ISOLATION, PURIFICATION, AND CHARACTERIZATION OF FERRITIN FROM HUMAN LIVER

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Ferritin from human liver has been isolated and purified by thermal denaturation and salt precipitation in combination with the methods of ion-exchange and gel permeation chromatography. The partial physicochemical characterization of this protein has been performed. It is proposed to use it in the creation of a radioimmunological method of determining the concentration of ferritin in human blood serum.

A considerable part of the iron in the human organism is present in the form of nonheme reserves in ferritin and hemosiderin [1]. The function of ferritin consists in the retention of ferric ions in nontoxic, soluble, and readily accessible form. Ferritin accumulates mainly in the liver, the spleen, and the bone marrow [2]. With the appearance of highly sensitive methods, it has been established that a definite part of this protein is constantly circulated in the serum and the formed elements of the blood [3], the concentration of this protein in the serum clearly correlating with the amount of reserve iron in the bone marrow [4] and the liver [5].

Since the concentration of ferritin in human blood serum is extremely low, highly sensitive radioimmunological or immunoenzyme methods are necessary for its assay. We have isolated and purified ferritin from the human liver and have also characterized this protein with respect to some physicochemical parameters. The ferritin obtained is being used as a basis for the development of a radioimmunological method of determining the amount of this protein in human blood serum.

To obtain the ferritin from a liver homogenate we used a well-known property of ferritin – its thermal stability. When the homogenate was heated to 70°C, it was possible to denature practically the bulk of tissue proteins. After centrifugation, the supernatant remained enriched with ferritin. When it was then treated with ammonium sulfate to 55% of the level of complete saturation we were able to convert the ferritin fraction into a precipitate quantitatively. However, as electrophoretic analysis in polyacrylamide gel showed, the ferritin precipitated by ammonium sulfate was not pure. For its further purification we used ion-exchange chromatography and gel permeation chromatography. Ion-exchange chromatography on carboxymethylcellulose (CM-52) in an acidic buffer system made it possible to free the ferritin satisfactorily from contaminating γ-globulins, hemoglobins, and aggregates. The use in the concluding stage of the purification of ferritin of gel filtration on a column of Sephadex G-200 permitted the purified enzyme to be obtained (Fig. 1).

The electrophoretic analysis of ferritin in polyacrylamide gel (PAAG) showed the presence of a single band corresponding in its electrophoretic mobility to a molecular weight of 460,000 daltons (Fig. 2). Electrophoretic analysis showed that the human liver ferritin was a less acidic protein than horse spleen ferritin. The molecular weight of a ferritin subunit was determined by electrophoresis in PAAG in the presence of the denaturing agent sodium dodecyl sulfate (Na-DDS) and the reducing agent 2-mercaptoethanol as 19,000 daltons, which agrees well with literature figures [6].

Quantitative analysis of the N-terminal amino acid confirmed the purity of the preparation of ferritin obtained from human liver. Qualitative analysis of the N-terminal acid showed that in the human liver ferritin it was serine. On the basis of isoelectric focusing in PAAG in the presence of markers, the isoelectric point of the ferritin obtained was calculated as

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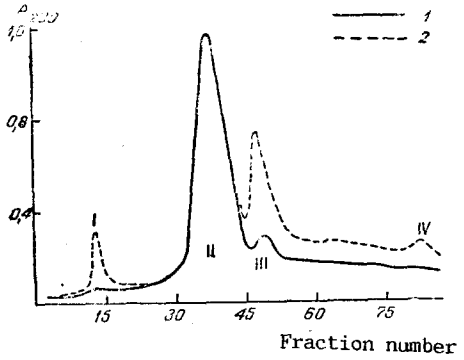

Fig. 1

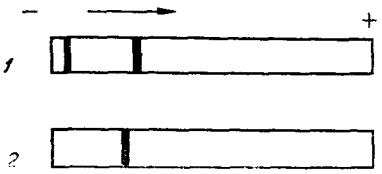

Fig. 2

Fig. 1. Gel filtration of the ferritin-containing fraction on Sephadex G-200 in Tris-HCl buffer, 0.05 M, pH 7.1, containing 0.1 M NaCl. Rate of elution 40 ml/h; 15-ml fractions collected. 1) Elution profile of ferritin after thermal denaturation, salt precipitation, and chromatography on CM-cellulose; 2) elution profile of ferritin after thermal denaturation and salt precipitation. I) Aggregates; II) ferritin fraction; III) γ-globulins; IV) hemoglobin impurities.

Fig. 2. Electrophoresis in 8% PAAG: 1) horse ferritin; 2) human ferritin. The electrode buffer was Tris-glycine, pH 8.6, and the time of electrophoresis 3 h at 5 mA per gel column.

5.3. The amino acid compositions of the human liver ferritin and horse ferritin are given below (moles of amino acid per mole of protein):

Amino acid	Human ferritin	Horse ferritin
Asp	19	21
Thr	6	7
Ser	11	11
Glu	25	29
Pro	5	3
Gly	11	12
Ala	12	17
Val	6	9
Met	3	4
Ile	3	4
Leu	21	31
Tyr	4	6
Phe	7	9
His	4	7
Lys	10	11
Arg	9	12

The analysis of the amino acid composition of the ferritin revealed a predominance of acidic amino acids, making up about 30% of the total, while basic amino acids accounted for about 12%.

EXPERIMENTAL

The ferritin was obtained from the liver of the cadaver of a healthy 58-year-old man. About 200 g of the liver was comminuted and ground in a porcelain mortar in the minimum volume of cold physiological solution to obtain a viscous homogenate. This procedure was performed on ice. By centrifugation at 5500 rpm for 30 min, a precipitate containing nuclei, mitochondria, and pieces of tissue was obtained. The precipitate was discarded, and the supernatant was subjected to thermal denaturation at 70°C for 15 min. After this, the suspension was again poured onto ice and was kept in the refrigerator for 12 h. The precipitate that had formed was collected by low-speed centrifugation at 3000 rpm for 20 min and was discarded. The ferritin-enriched supernatant was treated with ammonium sulfate.

Ammonium Sulfate Precipitation. The pH of the supernatant was first brought to 4.9 by the addition of 0.1 M HCl. Then a saturated solution of ammonium sulfate was added to the protein solution in an amount of 55% of that needed for complete saturation, and the mixture was left at 4°C for 12 h. The protein precipitate that had formed was collected by centrifugation at 5500 rpm for 20 min. The dark brown ferritin-containing precipitate was dissolved in 20 ml of 0.2 M sodium acetate buffer, pH 4.9, containing 0.05 M NaCl.

Chromatography on Carboxymethylcellulose. Whatman CM-52 CM-cellulose (United Kingdom), equilibrated with sodium acetate buffer, 0.2 M, pH 4.9, containing 0.05 M NaCl, was used for chromatography in a 2.6 × 40 cm column. The ferritin fraction, in 20 ml of sodium acetate buffer, was deposited on the column and was eluted with the same buffer at a rate of flow of 10 ml/h. Protein in the eluate was recorded from its absorption at 280 nm, with the aid of LKB Uvicord II instrument (Sweden).

Gel Filtration on Sephadex G-200. A 2.6 × 100 cm column filled with Sephadex G-200 (Sweden) equilibrated with Tris-HCl buffer, 0.05 M, pH 7.1, containing 0.1 M NaCl was used. The ferritin fraction that had issued in the first chromatographic peak in chromatography on CM-cellulose was deposited on the column and subjected to gel filtration at a rate of passage of 40 ml/h, which was maintained with the aid of peristaltic pump. The protein in the eluate was recorded from its absorption at 280 mm with the aid of the Uvicord II. The ferritin fraction was easily identified from the characteristic orange-brown coloration (see Fig. 1).

Electrophoresis in PAAG was performed in two-component gels comprising 8% separating and 3% concentrating gels in 0.8 × 11 cm transparent plastic tubes in Tris-glycine buffer, pH 8.6, at 5 mA per gel column for 3 h. The gel was fixed in 10% acetic acid for 1 h and was stained with Amido Black 10B (1.5 g of Amido Black in 250 ml of 2% acetic acid) for 1 h. The gel was washed free from excess of dye with 2% acetic acid solution for 12 h.

Electrophoresis in PAAG in the presence of Na-DDS was carried out in the 8% separating gel with Tris-glycine buffer, pH 8.6, containing 0.1% of Na-DDS as electrode buffer. Na-DDS to a final concentration of 0.1% and mercaptoethanol were added to the samples before they were deposited on the gel and they were heated in the boiling water bath for 2 min. Horse ferritin, cytochrome C, myoglobin, and chymotrypsinogen A (Serva) were used as marker proteins.

The N-terminal amino acid was determined by the dansyl method as proposed by Gray [7].

The amino acid analysis of the ferritin was performed on a Durrum D-500 automatic amino acid analyzer. The ferritin was hydrolyzed in glass tubes in vacuum in the presence of 5.7 N HCl for 24 h.

Isoelectric focusing was performed in PAAG using riboflavin as polymerization catalyst. We used 0.24 N H_2SO_4 as the anode solution and 0.48 N NAOH as the cathode solution. Isoelectric focusing was carried out for 24 h at an initial current of 2.5 mA per gel column. The gel was fixed in 10% trichloroacetic acid for 1 h and was then stained with a 0.1% solution of Coomassie in acetic acid–isopropanol–water (1:5:5) and was washed free from the excess of dye with the same mixture. Proteins with known isoelectric points – serum albumin, myoglobin, and cytochrome C (Serva) – were used as marker proteins.

SUMMARY

Electrophoretically homogeneous ferritin with an isoelectric point of 5.3 and a molecular weight of 460,000 daltons has been isolated from human liver by thermal denaturation, precipitation with ammonium sulfate, and ion-exchange and gel permeation chromatography.

The molecular weight of a subunit of the ferritin is 19,000 daltons and the N-terminal amino acid of the protein is serine.

A predominance of acidic amino acids is characteristic for the amino acid composition of human ferritin.

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CLEAVAGE OF THE NATURAL LIGNIN OF COTTON-PLANT STEMS WITH THIOACETIC ACID IN THE PRESENCE OF ANTHRAQUINONE

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The influence of additions of catalytic amounts of anthraquinone on the stages of hydrolysis in the cleavage of natural lignin by thioacetic acid has been investigated. The degree of delignification does not change under these conditions, but the yield of low-molecular-weight cleavage products increases, which indicates a profound degradation of the lignin macromolecule. The qualitative and quantitative compositions of the monomeric fractions of cleavage products do not undergo appreciable changes in the presence of anthraquinone.

The presence of anthraquinone (AQ) in alkaline cooks of lignin-containing materials intensifies the breakdown of the β-O-4-alkyl aryl ether bonds in the lignin macromolecule [1]. Since under the action of thioacetic acid (TAA) the same bonds are attacked selectively, we have investigated the influence of additions of AQ in the course of the cleavage with TAA of the natural lignin of the stems of the cotton plant, ssp. mexicanium and the variety Tashkent-6, on the yield and composition of the degradation products. The catalyst (in an amount of 0.1% of the weight of the raw material) was added at the stage of alkaline hydrolysis of the S-benzyl thioacetates formed under the action of TAA [2].

It was found that the degree of delignification determined from the change in the amount of Komarov lignin in the plant raw material before and after the reaction was not affected by the presence of AQ. The yield of low-molecular-weight lignin degradation products extractable by ether and by ethyl acetate increased in the presence of AQ from 35.4% [3] to 40.0% for the mexicanium cotton plant and from 45.5% [4] to 55.8% for the variety Tashkent-6. The more appreciable increase in the yield in the latter case corresponds to the higher amount of alkyl-aryl ether bonds in the lignin molecule [4].

To facilitate the identification of the products obtained, in accordance with [3] we extracted the monomer fraction with ether at pH 8 and the remaining low-molecular-weight substances with ethyl acetate at pH 2.

Part of the reaction mixture was acidified directly to pH 2 and was extracted with ethyl acetate, which made it possible to study the molecular-weight distribution of the degradation products. The amounts of oligomeric, tetrameric, trimeric, dimeric, and monomeric fractions determined with the aid of gel chromatography on Sephadex LH-20 [with methanol–water (9:1)

Fraction	Mexicanium variety				Variety Tashkent-6			
	without AQ		0.1% of AQ		without AQ		0.1 % Of AQ	
	A	B	A	B	A	B	A	B
Oligomers	2	0.7	3	1.2	9	4,1	3	1.7
Tetramers	11	3,9	4	1.6	6	2,7	9	5.0
Trimers	14	4,9	17	6.8	10	4,6	12	6.7
Dimers	40	14,2	46	18,4	28	12,7	33	18.4
Monomers	33	11,7	30	12,0	47	21.4	43	24,0
Total		35,4		40,0		45,5		55,8

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