A NEW TYPE OF MICROHETEROGENEITY OF ALBUMIN FROM BLOOD PLASMA

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UDC 612.124-086

A new type of microheterogeneity of the electrophoretically purified fraction of blood plasma albumin, concerned with its ability to dissolve in 80° ethanol after reprecipitation with 10% TCA, has been found. This microheterogeneity was confirmed by spectropolarimetry and peptide map methods, and in all probability it is connected with the possibility of complex formation between albumin and certain metabolic products in disease.

The writers have previously described changes in the peptide maps and conformation of albumin in diseases of the thyroid gland and diabetes [2, 6]. The suggestion was made that the mechanism of these changes is based on two possible factors: a disturbance of albumin biosynthesis and processes of complex formation, modifying the protein after it has been synthesized, and associated with its damage through interaction with chemically active metabolites.

An albumin with low constancy of optical rotation in conjunction with ordinary Moffit's parameters, characteristic of crystalline protein [12], has been found in several clinically healthy persons and animals.

The question thus arises whether some form of heterogeneity of albumin, due to its modified form, appears during disease. Albumin is known to dissolve [9] in 10% TCA and in 80° ethanol without losing its native characteristics [10, 11]. Albumin with abnormal optical rotatory dispersion (ORD) characteristics is not completely soluble in 80° ethanol after precipitation by TCA [7]. This property was used as a test of the heterogeneity of the electrophoretic fraction (EF) of albumin in healthy and sick persons and animals with abnormal ORD parameters of this protein. The problem was investigated by spectropolarimetric and peptide map methods.

EXPERIMENTAL METHOD

To obtain preparations of albumin with abnormal ORD characteristics rabbits received a subcutaneous injection of 1 ml cinnamic aldehyde or citral. Albumin from patients with diabetes, tuberculosis, and thyrotoxicosis (the choice of pathology was arbitrary) also was used. Albumin was isolated from the blood serum by preparative electrophoresis in agar [1] and with the aid of TCA [8] from the previously isolated EF. The purity of the preparations was verified by frontal electrophoresis in a Tiselius apparatus and in agar. Albumins isolated by both methods specified above were investigated spectropolarimetrically [4]. Peptide maps were obtained as described previously [3].

EXPERIMENTAL RESULTS

The mean values of the ORD parameters are given in Table 1. In every case before exposure of the animal to the pathogenic agent and in the healthy subjects the isolated EF of albumin had the conformational properties of the EF of normal albumin [5], which are slightly different from those for crystalline albumin [12]. This fraction, when treated with TCA and dissolved in ethanol, gave virtually no precipitate; i.e., it was homogeneous and indistinguishable from crystalline preparations of albumin. A considerable change

Department of Biochemistry, Crimean Medical Institute, Simferopol'. (Presented by Academician of the Academy of Medical Sciences of the USSR V. A. Éngel'gardt.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 8, pp. 48-50, August, 1973. Original article submitted July 9, 1971.

TABLE 1. Microheterogeneity of Blood Plasma Albumin

Test object	n	EF of albumin			Soluble part of EF of albumin after TCA		
		λ_c	a_0	b_0	λ_c	a_0	b_0
Rabbit serum albumin:							
normal	10	264±2	—290±2	—280±2	270±2	—300±2	—320±2
after injection of cinnamic aldehyde	5	245±5	—300±10	—160±20	270±2	—290±10	—320±10
after injection of citral	6	255±5	—310±10	—240±10	272±2	—300±10	—320±10
Human serum albumin:							
normal	10	264±2	—300±5	—290±5	272±2	—290±5	—320±5
of patient with thyrotoxicosis	6	264±2	—310±5	—290±10	270±2	—310±10	—320±20
of diabetic	3	252±2	—290±5	—180±10	274±2	—300±10	—330±10
of patient with tuberculosis	6	260±2	—300±10	—230±10	274±2	—290±5	—330±10

Legend: λ_c, a_0, b_0) ORD parameters from Moffit's equation [12]; n) number of experiments.

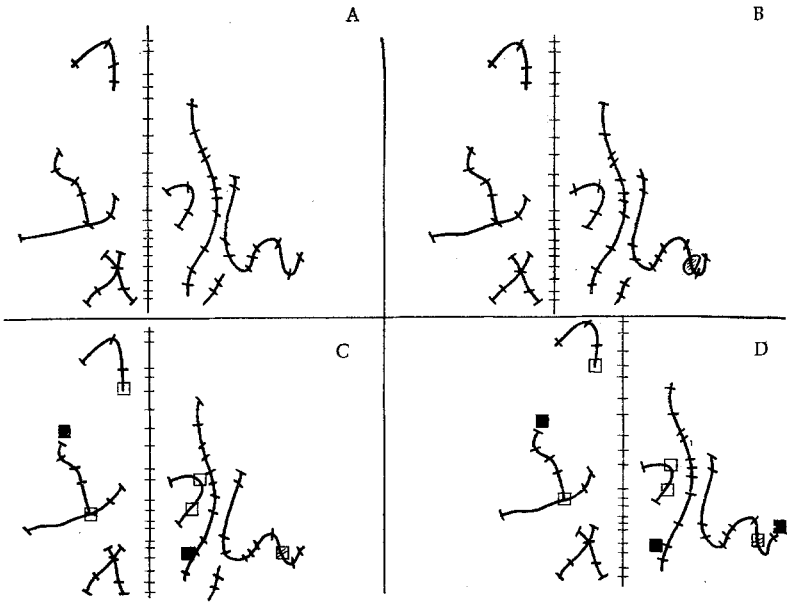

Fig. 1. Scheme of peptide maps of rabbit serum albumin: a) serum albumin of normal rabbit; B) part of EF of albumin from rabbit with inflammation, dissolved in ethanol; C) EF of serum albumin from rabbit with inflammation caused by citral; D) part of EF of albumin from rabbit with inflammation, not dissolving in ethanol.

in the ORD parameters (although electrophoretic homogeneity was preserved) of the rabbit albumin after inflammation and of the albumin from patients with severe forms of diabetes and tuberculosis was observed. Electrophoretically homogeneous albumin, isolated from the blood of patients and animals, when treated with TCA and ethanol gave a considerable precipitate. The part which dissolved in ethanol, however, regained all its native spectropolarimetric characteristics. So far as the albumin isolated from the blood of patients with thyrotoxicosis is concerned, in this series of experiments its EF was almost indistinguishable from normal according to the ORD parameters. However, after the chosen method of treatment a small precipitate always remained in these cases, and the dissolved albumin showed some improvement in its

ORD characteristics; i.e., they were closer to those of the crystalline preparations. Presumably, therefore, by reprecipitation of the albumin with TCA followed by dissolving in ethanol the albumin divides in these cases into two fractions: one with a changed and the other with an unchanged conformation. The albumin isolated by means of TCA and ethanol also had improved native ORD characteristics. A similar pattern was observed when albumin was isolated by this method from blood serum. These conformational changes characteristically were found only with the EF of albumin, for when albumin was isolated from the blood serum of patients and animals by means of TCA and ethanol, these preparations after dialysis were always native in their ORD characteristics.

To investigate the nature of these changes the method of peptide maps was used. As Fig. 1 shows, the soluble part of the EF of albumin from a rabbit with inflammation induced by citral (B) was virtually identical with the peptide map of normal rabbit serum albumin (A), whereas the EF untreated with TCA (C) differed appreciably from it and was identical with the peptide map of the precipitate (D) with the exception of one peptide which could be taken as variable.

The peptide maps thus showed conclusively that contamination by modified albumin occurs in the diseases listed above. The presence of conformational changes in albumin in disease can be detected not only spectropolarimetrically, but also by the direct chemical method after reprecipitation of the albumin with TCA by its ability to dissolve subsequently in ethanol. This conformationally changed form of albumin remaining in the precipitate can be assumed to be an albumin fraction loaded with various metabolites through complex formation.

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