SOME PHYSICOCHEMICAL PARAMETERS OF TRANSFERRIN ISOLATED FROM RAT BLOOD SERUM

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UDC 576.8097.543.544

Chromatography on ion-exchange resins has yielded a homogeneous iron-containing glycoprotein – transferrin. The amino acid composition of the protein has been shown, it has been established that the N-terminal amino acid of the protein is threonine, and the sedimentation coefficient has been calculated, being 5.8 S.

Serum transferrin (TF), which fulfills the function of transporting ferric iron ions into cells of the erythroid series where the biosynthesis of hemoglobin takes place, consists of a single glycopeptide chain with a molecular weight averaging 77,000 dalton [1]. The molecule of serum transferrin contains two specific centers binding Fe^{3+}, each of which can ensure the transport of the iron necessary for the biosynthesis of hemoglobin in the reticulocytes [2].

Transferrins form one of the most polymorphic systems of blood serum proteins. The molecular forms of transferrin differ by the magnitude of the charges, which depend on the number of sialic acid residues attached to the protein and, to a smaller degree, on the presence of one or two ferric iron ions attached to the protein molecule [3].

Recently, statements have appeared in the literature on the existence of a phenomenon of internal homology in the amino acid sequence of the transferrins [4], which is an indication of doubling of the structural gene for transferrin precursors in the course of phylogenesis.

We have isolated rat serum transferrin in two steps and have characterized it with respect to a number of physicochemical parameters in order to study its primary structure. The method of isolation that we used differs from that employed previously [5] by the higher purity of the given protein and a higher yield.

As the result of two successive chromatographic separations, first on DEAE-Sephadex A-50 and then on Sephadex G-100, we obtained homogeneous rat serum transferrin. The homogeneity of this protein was shown by electrophoresis in PAAG, by sedimentation, and by N-terminal amino acid analysis (Figs. 1 and 2). The molecular weight of the transferrin determined by electrophoresis in PAAG in the presence of sodium dodecyl sulfate was 76,000 dalton [5]. It was shown that the transferrin possesses a characteristic absorption spectrum in the visible region with a maximum at 465 nm. In the recording of the absorption spectrum the peak characteristic for heme-containing serum protein (hemoglobin, hemopexin) at 410 nm was not observed, which is an additional criterion of the purity of the transferrin isolated. The amino composition of the transferrin has been determined. A comparison of the amino acid compositions of differing transferrins has been made (Table 1), and the sedimentation coefficient has been calculated as 5.8 S, this value correlating well with that given in the literature for the sedimentation coefficients of the transferrins of other species of animals.

EXPERIMENTAL

Transferrin was isolated from rat blood serum in two steps: 1) chromatography on DEAE-Sephadex A-50 and 2) chromatography on Sephadex G-100.

In the experiments we used random-bred white rats of both sexes weighing 150-200 g. The blood was taken from the previously hexenal-anesthetized rats by cardic puncture. The blood was thermostated at 37°C for 1 h and after the retraction of the clot the serum was taken off by low-speed centrifugation at 3000 rpm for 30 min. Additional centrifugation at 6000 rpm for 20 min freed the serum from erythrocyte residues, and it was then stored at −20°C until it was used.

Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 89-92, January-February, 1980. Original article submitted July 13, 1979.

0009-3130/80/1601-0074 $07.50

Fig. 1

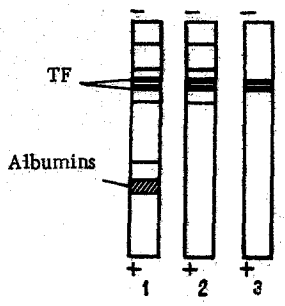

Fig. 2

Fig. 1. Sedimentation of transferrin (2 mg/ml) in 0.1 M tris-HCl buffer, pH 7.4, containing 0.1 M NaCl at 44,000 rpm (20°C, 60 min).

Fig. 2. Electrophoresis of transferrin in PAAG in the process of purification (the two bands on electrophoresis correspond to two isoforms of transferrin): 1) electrophoresis of TF in PAAG after salting out with $(NH_4)_2SO_4$ to 60% of the level of complete saturation; 2) electrophoresis of the TF in PAAG after chromatography on a column containing DEAE-Sephadex A-50; 3) electrophoresis of TF in PAAG after chromatography on a column of Sephadex G-100.

Chromatography on DEAE-Sephadex A-50. For chromatography we used DEAE-Sephadex A-50 (Sweden) equilibrated with sodium phosphate buffer (0.02 M, pH 6.6) on a K 26/40 column. The thawed-out serum (60 ml) was saturated with iron ions with the aid of a solution of $FeCl_3$, and then trace amounts of $NaHCO_3$ were added. After this, the serum was dialyzed in the cold against sodium phosphate buffer (0.02 M, pH 6.6) for 12 h. The precipitate that deposited was taken off, and the supernatant was deposited on a column of ion-exchange resin. The column was washed with sodium phosphate buffer (0.02 M, pH 6.6). The material not bound to the sorbent, containing mainly globulins, was discarded. The transferrins were eluted with sodium phosphate buffer (0.04 M, pH 5.9). The protein in the eluate as recorded automatically at 280 nm by means of a Spectrochrome F detector (France).

Chromatography on Sephadex G-100. For chromatography we used a column (100 × 3.3 cm) filled with Sephadex G-100 (Sweden) equilibrated with tris-HCl buffer (0.1 M, pH 7.3) containing 1 M NaCl. The transferrin fraction obtained after the preceding chromatography was dialyzed against tris-HCl buffer (0.1 M, pH 7.3) containing 1 M NaCl in the cold for 12 h before being deposited on the column of Sephadex G-100. The rate of passage through the column was 25 ml/h. The protein was recorded from its absorption at 280 nm with the aid of a Spektrokhrom F detector.

TABLE 1. Amino Acid Compositions of Human, Rabbit, Fish, and Rat Transferrins

Amino acid	Amino acid residues/mole of protein			
	human [6], mol. wt. 80,000	rabbit [6], mol. wt. 80,000	fish [6], mol. wt. 80,000	rat, mol. wt. 76,000
Asp	79	85	85	54
Thr	28	32	34	33
Ser	39	59	55	32
Glu	63	74	79	57
Pro	31	35	34	30
Gly	48	56	62	49
Ala	56	62	58	55
1/2 Cys	39	33	32	44
Val	43	50	44	36
Met	8	7	10	6
Ile	15	9	32	21
Leu	57	70	49	54
Tyr	22	26	36	21
Phe	28	32	29	29
Lys	60	61	61	58
His	20	20	18	14
Arg	27	29	29	25

Electrophoresis in PAAG. Electrophoresis was performed in "stacking" gels using an 8% separating and a 3% concentrating gel in tubes of transparent plastic, 0.8 × 11 cm, in tris-glycine buffer, pH 8.6 as the electrode buffer. The time of electrophoresis was 1.5 h at 4 mA per column of gel, and the fixation was carried out in 7% acetic acid for 1 h. The gels were stained with Coomassie Brilliant Blue P-230 (GFR) for 2 h and were washed free from excess dye with a mixture of glacial acetic acid, isopropanol, and water in a ratio of 1:5:5 for several hours.

Electrophoresis in PAAG in the presence of sodium DDS was carried out by the Weber-Osborn method [7]. As marker proteins we used bovine serum albumin, ovalbumin, chymotrypsinogen A, and cytochrome c (GFR).

Absorption spectra in the visible region were determined for 1% solutions of apotransferrin and of iron-saturated transferrin using a model 25 spectrophotometer with a cell having a working path-length of 1 cm. The samples were dissolved in 0.1 M tris-HCl buffer, pH 7.5, containing 0.015 M $NaHCO_3$, and were then dialyzed intensively against the same buffer with three changes to eliminate free iron. An aliquot of the dialysate was then used as control.

The sedimentation analysis of the rat serum transferrin was carried out on a Spinco model E analytical ultracentrifuge. On the sedimentation of a solution of a protein in a concentration of 2 mg/ml in 0.01 M tris-HCl buffer, pH 7.4, containing 0.01 M NaCl at 44,000 rpm for 60 min at 23°C using a synthetic cell one symmetrical schlieren peak was observed (Fig. 1). In the calculation of the sedimentation coefficient, a correction was introduced for the viscosity, density, and temperature of the solvent. The sedimentation coefficient was calculated as 5.8 S or 5.8×10^{-13} sec.

Amino acid analysis was carried out on a Multichrome automatic amino acid analyzer (USA). The hydrolysis of iron-saturated transferrin was carried out in glass tubes in vacuum in the presence of 6 N HCl for 24 h.

The N-terminal amino acid was determined by the Edman method [8]. It was found that the N-terminal amino acid of this protein is threonine.

SUMMARY

As the result of a two-step purification of the serum by chromatography on ion-exchange resins, homogeneous rat serum transferrin has been isolated.

The degree of purification of the resin was shown by electrophoresis in PAAG, centrifugation in an analytical ultracentrifuge, and N-terminal amino acid analysis.

The transferrin isolated possessed a characteristic absorption spectrum in the visible region with a maximum at 465 nm.

It was established that the N-terminal amino acid of the protein is threonine. The amino acid composition of the protein was determined and the sedimentation coefficient was calculated, being 5.8 S.

LITERATURE CITED

1. F. C. Greene and R. F. Feeney, Biochemistry, 7, 1366 (1968).
2. A. L. Scade, R. W. Reinhart, and H. Levy, Arch. Biochem., 20, 170 (1949).
3. G. A. Annenkov, Primate Serum Proteins [in Russian], Moscow (1974).
4. R. T. MacGillivray and K. Brew, Science, 190, 1306 (1975).
5. A. A. Buglanov, V. M. L'vov, T. A. Salikhov, and Kh. A. Aslanov, Khim. Prir. Soedin., 5 (1978).
6. H. G. van Eijk et al., Scand. J. Haemat., 9, 267 (1972).
7. K. Weber and M. Osborn, J. Biol. Chem., 244, 4406 (1969).
8. P. Edman, Ann. N. Y. Acad. Sci., 88, 602 (1960).