REVIEWS

Conformational Changes in Albumin Molecule: a New Response to Pathological Process

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A new fluorescent test developed at the Institute of Physicochemical Medicine (Ministry of Health of the Russian Federation) allows to determine not only blood concentration of albumin, but also to evaluate the state of its molecule. The test is feasible and enables express-analysis of the plasma and serum without fractionation and other preliminary procedures. This test reveals abnormalities in albumin molecule caused by toxic metabolites or conformational changes. Many diseases are accompanied by conformational changes in albumin, while its concentration often remains unchanged. Changes in albumin conformation can serve a diagnostic and prognostic criterion in some pathologies.

Key Words: *albumin; conformation; changes in pathology; fluorescent test*

Various diseases are accompanied by modifications at a molecular level, in particular, changes in the content or chemical structure of blood proteins. Some diseases are caused by genetically determined structural changes in polypeptide chains, while others, for instance, diabetes, are due to enhanced posttranslational glycosylation of normal protein chains.

Recently, nonchemical changes in protein molecules were described: primary structure is preserved and subsequent chemical modifications did not differ from normal, but their secondary or tertiary structure, *i. e.* conformation, is changed. Therefore, the protein molecule undergoes physicochemical, rather than true chemical transformations resulting in pathological conformations.

Prions are considered to be such proteins [24]. Although diseases caused by these proteins are relatively infrequent, there is a general problem: what is the role of physicochemical and, in particular, conformational changes in proteins and other macromolecules in the development of pathology?

It has been previously assumed that physicochemical properties of molecules are extremely important for normal body functions and their disturbances may cause diseases. Even more attention was paid to physicochemical than to true chemical aspects. As early as in 1914-1915 N. N. Anichkov proposed that poor water solubility of cholesterol promotes the formation of infiltrates in the vascular wall, thus causing inflammatory response and the formation of atherosclerotic plaques [7]. Phase theory of cell response to external stimulation proposed by D. N. Nasonov and V. Ya Aleksandrov in the 1930s should also be mentioned. [32]. However, clear understanding of the role of protein conformation in the pathogenesis of some diseases has come only recently in connection with the prion hypothesis.

Accumulating data indicated that physicochemical and conformational changes in macromolecules contribute to the pathogenesis of not only exotic prion diseases, but also to more common pathologies. In this

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review we consider conformational changes in albumin, the most common blood plasma protein.

Specific dyes, molecular probes sensitive to minor changes in the protein conformation and physicochemical properties, played an important role in the analysis of these parameters [10,20].

Dyes became widely practiced in cytology from the second half of XIX century. Most of those early dyes were simply absorbed by cell structures, *i.e.* were bound physically, rather than chemically. Thus, biological testing with physicochemical probes started approximately 140 years ago.

In the first half of the 20th century it was shown that albumin possesses the highest absorption capacity with respect to both dyes and medicines among plasma proteins [23,45,47,51,55]. In 1952, a fluorescent dye (ANS) binding to albumin was described. This dye fluoresced only after binding to albumin [52] and was named fluorescent probe.

The data published since 1951 showed that albumin-dye binding is disturbed in some diseases, in particular in jaundice [54,59]. This phenomenon was first explained by bilirubin competition for albumin binding centers [54]. Systematic investigation started by S. Cheger [39] and other researchers revealed that dye binding disturbances occur not only in bilirubinemia, but also in myocardial infarction [25] and in some other diseases [6,11,36, 37]. Binding of some drugs structurally similar to dyes was also changed in some pathologies, for instance, in renal insufficiency [46,48, 49], after surgical interventions [57], *etc.*

However, these findings were not applied in clinical practice because of relative complexity of the detection technique compared to very simple and rapid measuring of ANS fluorescence in diluted serum. Using this convenient probe, Yu. I. Miller found that even in jaundice the errors in albumin measuring with ANS do not correlate with serum bilirubin concentration [26,27]. It became clear that bilirubin can contribute, but not ultimately determines modifications in albumin molecule caused by bilirubin.

The data on conformational changes in albumin molecule under pathological conditions have been reported in 1975 [9,22]. These changes were revealed by other physical and physicochemical methods. They could imply the existence of a correlation between conformational changes in albumin molecule and abnormal dye binding.

B. M. Krasovitskii with co-workers synthesized a new fluorescent probe K-35 to analyze albumin abnormalities in details [28,29,53]. K-35 fluoresces only upon binding to albumin and this fluorescence can be immediately measured without serum fractionation or other additional procedures. This probe is much more sensitive to albumin abnormalities than ANS.

Specific test and diagnostic kits were developed. Fluorescence of K-35 mixed with the serum of healthy donors is directly proportional to the total albumin concentration (TAK). Thus, TKA can be determined by measuring K-35 fluorescence intensity (Fig. 1). However, sometimes in healthy subjects and very often in patients this index does not correspond to TAK. In this connection fluorescence intensity was named effective albumin concentration (EAK). This fluorescence reflects the concentration of normal "healthy" albumin and the EAK/TAK ratio in healthy donors is usually close to 1 [2], while in patients it is below 1, since EAK is lower than TAK (Fig. 1).

The test enables both EAK and TAK to be measured in the same sample [16,17,31]. Due to its simplicity hundreds of patients with different diseases were examined during a relatively short period [1, 2,11,40].

Analysis of K-35 binding and fluorescence showed that low EAK is due to structural changes in binding centers of the albumin molecule [15,28,42,44] probably associated with changes in body functions. Fluorescent probe allows to detect even minor alterations in the binding center: the fluorescence can be affected by dislocation of one or several atoms by fractions of an angstrom. Most of the other structural physical methods are less sensitive; besides, they require albumin isolation from the serum.

Serum TAK is an important clinical index. However, in most acute and chronic diseases it remains within the normal range (35-55 g/liter), and therefore these measurements are rarely helpful for physicians.

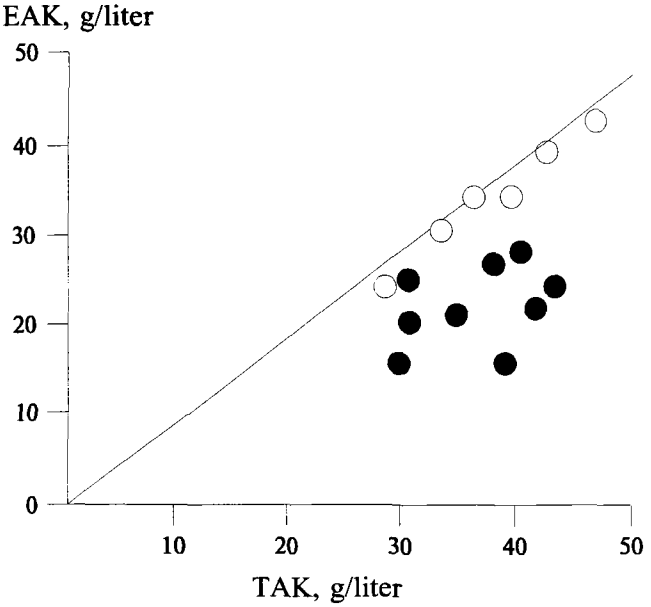

Fig. 1. Total (TAK) and effective (EAK) albumin concentrations in healthy volunteers (open circles) and randomly chosen patients with different diseases (filled circles). Dotted line marks EAK/TAK=1.

In contrast to TAK, EAK is very sensitive to pathology. This index depends on not only albumin concentration, but also its molecular state. EAK can be affected by three factors: decrease of total albumin (reduced TAK), saturation with metabolites (bilirubin, drugs, aromatic metabolites, *etc.*), and conformational changes.

Hence EAK reveals a pathological process in situation when TAK remains within the normal range. The EAK/TAK ratio does not depend on albumin concentration and characterizes only the state of its molecule.

If EAK is reduced due to the competitive inhibition of fluorescent probe binding by metabolites, an index directly proportional to their tissue concentration, index of toxicity (IT) can be calculated according to the formula (TAK/EAK)-1 [21]. IT increases with increasing tissue content of metabolites that normally should be bound and removed by albumin.

Thus, the fluorescent test makes it possible to measure two direct indices, TAK and EAK, and to calculate two derivatives, EAK/TAK and IT.

Changes in probe fluorescence turned out to be a typical albumin response to pathological processes. Low EAK/TAK (*i. e.* changes in albumin molecule) was first revealed in hepatitis, mechanical jaundice, thermal burns aggravated by liver insufficiency, and hyperbilirubinemia [28,29,53]. However, this reduction did not correlate with bilirubin concentration and hence cannot be explained by probe displacement with bilirubin.

A dramatic decrease in EAK/TAK was also noted in critical situations associated with pulmonary insufficiency [14].

It was found that many conditions and diseases (abscess, phlegmon, sepsis, renal insufficiency, multiple trauma, pneumonia, anemia, compression syndrome, and others) are accompanied by considerable decrease in EAK even in the absence of hyperbilirubinemia. [1,2,14]. Appendicitis, especially with suppurative complications and peritonitis, is also characterized by low EAK and EAK/TAK and high IT [12, 13,33-35].

Acute myocardial infarction was also accompanied by a gradual decrease in EAK and EAK/TAK and an increase in IT, TAK remained unchanged [3,4,19]. Surprisingly, in mental diseases, especially in some forms of depression the EAK/TAK index markedly decreased like in severe organic diseases [18,30].

These data suggest a new type of body reaction to pathological process associated with alterations in the state of serum albumin molecule. These alterations can result from albumin saturation with toxic products not eliminated by the liver and other organs (as in hyperbilirubinemia). However, in most cases they are caused by different factors, and changes in albumin molecule occur without its saturation with metabolites.

These changes can appear very rapidly (within 2-3 days or sometimes less than 1 h) and affect a considerable proportion of albumin molecules (50% or more). They are reversible and in some cases albumin conformation rapidly returns to normal [19,50]. It can be concluded that under acute conditions when albumin changes very rapidly, no chemical modifications occur in its binding centers. Changes in albumin polypeptide structure are even less possible: the modified pool is hardly synthesized *de novo,* since the period of albumin renewal is 20 days. Hence, changes in albumin conformation detected by fluorescent probe are caused by physicochemical, rather than chemical factors. Certainly, in chronic dysfunction lasting for months and years the possibility of chemical modification of albumin can not be excluded [39].

This novel reaction can be considered as a manifestation of recombination. According to D. S. Sarkisov, recombinations, *i. e.* rearrangements of the same structural elements, are an important mechanism of adaptive response. Indeed, albumin is a very labile molecule easily changing its conformation (it was not crystallized until recently). In this situation, it is more reasonable to use these conformational variants of albumin for instance in different pathological conditions, than to struggle against albumin variability. It can be suggested that an organism uses these conformations.

Changes in albumin conformation are nonspecific and accompany many diseases. However, EAK, EAK/TAK, and IT values change to a different degree in different diseases and at different stages of the pathological process. Thus, albumin indices measured on day 1 after operation allows to predict the progress of diffuse peritonitis, while routine biochemical indices or clinical scales give no such information [12, 13,33-35]. These indices retain their prognostic value during the treatment: if EAK exceeds 20 g/liter, the prognosis is favorable with 95% probability, the probability of unfavorable outcome increases to 40% at 8-20 g/liter and to 90% when EAK decreases below 8 g/liter. The critical values of EAK can be recorded several days before the manifestations of polyorganic insufficiency and its biochemical symptoms in patients.

It is of interest that all patients leaving the hospital after myocardial infarction still have low EAK or EAK/TAK indices, which implies that some important reparative processes are in progress despite cardiomyocyte death markers and common hematological and biochemical indices returned to norma. In severe heart insufficiency low EAK/TAK values are recorded for a long time (up to 6 months) [8].

Similar signs of incomplete recovery were observed in chronic hepatitis, when the level of bilirubin and other biochemical indices returned to normal [5].

Haloperidol, applied for the treatment of mental diseases exerts specific effect on EAK/TAK dynamics (albumin concentration remains unchanged); the character of EAK/TAK reaction to haloperidol allows to assess the individual sensitivity to this drug [17,30].

Thus, despite a nonspecific nature of changes in albumin conformation, this reaction is not the same in different situations and gives sufficient information for the assessment of patient's condition, his sensitivity to therapy and prognosis.

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