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RAPID UPTAKE BY LIVER SINUSOIDAL CELLS OF SERUM ALBUMIN MODIFIED WITH RETENTION OF ITS COMPACT CONFORMATION

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Summary

The clearance from the blood and the conformation of serum albumin modified by nitroguanidination and labeled with ^{125}I have been studied. Like formaldehyde-denatured albumin, but in contrast to native albumin, the nitroguanidinated derivative is rapidly cleared from the blood and taken up in lysosomes of liver sinusoidal cells. Although 94% of the free amino groups were blocked by nitroguanidination, we could not detect significant conformational changes using gel filtration, determination of reducible disulfide groups, and titration of tyrosine residues.

It is concluded that extensive denaturation is no prerequisite for the uptake of albumin derivatives in liver sinusoidal cells. It is suggested that the nitroguanidinated protein, in contrast to native albumin, is bound on membrane receptors of sinusoidal cells. The nitroguanidino groups themselves might be bound on these receptors, but it seems equally possible that the blocking of positive charges of the albumin molecule or minor, local conformational changes of the protein are sufficient for the binding on the receptors.

Introduction

Heat-denatured, aggregated plasma proteins are rapidly taken up by phagocytic cells in contrast to the native forms of these proteins [1—3]. Aggregation proper is not the cause of this fast clearance from the blood [4,5]. Recently, we described some characteristics of formaldehyde-treated albumin, which modification, though not aggregated, also disappears rapidly from the blood after injection, and is avidly taken up by liver sinusoidal cells. From a combination of results of ultracentrifugal and gel filtration experiments we concluded that formaldehyde treatment causes a partial unfolding of the albumin molecule.

In this paper, we describe the rapid endocytosis of nitroguanidinated albumin into liver sinusoidal cells after injection in rats. In this modification the positively-charged amino groups are replaced by neutral nitroguanido groups [6]. However, by the criteria we used, we could not detect any significant unfolding of this modified albumin molecule, whereas the same criteria clearly showed an unrolling of the peptide chain of formaldehyde-treated albumin.

Materials and Methods

Bovine serum albumin was obtained from Poviet Producten B.V., Amsterdam, The Netherlands. Formaldehyde treatment of albumin was carried out according to Mego and McQueen [7]. 1-Nitroguanyl-3,5-dimethylpyrazole, prepared by a two-step synthesis [6,8], was used for the introduction of nitroguanido groups into the lysine residues of the protein as described by Habeeb [6]. Bovine serum albumin at a concentration of 20 mg/ml was incubated for 6 h at room temperature with 75 mM 1-nitroguanyl-3,5-dimethylpyrazole in 0.2 M sodium borate buffer, pH 10.0. The reaction was stopped by neutralization with 1 M HCl and the residual 1-nitroguanyl-3,5-dimethylpyrazole was removed by centrifugation.

Native and modified albumin were labelled with ^{125}I according to Bocci [9]. About 100 mg protein was incubated for 30 min at room temperature in 2.7 ml 0.15 M Tris/acetate buffer, pH 8.0 (20° C) containing 2.5—5.0 mCi ^{125}I (carrier-free iodide, The Radiochemical Centre, Amersham, England) and 0.67 mM chloramin-T. The reaction was stopped by addition of an equivalent amount of $Na_2S_2O_5$, and the reaction mixture was subjected to gel filtration as described in the legend to Fig. 1, but using 0.15 M Tris/acetate buffer, pH 7.5 (4° C) instead of phosphate buffer. Monomer fractions were pooled, dialysed against cold 0.9% NaCl solution and concentrated to a concentration of 2 mg/ml with a Diaflo PM-10 ultrafilter (Amicon B.V., Oosterhout, The Netherlands). The monomer fractions used in the biological experiments had specific radioactivities of about $2 \cdot 10^7$ dpm per mg of protein.

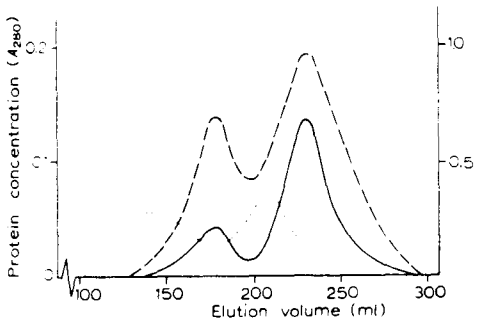
Determination of free amino groups was performed with trinitrobenzene sulfonic acid [10]. Tyrosine titration was done as described by Tanford [11]. Reducible disulfide groups were determined according to Habeeb [12]. Determination of protein and enzymes, measurement of radioactivity, liver fractionation, and autoradiography were done as described before [5].

Results

Chemical characterization of albumin modifications

Fig. 1 shows that, after gel filtration on Sephadex G-200, nitroguanidinated albumin and native albumin are eluted in the same volume. In accordance with our previous results, formaldehyde-treated albumin was eluted before the native protein. All experiments described below were done with monomer fractions only.

Some chemical characteristics of native, nitroguanidinated and formaldehyde-treated albumin are given in Table I. The number of free amino groups



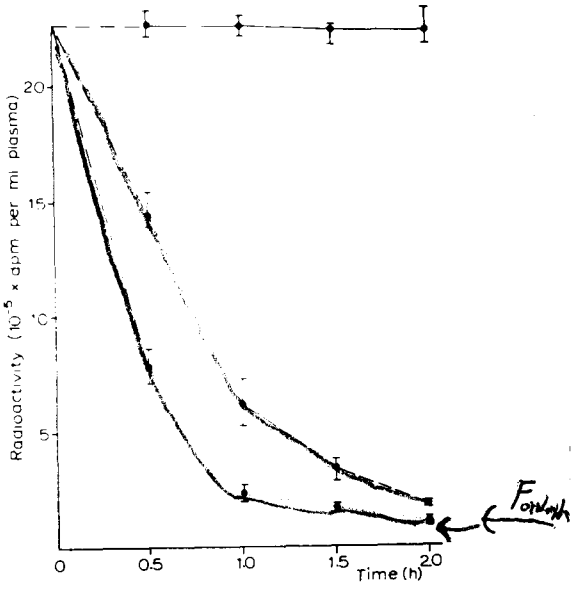


Fig. 1. Gel filtration of native (———), nitroguanidinated (- - - - -) and formaldehyde-treated (. . . . . .) albumin. About 40 mg of protein were applied to a Sephadex G-200 column of 3.2 X 43 cm. Elution was carried out at 4° C with 0.1 M phosphate buffer, pH 7.4. The absorbance of nitroguanidinated albumin is given on a reduced scale because of the strong absorbancy of the nitroguanido group at 280 nm.

Fig. 2. Disappearance from blood plasma of trichloroacetic acid precipitable radioactivity after intravenous injection of 1 mg ($2 \cdot 10^7$ dpm) of native (———), nitroguanidinated (- - - - -) and formaldehyde-treated (. . . . . .) ^{125}I-labeled albumin. Extrapolated (—·—). Male Wistar rats of 200 g body weight were used. Each point represents the mean value of three animals. Vertical bars give the range of individual values.

was determined with trinitrobenzene sulfonic acid. About 94% of the free amino groups had reacted with the nitroguanidination reagent. About half of the free amino groups had been blocked by formaldehyde treatment. Both treatments may be expected to lead to a decrease in the positive charge of the protein, i.e. an increase in the net negative charge at physiological pH. The degree of unfolding of albumin can be deduced from both the number of reducible disulfide groups and the titration of tyrosine residues. In native albumin the disulfide bridges can not be reduced [12] and only tyrosine resi-

TABLE I

CHEMICAL CHARACTERISTICS OF NATIVE AND MODIFIED ALBUMIN

Determinations were carried out as described under Materials and Methods. The number of groups per albumin molecule is given. Numbers between parentheses give the maximal deviation of the mean (three experiments). Results of tyrosine titrations refer to one series of experiments.

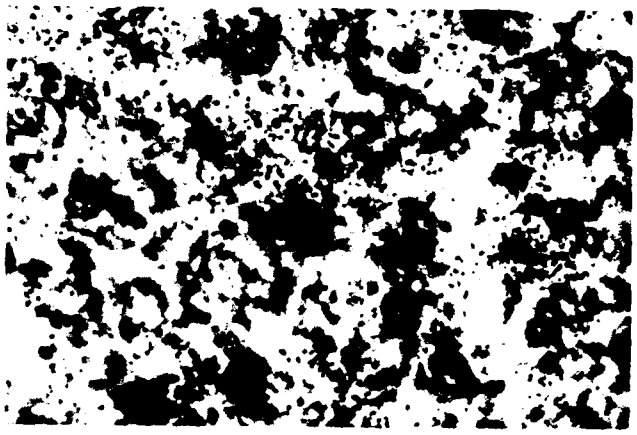
	Free amino groups	Reducible disulfides	Titratable tyrosine residues
Native	63 (2)	0.3 (0.1)	3.0
Nitroguanidinated	4 (1)	0.7 (0.1)	3.5
Formaldehyde-treated	34 (2)	5.7 (0.2)	6.2

dues present at the surface of the molecule can be normally titrated with a pK 10 [11]. Our results show that the number of reducible disulfide groups is not appreciably increased by nitroguanidination, whereas formaldehyde treatment causes considerable denaturation. These data are corroborated by the results of the tyrosine titrations. Since nitroguanidination does not block tyrosine residues [6], the titration behaviour of these residues is not affected directly by this modification procedure. Our failure to show significant conformational changes of albumin after nitroguanidination is in agreement with results obtained by Habeeb with sedimentation and diffusion experiments [6].

The results given above were obtained with protein that had not been subjected to the labeling procedure. In order to check a possible effect of this procedure on the proteins, samples of native and modified proteins were "labeled" with non-radioactive iodine by a method identical to that used for radioactive labeling. Determination of reducible disulfide groups showed that our iodination procedure did not change the conformation of the native and modified proteins. Gel filtrations were carried out with preparations labeled with radioactive iodine in order to isolate the monomer fractions used for the biological experiments. The elution patterns obtained were identical to those given in Fig. 1, providing additional evidence for the absence of conformational changes.

Fate of nitroguanidinated albumin after injection

Fig. 2 shows the plasma concentration of labeled native and modified



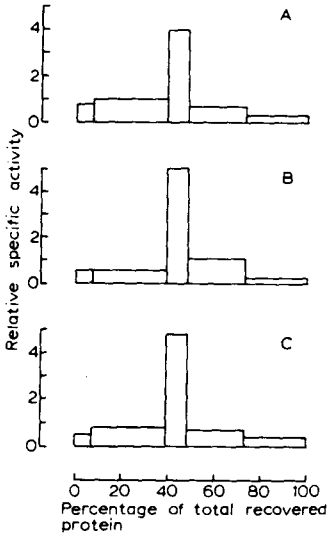


Fig. 3. Autoradiogram of liver section of rat 30 min after injection of ^{125}I-labeled nitroguanidinated albumin (see legend to Fig. 2). Exposure time 11 weeks. X 200.

Fig. 4. Distribution of trichloroacetic acid precipitable radioactivity (A), acid phosphatase (B) and cathepsin D (C) after differential centrifugation of liver homogenates 45 min after injection of ^{125}-labeled nitroguanidinated albumin. Livers were perfused with saline before excision and homogenates were subjected to differential fractionation [13]. Columns from left to right represent fractions in the order in which they are isolated: nuclear, mitochondrial, lysosomal, microsomal and supernatant fractions. Relative specific activity = percentage of total activity divided by percentage of total protein. Each diagram is the result of four fractionations. Mean recoveries: protein 94.0%; radioactivity, 93.8%; acid phosphatase, 92.6%; cathepsin D, 85.3%.

albumin in the first hours after injection. Although the conformation of nitroguanidinated albumin closely resembles that of native albumin its clearance behaviour is quite similar to that of the denatured formaldehyde-treated derivative. Between 28% and 33% of the administered dose was recovered in the liver 45 min after injection. From the autoradiograph shown in Fig. 3 it is clear that the label was predominantly present in sinusoidal cells. The subcellular localization of the protein was determined by differential centrifugation. A comparison of the distribution of the label with that of the lysosomal enzymes acid phosphatase and cathepsin D (Fig. 4) shows a clearly lysosomal localization for nitroguanidinated albumin.

Discussion

As work by Ashwell and Morell [14,15] has demonstrated a slight change in certain serum glycoproteins, viz. unmasking of galactose units by removal of terminal sialic acid residues, causes binding of the molecules to receptors on cell membranes of hepatocytes. Following this binding to the cell membrane the glycoproteins are endocytosed and taken up into lysosomes. The binding and endocytosis of glycoproteins which have not been treated with sialidase, is negligible.

A similar situation may occur when proteins are taken up by sinusoidal cells. Evidently, unmodified albumin has no groups on its surface which can be bound to receptors on the membranes of these cells. Modification of albumin either by formaldehyde treatment or by nitroguanidination leads to rapid uptake of the molecule. These modification procedures might produce a handle at the surface of the molecule with which it is withdrawn from the circulation by membrane receptors of sinusoidal cells.

A common characteristic of our modifications is the increase in net negative charge of the molecule. It is possible that positively-charged lysine residues of certain stretches of peptide chain on the surface of the native albumin molecule hamper the binding of these exposed stretches to receptors on the membranes of the sinusoidal cells.

An alternative explanation is that suitable groups, which in the native protein are buried inside, are brought to the surface of the molecule by the modification procedures. In the case of formaldehyde treatment, which causes extensive denaturation, this possibility seems quite obvious. The nitroguanidino group might mimic such buried groups, or its introduction might lead to their exposure. It is, however, clear that in the latter case a local, minor change would be involved, which cannot be shown by the methods we used to study the conformation of the protein molecule.

Acknowledgements

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Proteolysis of formaldehyde-treated albumin in Kupffer cells and its inhibition by suramin.