# TRYPTIC PEPTIDES OF RAT SERUM TRANSFERRIN

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Limited hydrolysis has given two peptide fractions bearing iron-binding centers. A partial characterization of them is presented.

Transferrins represent a class of single-chain glycoproteins capable of reversible binding ferric iron ions which are necessary for the biosynthesis of hemoglobin in reticulocytes, and also some other metal ions, for which there are two specific binding centers in the molecule of the protein. On an average, the molecular mass of the transferrins is 77,000 daltons [1].

The so-called electrophoretic polymorphism of the transferrins is due to different amounts of sialic acid residues or the presence of one or two ferric iron ions added to the protein molecule [2].

Some years ago, MacGillivray and Brew [3] described two sections of the primary structure of human serum transferrin consisting of, respectively, 87 and 57 amino acid residues. When the sections were superposed upon one another in a suitable manner, about 40% of the amino acid residues in the corresponding positions proved to be identical. Such a phenomenon of internal homology in a protein structure suggests that in the course of phylogenesis a doubling of the chain for transferrin precursors took place. Recently, structural homology has also been shown for human lactotransferrin and ovotransferrin (conalbumin) [4].

As long ago as 1968, Fletcher and Huehns [5] showed that transferrin at 50% saturation was a less effective donor of iron to reticulocytes than 100%-saturated transferrin. Furthermore, it was noted that the two-iron-binding centers differed in their capacity for liberating iron. On the basis of these facts, it was assumed that the transferrins are not simply passive iron carriers but play a key role not only in the transport of iron but also in the regulation of its absorption and distribution in the organism.

It is quite obvious that great interest is roused by the possibility of cleaving the transferrin molecules into two monoiron fragments and studying each iron-binding center separately. Certain difficulties in this respect result from the fact that iron-saturated transferrin (Fe-TF) is fairly resistant to proteolysis, and the apo form (apo-TF) of the protein rapidly degrades into small peptides incapable of binding iron [6].

However, we have succeeded in showing that by treating iron-saturated serum transferrin trypsin it is possible to cleave the transferrin molecule into two peptide fragments — C-terminal and N-terminal, respectively — which can then be separated satisfactorily on a column of Sephadex G-100. The pattern of gel filtration is shown in Fig. 1a and b. On intensification, the material present in the first chromatographic peak proved to be transferrin that had not been cleaved under the action of the enzyme. The mixture of peptide fragments present in the fraction of the second chromatographic peak could then be separated by repeating gel filtration.

To obtain the molecular-weight characteristics of the tryptic peptides obtained, we used electrophoresis in polyacrylamide gel (PAAG) in the presence of a denaturing agent — sodium dodecyl sulfate (SDS) — in comparison with marker proteins of known molecular weights. We calculated the molecular weights of these fragments as 33,000 and 38,000 daltons, respectively. On identification, the peptide fragment with the higher molecular weight (38,000 daltons) proved to be N-terminal. On analysis of the N-terminal amino acids, they proved to be identical in the native protein molecule and in the N-terminal tryptic fragment (38,000 daltons) (valine residue).

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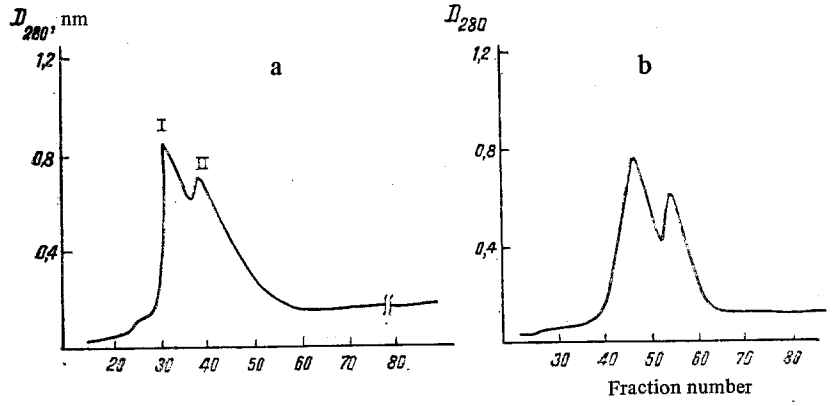


Fig. 1. Gel filtration of a trypsin hydrolysate (a) and of a mixture of neutral fragments (b) on a column (2.5 × 100 cm) of Sephadex G-100: I) peak containing uncleaved transferrin; II) peak containing a mixture of peptide fragments.

An analysis of the spectroscopic properties of the tryptic peptides obtained showed that each contained an iron-binding center, as a result of which the peptides exhibited characteristic absorption in the visible region of the spectrum with a maximum at about 450-465 nm (Fig. 2). On comparing the recorded absorption spectra in the visible region for the native transferrin molecules and for the mixture of tryptic peptide fragments obtained, we observed no appreciable differences whatever between them.

A comparison of the amino acid composition of the N-terminal and C-terminal tryptic peptides (Table 1) gave extremely similar results. There are some differences in the amounts of residues of the amino acid alanine, phenylalanine, and histidine. The sum of the amino acid residues of both fragments taken together give the pattern of the amino acid composition of the native transferrin.

Apparently, the cleavage of the native transferrin under the action of a proteolytic enzyme — trypsin — into two fragments differing only slightly from one another in molecular mass and electrophoretic mobility does not cause appreciable conformational changes or changes in the capacity for binding iron in the two peptide fragments formed.

## EXPERIMENTAL

The rat serum transferrin was isolated by a method which we have described previously [7]. To prove the homogeneity of the purified transferrin preparation we used methods of electrophoresis in polyacrylamide gel (PAAG) of the native protein and electrophoresis in PAAG in the presence of a denaturing agent — sodium dodecyl sulfate (SDS). The protein obtained had a $E_{280}/E_{465}$ ratio of 24.9, which is close to the value of this ratio for homogeneous transferrin saturated with iron ions [8].

Tryptic Hydrolysis of Transferrin. The rat serum transferrin was saturated with iron ions with the aid of a solution of $FeCl_3$, and then trace amounts of $NaHCO_3$ were added and the mixture was incubated with "Serva" trypsin for 10 h (7 mg/ml of transferrin, 0.5 mg/ml of trypsin in 0.3 M Tris-glycine buffer, pH 8.3).

Chromatography on Sephadex G-100. The trypsin hydrolysate was chromatographed on a column (2.5 × 100 cm) filled with Sephadex G-100 (Sweden) in a 0.1 M Tris-HCl buffer system, pH 7.3, containing 1 M NaCl. The protein eluate was recorded from its absorption at 280 nm. Chromatography gave two fractions. The material present in the fractions was dialyzed against distilled water and was then made alkaline to pH 7.5, concentrated, and freeze-dried.

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

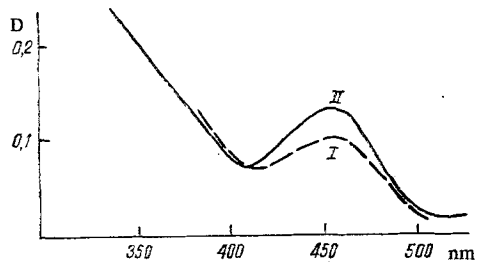


Fig. 2. Absorption spectra of the peptide fragments bearing iron-binding centers (Beckman M25 spectrometer): 1) fragment with a molecular mass of 33,000 daltons; 2) fragment with a molecular mass of 38,000 daltons.

TABLE 1. Amino Acid Composition of the Fragments and of Native Rat Transferrin

| Amino acid | Moles amino acid/mole of protein | | | |
|---|---|---|---|---|
| | fragment w/ mol. mass 33,000 | fragment w/ mol. mass 38,000 | sum of the fragments | rat serum transferrin |
| Asp | 29 | 34 | 64 | 58 |
| Thr | 21 | 16 | 37 | 33 |
| Ser | 28 | 28 | 56 | 34 |
| Glu | 26 | 20 | 46 | 57 |
| Pro | 21 | 26 | 47 | 30 |
| Gly | 29 | 28 | 57 | 51 |
| Ala | 24 | 32 | 56 | 55 |
| Val | 19 | 23 | 42 | 36 |
| Met | 2 | 3 | 5 | 4 |
| Ile | 8 | 8 | 18 | 21 |
| Leu | 30 | 32 | 62 | 54 |
| Tyr | 9 | 12 | 21 | 21 |
| Phe | 8 | 16 | 24 | 29 |
| His | 7 | 12 | 19 | 14 |
| Lys | 37 | 32 | 69 | 58 |
| Arg | 12 | 14 | 26 | 25 |

Electrophoresis in PAAG in the presence of SDS was carried out by the method proposed by Weber [9]. Bovine serum albumin, ovalbumin, chymotrypsinogen A, and cytochrome C, all from "Serva," were used as marker proteins.

Investigation of Absorption Spectra in Visible Light. To record the absorption spectra in the visible region we used a Beckman M-25 spectrophotometer with a cell working space of 1 cm. The samples were dissolved in 0.1 M Tris-HCl buffer, pH 7.5, containing 0.015 M $NaHCO_3$ and the solutions were then dialyzed intensively against the same buffer with three changes in order to eliminate free iron. An aliquot of the dialysate was then used as control.

The N-terminal amino acids were determined by Edman's method [10].

Amino acid analysis was carried out on a Durrum-500 instrument. The hydrolysis of the samples of the peptide fragments and of the iron-saturated transferrin was carried out in sealed glass ampuls in vacuum in 5.7 N HCl at 110°C for 24 h.

## SUMMARY

Two monoiron fragments of rat serum transferrin have been obtained by tryptic proteolysis and separated by gel filtration on Sephadex G-100.

The degree of purification of the N- and C-terminal fragment was shown by electrophoresis in PAAG under denaturing conditions.

The molecular weights of the peptide fragments have been determined and their amino acid compositions have been established.

It has been shown that each peptide fragment has an iron-binding center giving, in the recording of the absorption spectrum in the visible region, a characteristic maxima at 465 nm.

No appreciable differences have been observed in the absorption spectra of the two peptide fragments.

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# IODINE-CONTAINING COMPLEXES OF THE BLACK SEA ALGA *Phyllophora nervosa*


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Iodine-containing complexes have been isolated from a Black Sea red alga. Information is given showing their carbohydrate nature. The causes of the hydrophobic peptide–peptide interaction in the complex are discussed.


The Black Sea red alga *Phyllophora nervosa* (DC) Grev. accumulates considerable amounts of iodine from seawater (up to 0.5% on the dry weight) and contains it in the form of organoiodine compounds which are not extracted by water, weak solutions of acids and alkalis, solutions of salts, or organic solvents [1, 2]. On the isolation from this alga of albumin, globulin, glutelin, and other soluble nitrogenous compounds by known methods [3], the iodine again remains in the insoluble residue. It is logical to assume that the soluble nitrogenous compounds of the alga do not contain iodine. At the same time, attention is attracted by the high amount of insoluble nitrogenous substances in the alga [3], which can be converted into soluble forms as the result of the alkaline degradation of its biomass [4, 5]. Under these conditions, the iodine compounds also pass into a water-soluble state and are detected in the form of iodinated peptides. This suggests that the alga contains insoluble and stable iodine–protein complexes.

We give the results of investigations of the iodine-containing fragments (peptides) obtained by the incomplete alkaline hydrolysis of insoluble iodine–protein complexes of *P. nervosa* which, in our opinion, permit definite information to be obtained about their structure and properties. The iodinated fragments were isolated by means of the following scheme (see scheme on following page.)

The water-soluble nitrogenous compounds were eliminated from the initial air-dry raw material by successive extraction with acetone, 70% ethanol, 7% NaCl solution, and 0.2% NaOH at 4°C and then the water-soluble polysaccharides (the bulk of the mass) were eliminated by treatment with hot water (90°C). The seaweed residue was treated with 1% caustic soda at 90-95°C for 4 h. As a result of the partial hydrolysis of the insoluble iron-protein complexes, alkali-soluble, iodine-containing compounds were obtained which were isolated by precipitation at pH 3.5. The precipitate was purified by repeated reprecipitation and was studied. The presence in the IR spectrum of the iodine compounds obtained of absorption bands (1660,


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