LITERATURE CITED

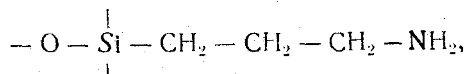
1. L. I. Brutko and P. S. Massagetov, Khim. Prirodn. Soedin., 21 (1967).
2. M. S. Yunusov, Ya. V. Rashkes, V. A. Tel'nov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 515 (1969).
3. A. S. Narzullaev, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 497 (1973).
4. S. W. Pelletier, L. H. Keith, and P. C. Parthasarathy, J. Am. Chem. Soc., 89, 4146 (1967).
5. M. S. Yunusov and S. Yu. Yunusov, Khim. Prirodn. Soedin., 90 (1970).

ISOLATION AND PURIFICATION OF gamma-GLOBULIN FROM BLOOD PLASMA AND SERUM BY ABSORPTION ON AMINOSILOCHROME

M. A. Tel'nova, T. S. Kotova,
S. V. Belyaev, and V. M. Stepanov

UDC 576.8.097:543.544

Aminosilochrome [1, 2] — a derivative of macroporous silica containing covalently bound alipatic amino groups



— may be used in the chromatography of proteins as an anion-exchange resin which is favorably distinguished from the ion-exchange resins based on cellulose that are usually used by simplicity of preparation, stability to a number of chemical and microbiological effects, and, which is particularly important, by a low hydrodynamic resistance. Another feature of aminosilochrome that is of practical importance is the very high rate of settling of aqueous suspensions of this sorbent which make it particularly convenient for the sorption of proteins under static conditions — a process which is distinguished by rapidity and is applicable to mixtures of extremely complex composition.

In the present work we consider the use of selective sorption on aminosilochrome under static conditions to isolate and purify the gamma-globulins of blood.

Chromatography on columns of DEAE-cellulose [3] or other ion-exchange resins or sorption from solutions on the same ion-exchange resins under static conditions [4] is frequently used to obtain pure gamma-globulins.

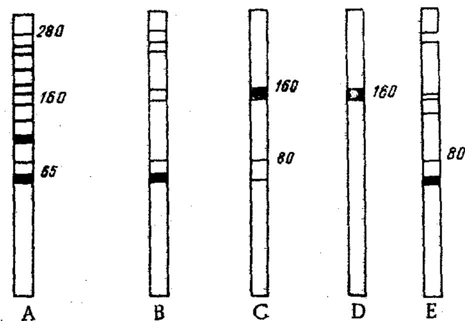
The conditions for the sorption of the gamma-globulins on aminosilochrome under static conditions are basically similar to those developed for the column chromatography of these proteins on aminosilochrome [2]. The sorption of the gamma-globulins on aminosilochrome is performed at pH 7.5, and the sorbent binds not only the gamma-globulins but the albumins of the serum and some other proteins. At the same time, a considerable part of the proteins — fraction I, amounting to 46% of their total amount — is not bound at this pH and is readily

TABLE 1. Chromatography of Human Blood Serum on Aminosilochrome C-80

Fraction	Desorption conditions	Volume, ml	Protein content	
			opt. units	%
Initial serum	—	0.7	24.5	100
I	0.01 M phosphate buffer, pH 7.5	69	11.3	46
II	0.01 M acetate buffer, pH 4.5	40	6.0	24.6
III	1 M HCl	65	7.3	29.6
Total				100.2

All-Union Scientific-Research Institute of the Genetics and Selection of Industrial Microorganisms, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 838-842, November-December, 1977. Original article submitted August 16, 1977.

 0009-3130/77/1306-0706$07.50 © 1978 Plenum Publishing Corporation



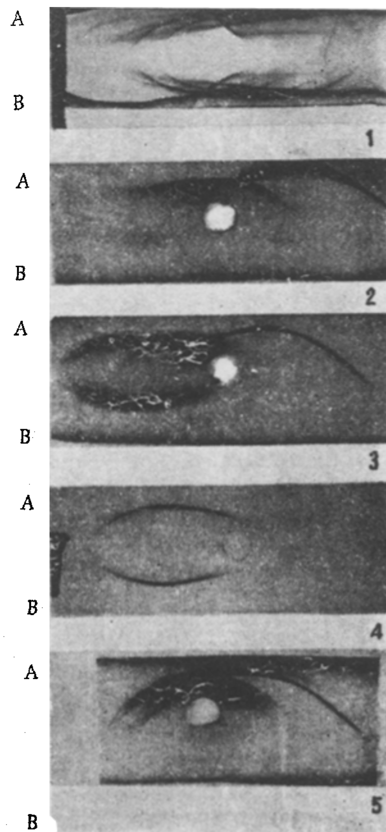


Fig. 1 Fig. 2

Fig. 1. Electrophoresis of protein fractions in polyacrylamide gel: A) blood serum; B) fraction I; C) fraction II before salting out; D) fraction II after salting out with sodium sulfate; E) fraction III. The molecular weights of the proteins are given in thousands.

Fig. 2. Immunoelectrophoresis of the protein fractions: 1) human blood serum; 2) fraction I; 3) fraction II before salting out with sodium sulfate; 4) fraction II after salting out with sodium sulfate; 5) fraction III; A) ovine antiserum to whole human serum; B) ovine antiserum to human gamma-globulin. In the immunoelectrophoretogram of fraction (II) before salting out (No. 3) can be seen from left to right: gamma-globulin, transferrin, albumin.

separated from the sorbent by simple decantation (Table 1). According to electrophoresis in polyacrylamide gel and immunoelectrophoresis, this fraction contains almost all the protein of the blood plasma or serum in various ratios. Here is concentrated the bulk of the albumin, and the gamma-globulins are present only in trace amounts (Fig. 1b; Fig. 2, part 2). After repeated washing of the sorbent by the decantation method, which greatly accelerates all operations, the gamma-globulins are desorbed by changing the pH to 4.5 — a region close to the isoelectric point of these proteins. Fraction (II) so obtained (see Table 1) contains mainly the gamma-globulin together with small contaminating amounts of other proteins (Fig. 1c). Serum albumin and transferrin have been identified among them by immunoelectrophoresis (Fig. 2, part 3). Fraction (II) makes up 20-25% of the total amount of proteins in the blood plasma or serum. When the amount of blood serum deposited on 2 g of dry aminosilochrome was changed from 0.2 ml to 1.5 ml, the proportion of fraction (II) scarcely changed, amounting to 24%, which shows the high capacity of the sorbent. At pH 4.5, part of the proteins of the serum remained bound to the ion-exchange resin and was eluted only when it was treated with 1 M HCl (fraction III, Fig. 1e; Fig. 2, part 5); it makes up 29.6% of the total amount of protein.

In order to obtain immunochemically pure gamma-globulin, it was precipitated by adding sodium sulfate to fraction II to give a final concentration of 17%. The precipitate obtained

was dissolved in 0.1 M phosphate buffer, pH 7.5, and was dialyzed against the same buffer, and the purity of the preparation obtained was checked by electrophoresis in polyacrylamide gel and by immunoelectrophoresis (Fig. 1d; Fig. 2, part 4). The gamma-globulin isolated amounted to 37% of the protein contained in faaction (II).

Similar results were obtained in the fraction of antitetanic plasma and horse blood serum. In this case, the pure gamma globulin amounted to 50% of the protein present in fraction (II).

Thus, the use of sorption on aminosilochrome C-80 under static conditions in combination with precipitation by means of sodium or ammonium sulfate enables a high-purity preparation of gamma-globulin to be obtained from blood plasma or serum. It must be noted that the operation to obtain the enriched gamma-globulin of fraction (II) occupies about four hours under laboratory conditions.

The method of isolating gamma-globulin on a sorbent consisting of modified macroporous silica — aminosilochrome C-80 — under static conditions can be used to isolate other proteins. Thus, it has been used to obtain highly purified porcine pepsin [5].

EXPERIMENTAL

Preparation of Aminosilochrome C-80. Silochrome C-80 (granule 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. The aminosilochrome obtained was washed with ethanol (3 × 1.5 liter) and was dried at 120°C for a day. A determination of the total nitrogen by a modified Kjeldahl method [6] showed that 1 g of aminosilochrome C-80 contained 518 mole of nitrogen.

Blood Serum. The work was carried out with native human donor plasma and the serum obtained from it by Perkins' method [7]. The serum was desalted by 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, the precipitate forming during this period being separated by centrifuging at 6000 rpm on a High Speed 25 centrifuge. The fraction obtained was treated with 0.02% of sodium azide.

Isolation of the gamma-Globulin Fraction (see Table 1). In a 50-ml beaker, 40 ml of 0.01 M phosphate buffer, pH 7.5 was added to 2 g of aminosilochrome C-80. The buffer solution was removed by decantation so that the anion-exchange material remained moist. Then 0.7 ml of human donor blood serum or plasma was deposited on its surface and the mixture was stirred at 20°C for 15 min. After this, the sorbent was washed repeatedly with 10-ml portions of the initial buffer until the wash-waters ceased to show appreciable absorption at 280 nm. The wash waters with absorption greater than 0.100 optical unit/ml were combined into fraction (I) (see Table 1). The aminosilochrome was transferred to a glass filter and the gamma-globulin fraction was immediately eluted with 10-ml portions of 0.1 M acetate buffer, pH 4.5 (fraction II, Table 1). Then the aminosilochrome was washed with 1 M HCl until absorption in the eluate at 280 nm had disappeared (fraction III), with 100 ml of 0.01 M NaOH, and with deionized water to pH 5.6. The same sample of aminosilochrome C-80 was used repeatedly (up to 10 times) with no deterioration of its properties.

Preparation of Pure gamma-Globulin. To obtain immunochemically pure gamma-globulin, 50 ml of fraction (II) (optical density at 280 nm — 0.120 opt. units/ml) was treated with sodium sulfate to a final concentration of 17%. After stirring for 1.5 h, the precipitate containing the gamma-globulin was separated by centrifuging at 6000 rpm for 20 min and the supernatant (47 ml with an absorption at 0.075 opt. units at 280 nm) was discarded. The precipitate was dissolved in 1 ml of 0.1 M phosphate buffer, pH 7.5 and the solution was dialyzed against the same buffer for 24 h. This gave 1 ml of a solution of gamma-globulin containing 2.22 opt. units of protein, which corresponds to 37% of the total protein of fraction II.

Electrophoresis in Polyacrylamide Gel. To estimate the protein compositions of fractions I, II, and III, they were dialyzed against 0.01 M phosphate buffer, pH 7.0 and were analyzed by electrophoresis in 5% polyacrylamide gel in the presence of 0.1% sodium dodecyl sulfate in a Savant instrument at a current strength of 14 mA per tube. The gels were stained with 0.25% Coomassie Blue in 7% acetic acid and were washed with 7% acetic acid. The molecu

lar weights of the proteins revealed were determined by the method of Weber and Osborn [8], using a calibration curve plotted with the following standards: rat gamma-globulin (mol. wt. 160,000), human and bovine serum albumins (mol. wts. 65,000 and 67,000, respectively), catalase (mol. wt. 57,500), egg albumin (mol. wt. 43,000), aldolase (mol. wt. of a subunit 40,000), porcine pepsin (mol. wt. 35,000), chymotrypsinogen (mol. wt. 25,000), ferritin (mol. wt. of a subunit 18,500), horse myoglobin (mol. wt. 17,800), and horse hemoglobin (mol. wt. of a subunit 16,000). Before deposition on the gel, the standard samples, apart from the rat gamma-globulin, were incubated at 100°C with a 1% solution of mercaptoethanol and a 1% solution of sodium dodecyl sulfate for 2 min. The samples investigated, and also the rat gamma-globulin were incubated under the same conditions without the addition of the mercaptoethanol.

Other Methods. The fractions isolated were investigated by immunoelectrophoresis [9]. To reveal the proteins isolated we used ovine antisera to whole human blood serum (No. 22) and to human gamma-globulin (No. 43) (provided by E. V. Chernokhvostova, Moscow Municipal Institute of Epidemiology and Microbiology).

SUMMARY

An accelerated method has been developed for isolating gamma-globulin from blood plasma and serum which is based on sorption on aminosilochrome C-80 under static conditions.

LITERATURE CITED

1. L. F. Matyash, T. L. Voyushina, S. V. Belyaev, and V. M. Stepanov, Prikl. Biokhim. Mikrobiol., 11, 604 (1975).
2. M. A. Tel'nova, S. V. Belyaev, T. S. Kotova, and V. M. Stepanov, Khim. Prirodn. Soedin., 261 (1977).
3. C. A. Williams and M. W. Chase, Methods in Immunology and Immunochemistry, Academic Press, New York (1968).
4. A. E. Reif, Immunochemistry, 6, 723 (1969).
5. T. A. Solov'eva, S. V. Belyaev, and V. M. Stepanov, Khim. Prirodn. Soedin., 398 (1977).
6. V. Kh. Akrapov and V. M. Stepanov, Prikl. Biokhim. Mikrobiol., 13, 141 (1977).
7. M. Perkins, in: Manual of Tissue Typing Techniques (ed. J. G. Ray, D. B. Hare, and E. Donald), Bethesda, Maryland (1973).
8. K. Weber and M. Osborn, J. Biol. Chem., 244, 4406 (1969).
9. P. Grabar and P. Burton, Immunoelectrophoretic Analysis [Russian translation], Moscow (1963).