acetone cyanohydrin) to the double bond of the methylene group of the α-hydroxymethylene- and α-dimethylaminomethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-ones has led to α-[cyano-(hydroxy)methyl]- and α-[cyano(dimethylamino)methyl]-2,3-trimethylene-3,4-dihydroquinazolin-4-ones.

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ISOLATION OF TRANSFERRIN FROM RAT BLOOD AND ITS PURIFICATION AND SOME PHYSICOCHEMICAL PROPERTIES

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Transferrin (Tf — synonym: siderophilin) is a metal-containing glycoprotein which is responsible for the transport of iron into the cells of the erythroid series, where the biosynthesis of hemoglobin is performed. It consists of one polypeptide chain with a molecular weight of 70,000-90,000 daltons and has two specific sites binding two iron ions [2].

The transferrins form one of the most polymorphic systems of blood serum proteins. In man no less than 17 alleles forming an autosomic two-allele system controlling a single locus and inherited codominantly have been found. The molecular forms of transferrin differ in the magnitude of the charges, which depend on the number of sialic acid residues attached to the protein and, to a smaller extent, on the presence of one or two ferric iron ions attached to the protein molecule [1].

On electrophoresis in polyacrylamide gel (PAAG), the transferrins of different species of animals migrate in the form of several zones, which permits us to speak not of transferrin but of transferrins [4].

There is no information in the literature on the mechanism of the biosynthesis of this protein and its regulation at the molecular and genetic levels. We have isolated and partially characterized the transferrin from rat blood serum in order to study the molecular mechanisms of the regulation of its biosynthesis.

As a result of precipitation with ammonium sulfate and two subsequent stages of chromatography on ion-exchange resins (Fig. 1), the transferrin was purified to the state of electrophoretic homogeneity (Fig. 2b).

The molecular weight of the transferrin determined by electrophoresis in PAAG in the presence of sodium dodecyl sulfate (NaDDS) was 76,500 daltons, which agrees well with the molecular weight of this protein for many other species of animals and man [4-7]. The absorption spectrum in the visible region after rechromatography showed a peak at 465 nm, and there was a second peak with a maximum of 410 nm which appeared after chromatography on DEAE-Sephadex A-50, apparently representing a hemopexin impurity [6]. Isoelectric focusing in PAAG with the use of 40% ampholine (pH 3-10) showed two bands corresponding to two isoforms of transferrin (Fig. 2c), which indicates the polymorphic nature of this system of blood proteins

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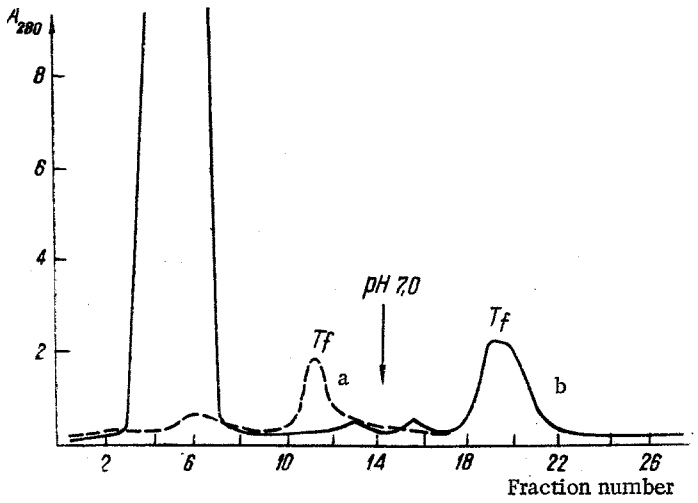

Fig. 1. Diagram of the elution of Tf; a) on a column of CM-cellulose. Charging buffer ($CH_3COONa - CH_3COOH$), 0.05 M, pH 5.0. Eluting buffer ($CH_3COONa - CH_3COOH$) 0.05 M, pH 7.0, rate of elution 60 ml/h. Fraction volume 18 ml. The first peak corresponds to the albumins and the fourth to transferrin. The materials of the second and third peaks have not been identified; b) on a column of DEAE-Sephadex A-50. Charging buffer (Tris-HCl) 0.05 M, pH 8.6. Eluting buffer with a linear ionic strength gradient (Tris-HCl, 0 M NaCl – Tris-HCl, 0.5 M NaCl). Rate of elution 60 ml. Fraction volume 20 ml. The second peak corresponds to Tf.

differing in the sialic acid contents [1]. From the graph of Fig. 3 we calculated the isoelectric points. equal to 6.0 and 6.3, which corresponds to the values of the isoelectric points of the transferrins of other higher organisms [3, 8].

EXPERIMENTAL

The isolation of the transferrin from rat blood serum was carried out by the method of Guerin et al. [8], with some modifications. The blood was taken from the caudal veins of random-bred albino rats of both sexes weighing 150-200 g. All the operations were performed in the cold to prevent hemolysis. The blood was thermostated at 37°C for 1 h and after retraction of the clot, the serum was taken off by low-speed centrifuging at 3000 rpm for 20 min. It was freed from residues of erythrocytes by additional centrifuging at 6000 rpm for 30 min, and the serum was stored at –20°C.

Precipitation with $(NH_4)_2SO_4$ was begun by bringing the pH of the serum to 8.0 and it was saturated with iron ions with the aid of a 0.6 M solution of ferrous chloride, in order to achieve the optimum stability of the protein [8, 9]. Then a saturated solution of ammonium sulfate was added to the serum to 60% saturation and the mixture was left at +4°C overnight. The protein precipitate was centrifuged at 6000 rpm for 30 min and was then dialyzed against sodium acetate buffer (0.05 M, pH 5.0) with three changes of the buffer solution.

Chromatography on CM-Cellulose. For chromatography we used Whatman CM-32 CM-cellulose (United Kingdom) equilibrated with sodium acetate buffer (0.05 M, pH 5.0) on a K 26/40 column (Sweden). After salt precipitation, the protein solution was deposited on the column of CM-cellulose which was then washed with the charging buffer until the background line on a LKB Uvicord P recorder (Sweden) was stable, after which elution was carried out with sodium acetate buffer, 0.05 M, pH 7.0 (see Fig. 1).

Chromatography on DEAE-Sephadex A-50. We used Pharmacia DEAE-Sephadex A-50 (Sweden) equilibrated with Tris-HCl buffer (0.05 M, pH 8.6) on a K 26/40 column. The fraction containing the transferrin, after chromatography on a column of CM-cellulose, was dialyzed against the buffer with three changes. Elution was carried out with a linear ionic strength gradient (from Tris-HCl, 0.1 M NaCl, to Tris-HCl, 0.05 M NaCl), the protein in the eluate being recorded with the aid of the Uvicord P (see Fig. 1). Analysis of the homogeneity of the protein

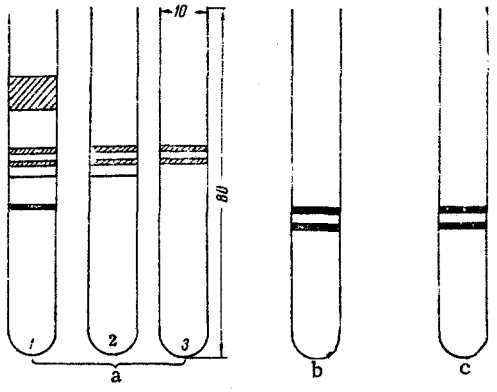

Fig. 2. Electrophoresis of transferrin in the process of purification. a: 1) Gel electrophoresis of Tf in PAAG after precipitation with $(NH_4)_2SO_4$ to 60% saturation; 2) gel electrophoresis of the Tf fraction after chromatography on CM-cellulose (Whatman CM 32); 3) gel electrophoresis of the Tf fraction after chromatography on DEAE-Sephadex A-50. b: Electrophoretically homogeneous Tf obtained after precipitation by $(NH_4)_2SO_4$ and ion-exchange chromatography (the two bands correspond to two isoforms of Tf). c: Isoelectric focusing of the two isoforms of transferrin in PAAG, ampholines pH(3-10).

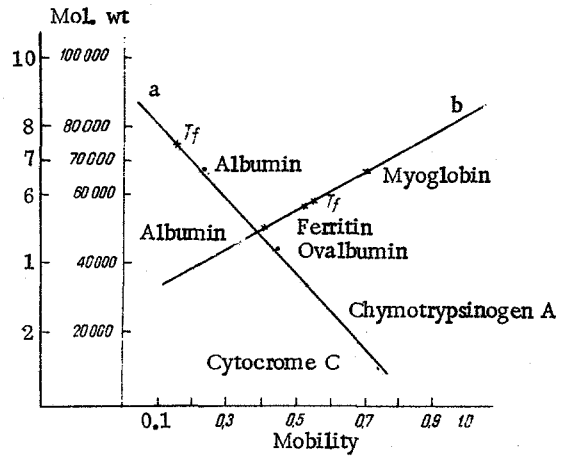

Fig. 3. Dependence of the electrophoretic mobility of Tf and of marker proteins in the presence of SDS on the molecular weight (a) and the isoelectric points (b).

by electrophoresis in PAAG is shown in Fig. 2. The concentration of the protein in the samples was determined from the absorption at 280 nm [2] and by Lowry's method [10].

Electrophoresis in PAAG. Electrophoresis was performed in "stacking" gels using an 8% separating gel and a 4% concentrating gel in 0.8 × 11 cm tubes of transparent plastic in Tris-glycerol buffer, pH 8.6, as the electrode buffer. The time of electrophoresis was 2 h at 4 mA per tube, and fixation was carried out in 7% acetic acid for 1 h. The gels were stained with Serva (GFR) Coomassie Brilliant Blue for 2 h and were washed free from excess of dye by being kept in a 1:5:5 mixture of glacial acetic acid, methanol, and water for several hours.

Electrophoresis in PAAG in the presence of SDS was carried out by the method of Weber and Osborne [11]. Bovine serum albumin, ovalbumin, chymotrypsinogen A, and cytochrome C [all Serva products (GFR)] were used as marker proteins.

Isoelectric focusing was carried out in acrylamide gels using riboflavin as polymerization catalyst. The sample was added directly to the gel in a concentration of 2.5 mg/ml.

For isoelectric focusing in PAAG we used a normal instrument for electrophoresis with 0.24 N H_2SO_4 as the anode solution and 0.48 N NaOH as the cathode solution. The process was continued for 24 h at an initial current of 2.5 mA per tube of gel. The gels were fixed in 10% trichloroacetic acid for 1 h and were then stained with a 0.1% solution of Coomassie Brilliant Blue in a 1:5:5 mixture of acetic acid, methanol, and water, and were washed free from superfluous dye with the same mixture. As marker proteins we used Serva (GFR) proteins with known values of the isoelectric points: bovine serum albumin, ferritin, and sperm whale myoglobin.

The absorption spectrum in the visible region was determined for a 1% solution of transferring on a Hitachi spectrophotometer (Japan). The samples were dialyzed against 0.1 M Tris-HCl buffer, pH 7.5, containing 0.015 M $NaHCO_3$ for binding free iron.

SUMMARY

As the result of a purification process consisting of precipitation and successive stages of chromatography on ion-exchange resins, electrophoretically homogeneous rat transferrin has been obtained with a molecular weight of 76,500 daltons, a maximum in the absorption spectrum at 465 nm, and isoelectric points of the two isoforms of 6.0 and 6.3.

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THE GLOBULINS OF COTTON SEEDS

XIX. CHYMOTRYPTIC AND TRYPTIC PEPTIDES OF SUBUNIT C OF THE 11S GLOBULIN

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In order to determine the primary structure of subunit C of the 11S globulin of cotton seeds [1], we have cleaved it with chymotrypsin and trypsin [2, 3]. From the acid-soluble fraction of the chymotryptic hydrolyzate, by chromatography on an ion-exchange column we obtained 33 combined fractions. Of them, fractions 1, 2, 3, 4, 10, 11, 19, 32, and 33 represented pure peptides. The homogeneity of the peptides was checked by paper chromatography, thin-layer electrophoresis, and determinations of the N-terminal amino acids. The other fractions were separated by paper chromatography and also by preparative electrophoresis in a thin layer of cellulose. The acid-insoluble fraction (XT P1) consisted mainly of a single peptide (its final purification was performed by gel filtration on a column of Sephadex G-50) containing carbohydrate. Table 1 gives the amino acid compositions of the chymotryptic peptides isolated in the pure state.

We may note that Table 1 gives only 51 peptides, although another 28 peptides were isolated. However, because of their small amount we determined only their N-terminal amino acids.

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