CHANGES IN OPTICAL ROTATORY DISPERSION CHARACTERISTICS OF SERUM ALBUMIN IN ACUTE APPENDICITIS AND CHOLECYSTITIS

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Optical rotatory dispersion of serum albumin of patients with cholecystitis and appendicitis was investigated. Conformational changes characterized by uncoiling were found in these diseases. A method of purifying albumin from its modified forms, probably responsible for these changes in albumin structure, is suggested.

KEY WORDS: albumin and its structure; appendicitis; cholecystitis; protein conformation under normal and pathological conditions.

The writers previously showed that in certain endocrine diseases changes are observed in the optical rotatory dispersion (ORD) of albumin, which may be evidence of conformational changes in its structure. These changes were combined with changes in the peptide maps [1, 3, 7]. Changes in the peptide maps of hemoglobin in diabetes [9] and of muscle aldolase in thyroid disorders [4] also are known. These changes may be connected with the modification of albumin, as a result of which a component consisting of modified albumin appears in its electrophoretic fraction [1].

The object of this investigation was to extend the study into a group of diseases not directly connected with endocrine pathology and also to elucidate the causes of conformational changes previously observed in albumin.

EXPERIMENTAL METHOD

Altogether 20 patients with acute cholecystitis and acute appendicitis, with a well-marked clinical picture, were investigated. Serum albumin was isolated from these patients by preparative electrophoresis [2] in 0.075 M veronal buffer, pH 8.6, with a voltage gradient of 10 V/cm for 7-8 h. The homogeneity of the isolated albumin was verified by microelectrophoresis in agar gel and by frontal electrophoresis in a Tiselius' apparatus. The serum albumin was treated with allowance for its heterogeneity in certain forms of pathology [1, 3]. For this purpose, 2-3 ml of 20% TCA was added drop by drop to 5 ml of a solution of the electrophoretic albumin fraction (EAF) with stirring, and the residue was washed twice with 5% TCA and dissolved in 80% ethanol at 0-4° C. Spectropolarimetric investigations were carried out as described previously [6].

EXPERIMENTAL RESULTS AND DISCUSSION

The spectropolarimetric characteristics of EAF before and after reprecipitation with TCA are shown in Table 1. The albumin isolated electrophoretically from patients both with acute cholecystitis and with acute appendicitis clearly had abnormal ORD characteristics reflecting uncoiling processes. Changes were more marked in the b_0 than in the parameters λ_c and a_0. The changes in the a_0 and b_0 parameters were statistically significant. After reprecipitation with TCA and solution in ethanol, all EAF with $\lambda = 260$ nm

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TABLE 1. Spectropolarimetric Characteristics in Acute Appendicitis and Cholecystitis (M ± m)

Disease	Conditions	λ_c	$-a_0$	$-b_0$
Acute cholecystitis	I	258±1,4*	297±3,4	238±12,3*
	II	9,9±1,7*	5,5±4,93	81,0±10,5*
Healthy	I	264±0,63	290±3,12	300±1,56
Acute appendicitis	I	259,6±0,9*	283,5±9,9	244,0±8,2*
	II	10,0±0,8*	2,5±1,12	77,0±6,5*

Legend: $-a_0$, $-b_0$) Parameters of ORD from Moffitt's equation (see [6]); λ_c wavelength, in nm. Asterisk indicates $P < 0.05$. Number of subjects in group 10. I) EAF before reprecipitation with TCA; II) after reprecipitation with TCA (in this case difference between parameters of protein in healthy subjects and patients).

(λ_c is the constant of a simple Drude equation reflecting the degree of uncoiling of the protein molecule [6]) formed insoluble residues, whereas the soluble part had native ORD characteristics, similar to those of crystalline preparations of albumin [10] with $\lambda_c > 264$ nm. Whereas before reprecipitation with TCA λ_c = 256 and $b_0 = -238$, after reprecipitation $\lambda_c = 270$ and $b_0 = -320$. Changes in the parameters a_0 and b_0 of ORD in this case also were statistically significant.

Treatment of albumin from patients with acute appendicitis and cholecystitis with TCA and ethanol thus enables this protein to be separated in these diseases into fractions, one conformationally unchanged, remaining in solution, and the other with a changed conformation, irreversibly precipitated, probably because of uncoiling of this part of the albumin. The writers showed previously that peptide maps of residues from TCA–ethanol are identical with the peptide map of albumin [5].

In both cases the changes in conformation of EAF toward uncoiling were equal in degree. It is interesting to note that albumin with abnormal ORD characteristics discovered in patients with acute appendicitis and cholecystitis can be purified with the aid of TCA–ethanol from its modified form; in all probability this fact reflects a change in the conformation of the protein in these diseases.

The results evidently show that conformational changes observed previously in endocrine disorders are not specific for those conditions but may also arise in acute inflammatory diseases. Conformational changes discovered by the writers and described in the literature [8] in disease are, it will be noted, facts of a fundamentally novel order and their study may perhaps shed light on the features of specificity that distinguish diseases from each other.

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