1-acid glycoprotein after the two chromatographic steps. Electrophoresis was carried out on polyacrylamide plates (Cellacryl) with 25 m*M* Tris—glycine buffer (pH 8.3). A current of 20 V cm^{-1} was applied for 90 min. Proteins were stained with Coomassie blue. Peak II from the DEAE-Trisacryl column (b) contains α_1-acid glycoprotein together with albumin, haptoglobin and α_2-macroglobulin. The two-step chromatographic fraction (c) is pure.

Fig. 3. Two-dimensional immunoelectrophoresis on agarose gel of purified α_1-acid glycoprotein. Electrophoresis in the first dimension was carried out on 1% agarose gel plates (9.5 × 1.5 × 0.1 cm) with 25 m*M* Tris—glycine buffer (pH 9.2). A current of 20 V cm^{-1} was applied for 45 min. Electrophoresis in the second dimension was carried out on a gel plate (9.5 × 9 × 0.1 cm) in which rabbit antiserum to human serum proteins was incorporated before migration. A current of 17 V cm^{-1} was applied for 3 h. The gel was then washed and dried and proteins were stained with Coomassie blue.

was used, the percentage of sialic acid was the same before and after chromatography (10.5%).

Other techniques using ion exchangers, particularly that of Bezkorovainy and Winzler [13], involved DEAE-Sephadex chromatography during which α_1-acid glycoprotein was eluted with a strongly acidic buffer (pH 2.8). Under these conditions, desialylation occurred and the sialic acid content of the purified glycoprotein was only 8% [14].

In the procedure described, exposure to acidic pH can be avoided by eluting with buffered 100 m*M* disodium phosphate solution. Citrate and phosphate ions were chosen because they have a good affinity for DEAE-Trisacryl and the separation obtained was better than that using chloride ions.

Some techniques use only one chromatographic step [15—19], but they need preliminary fractionation of plasma, which can be denaturing such as acidic precipitation [15, 16] or salt precipitation [18, 19].

We must also point out the importance of our choice of DEAE- and CM-Trisacryl as chromatographic ion exchangers. It allows high flow-rates, hence limiting the denaturation risks. This seems to be a decisive advantage in comparison with the recently published three-step purification of α_1-acid glycoprotein [20] in which chromatography is followed by a preparative isoelectric focusing step.

The two-step purification described here provides an α_1-acid glycoprotein without apparent desialylation. Hence it would be a valuable tool in studying pathological variations in the sialic acid content of this glycoprotein.

REFERENCES

1 A.M. Baskies, P.B. Chretien, J.F. Weiss, R.W. Makuch, R.A. Beveridge, W.J. Catalona and H.E. Spiegel, Cancer, 45 (1980) 3050—3060.
2 C.Y.T. Chu, L.T.Y. Lai and H.P. Pokala, J. Natl. Cancer Inst., 68 (1982) 75—79.
3 P.G. Gobbi, G. Merlini, G.A. Parrinello, P. Cavalli and E. Ascari, Acta Haematol., 67 (1982) 255—262.
4 P.A. Ganz, W.E. Shell and Z.A. Tökes, J. Natl. Cancer Inst., 71 (1983) 25—30.
5 P.P. Asses and G.D. Tracopoulos, Amer. J. Clin. Pathol., 76 (1981) 437—441.
6 A. Bourguignat, A. Albert, G. Férard, A. Tulasne, I. Kempf and P. Métais, Clin. Chem., 29 (1983) 1904—1907.
7 C.M. Colley, A. Fleck, A.W. Goode, B.R. Muller and M.A. Myers, J. Clin. Pathol., 36 (1983) 203—207.
8 B. Fellahi, P. Bardos, J.P. Muh, H. Mouray and J. Weill, Ann. Biol. Clin., 31 (1973) 421—429.
9 S.J. Smith, G. Bos, M.R. Esseveld, H.G. Van Eijk and J. Gerbrandy, Clin. Chim. Acta, 81 (1977) 75—85.
10 J.P. Chapelle, A. Albert, J.P. Smeets, C. Heusghem and H.E. Kulbertus, Clin. Chim. Acta, 115 (1981) 199—209.
11 R.W. Jeanloz, in A. Gottschalk, (Editor), Glycoproteins, Elsevier, Amsterdam, 2nd ed., 1972, pp. 565—611.
12 L. Warren, J. Biol. Chem., 234 (1959) 1971—1975.
13 A. Bezkorovainy and R.J. Winzler, Biochim. Biophys. Acta, 49 (1961) 559—565.
14 E. Athineos, J.C. Kukral and R.J. Winzler, Arch. Biochem. Biophys., 106 (1964) 338—342.
15 S. Delong Bolmer and E.A. Davidson, Biochemistry, 20 (1981) 1047—1054.
16 G. Biserte, R. Havez, J. Laturaze and A. Hayem-Lévy, Pathol. Biol., 9 (1961) 1081—1088.
17 K. Schmid, M.B. McNair and F. Burgi, J. Biol. Chem., 230 (1958) 853—864.
18 P.A. Charlwood, M.W.C. Hatton and E. Regoeczi, Biochim. Biophys. Acta, 453 (1976) 81—92.
19 I. Nicollet, J.P. Lebreton, M. Fontaine and D. Hiron, Biochim. Biophys. Acta, 668 (1981) 235—245.
20 P. Laurent, L. Mirbel, J. Bienvenu, C. Vallve and P. Arnaud, FEBS Lett., 168 (1984) 79—83.