ISOLATION OF γ-GLOBULIN FROM BLOOD SERUM BY CHROMATOGRAPHY ON AMINOSILOCHROME C-80

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One of the widely used methods for isolating immunoglobulins from blood serum is ion-exchange chromatography on DEAE-cellulose [1, 2]. It has been shown previously [3] that in a number of cases, particularly in the chromatography of porcine pepsin, the DEAE-cellulose can successfully be replaced by an anion-exchange resin based on macroporous silica – aminosilochrome. The present work was devoted to obtaining purified preparations of γ-globulins from human placental blood serum by chromatography on aminosilochrome C-80.

In preliminary experiments we studied the sorption of a commercial preparation of immunoglobulin on aminosilochrome C-80 under static conditions (Fig. 1). At pH 7.5, 20 min after the deposition of the sample almost complete sorption of immunoglobulin on the aminosilochrome is observed, while at pH 4.5 sorption is minimal. On this basis, we selected the following conditions for chromatographing the blood serum (Fig. 2):

Fraction	Elution conditions	Volume, ml	Amount of protein, optical units	Yield on protein
Initial serum		0.5	15	100
I	0.01 M phosphate buffer, pH 7.5	60.5	7.25	48.3
II	0.1 M acetate buffer, pH 4.5	24.5	3.25	21.7
III	1 M HCl	27.7	2.49	16.6
IV	H_2O	26	1.82	12.1
Total				98.7

After the deposition of the serum and the washing of the column with 0.01 M phosphate buffer, pH 7.5, the proteins not sorbed under the given conditions (fraction I) were separated. The results of electrophoresis show the presence in this fraction of almost the whole set of serum proteins (Fig. 3). Then 0.1 M acetate buffer, pH 4.5, close to the isoelectric point of the γ-globulins, by suppressing the ionic interactions of the γ-globulins with the positively-charged amino groups of the aminosilochrome, eluted the bulk of the γ-globulins (fraction II, making up 20-25% of the total amount of proteins).

Electrophoresis in polyacrylamide gel shows that this fraction contained the γ-globulin (main band corresponding to a molecular weight of 130-160 thousand, containing 90% of the protein) and impurity proteins (bands corresponding to molecular weights of 60-80 thousand). Fraction II was investigated by immunochemical methods – immunodiffusion and immunoelectrophoresis. Gel immunodiffusion showed one precipitation band with antiserum to human γ-globulin and three bands on treatment with antiserum to whole human serum (Fig. 4, A and B). On immunoelectrophoresis, antiserum to whole human serum showed, in addition to a γ-globulin band, two other precipitation bands – albumin and transferrin bands, which confirm the presence of albumin and transferrin as impurities in fraction II (Fig. 5A).

On contact with ovine antiserum to human γ-globulin, one precipitation band appeared (Fig. 5, B). Fraction III, containing both the high- and low-molecular-weight proteins of the blood serum (see Fig. 3) was de-

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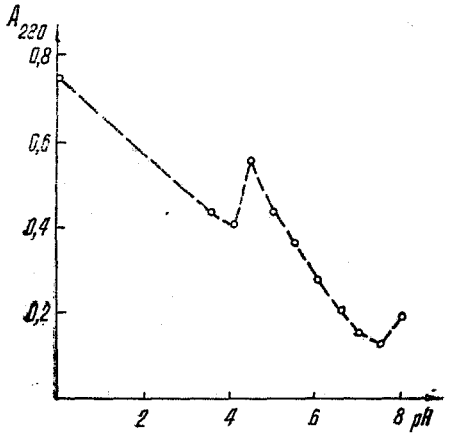

Fig. 1. Dependence of the sorption of human placental γ-globulin on aminosilochrome C-80 on the pH (A_{280} – optical density at 280 nm).

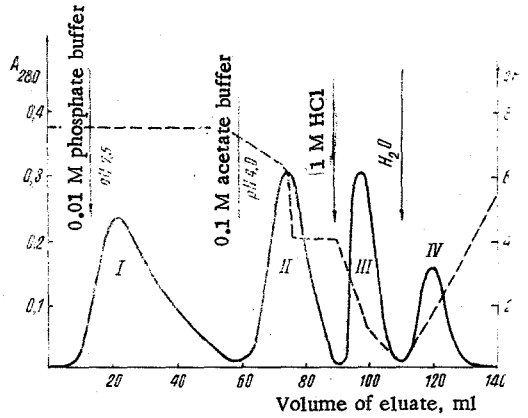

Fig. 2. Chromatography of human blood serum on aminosilochrome C-80.

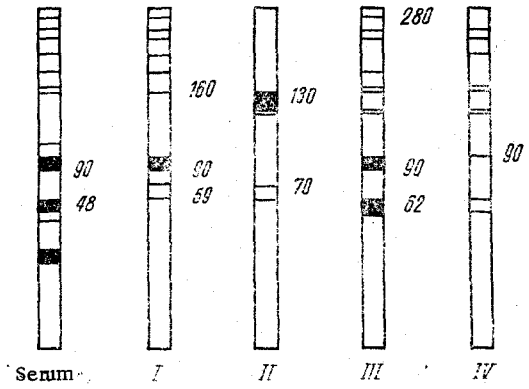

Fig. 3. Disk electrophoresis of human blood serum and its fractions in polacrylamide gel (molecular weights given in thousands).

sorbed under severe conditions – 1 M HCl (see Fig. 2). After this, on washing with deionized water, probably as the result of the change in the pH and the ionic strength, the protons most strongly bound to the ion-exchange resin, constituting fraction IV, were eluted from the column (see Figs. 2 and 3).

Thus, as a result of the fractionation of human placentary serum on aminosilochrome C-80 in one operation we obtained an enriched γ-globulin fraction containing small amounts of serum albumin and transferrin as impurities. The proposed method of isolating the γ-globulin fraction has a number of advantages in comparison

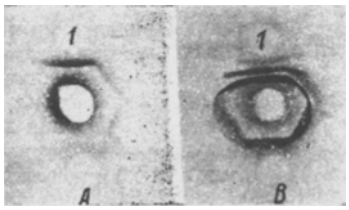

Fig. 4. Gel immunodiffusion of fraction II. In the central well: A) ovine antiserum to human γ-globulin (dilution 1 : 1); B) ovine antiserum to whole human serum (1 : 1). In the side wells the following dilutions of fraction II beginning with No. 1 and running clockwise: 1 : 10, 1 : 100, 1 : 200, 1 : 400, 1 : 800, 1 : 1600.

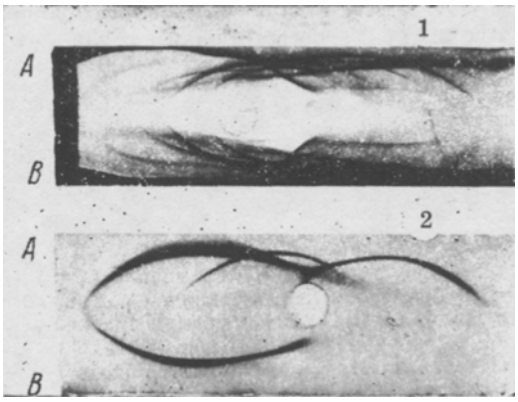

Fig. 5. Immunoelectrophoresis of human blood serum and of fraction II: 1) human blood serum; 2) fraction II; A) ovine antiserum to whole human serum (No. 22); B) ovine antiserium to human γ-globulin (No. 43). In fraction II the following appear from left to right, i.e., from cathode to anode: γ-globulin, transferrin, albumin.

with the method of chromatographing blood serum on DEAE-cellulose that is widely used at the present time. These include the availability of the support, the ease and facility of its treatment, its resistance to microbiological attack, and the possibility of repeated utilization. The results obtained show that aminosilochrome may find wide application as an anion-exchange material for the chromatography of a number of proteins.

EXPERIMENTAL

Preparation of Aminosilochrome C-80. Silochrome C-80 (grain size 0.35-0.5 mm, specific surface 80-130 m^2/g) (200 g) was added to 20 g of γ-aminopropyltriethoxysilane in 600 ml of ethanol, and the mixture was heated at 37°C for 72 h. Then the aminosilochrome was washed with ethanol (1.5 liters ×3) and was dried at 120°C for 24 h. The results of the determination of total nitrogen by a modified Kjeldahl method [4] showed that 1 g of the aminosilochrome obtained contained 360 μmole of amino groups.

Study of the Sorption of Immunoglobulin on Aminosilochrome C-80. For this purpose, we used a commercial preparation of immunoglobulin from human placenta obtained in the I. I. Mechnikov Institute of Vaccines and Sera. Each of a number of 50-ml flasks was charged with 500 mg of aminosilochrome equilibrated with 0.01 M phosphate or acetate buffer with pH values from 3.5 to 8.0, and to each was added 0.5 ml of the appropriate buffer and 0.1 ml of the immunoglobulin solution diluted fivefold. With continuous stirring, samples of the supernatant liquid were taken every 5 min and the absorption at 280 nm was measured against the corresponding buffer.

Preparation of the Blood Serum for Sorption. The work was performed with human placentary blood serum obtained by Perkins' method [5] to which 0.02% of sodium azide had been added for preservation. The serum was subjected to gel filtration on a column of Sephadex G-25 (2.6 × 33 cm). The protein fraction was collected and was kept overnight at 4°C, and then the precipitate was separated by centrifuging at 6000 rpm at +4°C on a High Speed 25 centrifuge. Sodium azide was added to the serum to a final concentration of 0.02% for preservation.

Isolation of the Immunoglobulin Fraction. The serum prepared as described above (0.5 ml) was deposited on a column containing 5 ml of aminosilochrome equilibrated with 0.01 M phosphate buffer, pH 7.5. The column was washed with 65 ml of the same buffer and then the immunoglobulin fraction was eluted with 0.1 M acetate buffer at the rate of 0.6 ml/min at 4°C, using a peristaltic pump. Fractions (4.5 ml each) were collected in a cooled receiver and their optical densities at 280 nm were measured. The column was washed with 1 M HCl and then with deionized water until absorption at 280 nm had disappeared from the eluate. All the operations were performed at +4°C. The aminosilochrome C-80 was regenerated by being washed with 100 ml of 0.01 M NaOH and then with deionized water to pH 5.6, and it was equilibrated with 80-100 ml of 0.01 M phosphate buffer, pH 7.5. The experiments on the isolation of the immunoglobulin fraction were repeated several times using the same sample of aminosilochrome without appreciable worsening of the properties of the ion-exchange material.

Disk Electrophoresis of the Fractions. The fractions were dialyzed against 0.01 M phosphate buffer, pH 7.0. Their molecular weights were determined by Weber's method [6] in 5% polyacrylamide gel in the presence of a 0.1% solution of sodium dodecyl sulfate in a Savant instrument at a current strength of 14 mA per tube. To plot the calibration curve we used the following proteins: rat immunoglobulin (mol. wt. 160,000), bovine and human serum albumins (mol. wts. 67,000 and 65,000, respectively), catalase (mol. wt. 57,500 ×4), egg albumin (mol. wt. 43,000), aldolase (mol. wt. 40,000 ×4), porcine pepsin (mol. wt. 35,000), chymotrypsinogen (mol. wt. 25,000), ferritin (mol. wt. 18,500 ×24), horse myoglobin (mol. wt. 17,800), and horse hemoglobin (mol. wt. 16,000 ×4). Before deposition of the gel, the standard samples were incubated at 100°C with a 1% solution of 2-mercaptoethanol for 2 min. The samples investigated were incubated under the same conditions without the addition of the 2-mercaptoethanol.

Other Methods. The fractions isolated were investigated by gel immunodiffusion by Ouchterlony's method [7]. Fractions concentrated by ultrafiltration were also investigated immunoelectrophoretically according to Grabar and Burtin [8]. In both cases ovine antiserum to whole human serum (No. 52) and ovine antiserum to human γ-globulin (No. 43), obtained from the Moscow Municipal Institute of Epidemiology and Microbiology through E. V. Chernokhvostova was used for detection. A. N. Mats and N. P. Perepechkina of the Institute of Vaccines and Sera provided the samples of γ-globulin and blood plasma.

SUMMARY

A method has been developed for isolating a fraction enriched in γ-globulin from human blood serum on a column of aminosilochrome C-80.

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