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ORIGINAL PAPER

Fluorescent Study of Human Blood Plasma Albumin Alterations Induced by Ionizing Radiation

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Abstract The use of hydrophobic fluorescent probe ABM (benzanthrone derivative) and albumin autofluorescence allowed show conformational alterations in Chernobyl clean-up workers blood plasma. Results obtained in 1996–1997 suggest that acidic expansion of plasma albumin takes place. Latest data (2006–2008) result in splitting of albumin alterations onto two stages - acidic expansion and N-F transition. The N-F transition is accompanied by the blue shift of fluorescence spectra and dehydration of tryptophanyl region of albumin molecule. In 2007 obtained.patterns of ABM spectra had never been previously seen in examined healthy individuals or patients with tuberculosis, multiple sclerosis, rheumatoid arthritis, etc. Patterns of ABM fluorescence spectra are associated with conformational changes of blood plasma albumin. The use of probe ABM and albumin auto-fluorescence allowed show conformational alterations in albumin of Chernobyl clean-up workers blood plasma. It is necessary to note that all investigated parameters significantly differ in observed groups of patients. These findings reinforce our understanding that the blood plasma albumin is a significant biological target of radiation. It may be concluded that fluorescence characteristics are representative of radiation induced albumin alterations and its carrier function.

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Introduction

Exposure of biomolecules to ionizing radiation results in the damage that is initiated by free radicals and progresses through a variety of mechanisms. Radiation-induced cell membrane damage may be a consequence not only of lipid peroxidation but also of protein alterations [1].

Albumin is most abundant in the blood plasma (about 60% of the total serum content) and acts as a transport carrier of endogenous and exogenous ligands poorly soluble in water. This protein is composed of three homologous domains each of which is divided into two sub-domains. Albumin has a high affinity for fatty acids, hematin, and bilirubin and a broad affinity for small negatively charged aromatic compounds [2–4]. The principal function of serum albumin is to transport a wide variety of fatty acids and metabolites via the main binding regions. Now it is recognized that there are at least six binding regions with the presence of one or two high affinity binding sites and a number of sites with a lower affinity [5].

Fluorescence spectroscopy became a valuable tool in the study of proteins due to its great sensitivity, and fluorescent dyes are being increasingly used in clinical and medicinal applications [6]. Fluorescence serves as very sensitive and convenient means indicating the alterations of the fluorophore environment, which can provide abundance of useful information. Alterations of proteins molecule can be followed via intrinsic (by emission of tryptophan residue) or extrinsic (fluorescent probe) emitters attached to the protein. Non-covalent extrinsic fluorescent dyes are applied I various fields of protein study – to characterize folding

and denaturation processes, measure hydrophobicity of surface and binding sites etc. [7, 8].

The aim of the present work was to determine by fluorescence spectroscopy several aspects of alterations of blood plasma albumin molecule of Chernobyl clean-up workers 20 years after accident.

Experimental

Study subjects

The study subjects were randomly selected from Latvia's residents who had participated in the accident cleaning-up works in Chernobyl in 1996–1997. The same individuals (male, $n=54$) were tested during April 2006–June 2008. Individuals who had been under treatment for acute infections, active autoimmune diseases malignancy were excluded. Control group ($n=17$, male) consists of healthy individuals of the corresponding age, who have never had professional contact with radioactivity.

Blood plasma collection

The Ethics Committee of the Medical Academy of Latvia approved the protocol of the present study design. Peripheral blood samples were collected from vein into disposable vacuum tubes and then were centrifuged for blood cell separation and blood plasma collection.

The fluorescent probe ABM

The hydrophobic fluorescent probe ABM is environment-sensitive fluorophore, which consists of morpholine moiety acting as electron donor, benzanthrone unit as electron spacer, and carbonyl group as electron acceptor (Fig. 1).

This dye was synthesized and characterized at our previous works [9, 10]. It was established that ABM spectral parameters reflect a wide range of interrelated (interdependent) characteristics of peripheral blood mononuclear cells - fluidity and microviscosity of membrane, proliferate and lipid metabolic activity of cells, distribution of lymphocyte subsets. The observed changes of the studied ABM spectral parameters reflect alterations of the cellular mechanisms of immunity, and can used in preliminary screening test in immune diagnostics [11, 12].

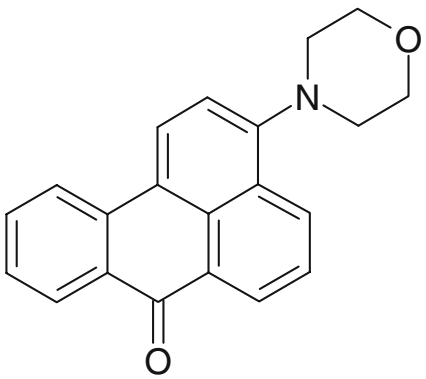

Fig. 1 The chemical structure of fluorescent probe ABM

Sample preparation and fluorescence measurements

Investigations were performed by means of the fluorescent probe ABM. The fluorescent dye ABM was dissolved in ethanol, with a resulting 0.1% concentration of ethanol in probe. The "blank" sample for each experiment was prepared by ABM titration without blood plasma. The blood plasma (diluted 200 fold) was incubated with ABM (it resulting concentration was 19.6 nmol/l) at room temperature for 5 min. The interval between plasma preparation and the fluorescence measurement, 3 h, was constant for all samples. Fluorescence parameters were registered on the spectrofluorimeter Spectrofluo JY3 (ISA Jobin Yvon Instruments S.A., France) at excitation wavelength 470 nm and emission wavelength of 500–700 nm. To register fluorescence from the surface of its excitation, the sample was placed into $1\times10\times40$ mm^3 cuvette fixed at an angle of 30° to the excitation light beam. Fluorescence intensity (F) was measured in arbitrary units (F, a.u.).

In albumin auto-fluorescence investigations fluorescence spectra were measured at the excitation wavelength 286 nm. It is known the human plasma albumin fluorescence is dominated by tryptophane residues emission (330 nm) [2, 4].

Statistical analysis

Statistical differences among groups having different spectral characteristics were calculated using the Student's t-test and Whitney-Mann's U test [13].

Results and dicussion

ABM binding with human serum albumin (HSA)

It is known that lipophilic fluorophores (such as ANS, TNS etc.) bind to albumin in up to five different binding sites in hydrophobic region of domain II near the tryptophan residue [14] (see Fig. 2).

It was established in our previous work that the fluorescent probe ABM binds to protein. When adding solution of ABM to albumin no fluorescence zone shift was observed, although visible increase of fluorescence intensity was revealed in comparison with the buffer solution [16].

Changes of pH in the region 3–12 strongly affected the quantum yield and maximum position of fluorescence of

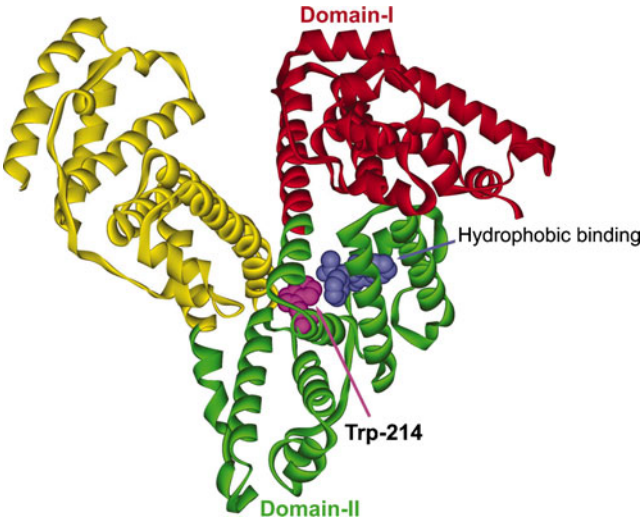

Fig. 2 The structure of human serum albumin and the location of tryptophan residue [15]

ABM bound with albumin [12]. Areas of greatest changes correspond to the known conformational transitions in proteins. ABM can be used as a probe which is sensitive to conformational changes of albumin.

In human blood plasma ABM is non-covalently bonded to albumin and has one emission maximum at 650 nm.

The 1996–1997 data for the studied patients were similar to those obtained at pH 1–2: the fluorescence zone shifted to the short wave region (600 nm–630 nm) as compared to the spectrum at pH 7.4 (650 nm); fluorescence intensity decreases by 24% in comparison with healthy donors.

Screening of ABM labeled blood plasma samples in 2006–2008 revealed that in 83% of patients (Group 1) the fluorescence zone had shifted to the short wave region, but in 17% (Group 2) it had not changed (contrary to the spectrum for healthy donors). We obtained 3 patterns of ABM fluorescence spectra in observed groups of patients (Fig. 3):

1A. Fluorescence zone shifted to short wave region by 20–30 nm (620–630 nm).
1B. Fluorescence zone shifted to short wave region by 30–50 nm (600–620 nm).
2. Fluorescence maximum at 650 nm observed for blood plasma of healthy donors.

These observations may be consistent either with the changed binding of ABM or conformational alterations of albumin molecule. Level of structural heterogeneity could also follow a non-uniform pattern of fluorescence spectra significantly differing in groups of patients. Not so significant albumin structure alterations are obtained in Group 2 as compared with Group 1. Data in Group 1 results in splitting of albumin alterations onto two stages:

Group 1A. Acidic expansion stage (620–630 nm); fluorescence intensity decreases more significantly (by 17% as compared to the results obtained in 1996–1997).

Group 1B. N-F transition stage (600 nm–620 nm). This well-known N-F transition takes place at pH values, lower than 4.3 and probably under ionizing radiation, and involves separation of domain III from the rest of the albumin molecule as well as the separation of the subdomains of domain III from each other (a change from a native or "N" form to a faster or "F" migrating form of albumin) [17–19]. The F form is characterized by a substantial increase in viscosity and much lower solubility. In our experiments, this stage is characterized by an increase of fluorescence intensity as compared to healthy donors (Table 1). It may be explained by an increased capacity of albumin binding sites. In human serum albumin for ligands exists multiple binding sites differed in affinity for this probe.

It is known that in acidic expansion stage relaxation of protein groups and bound water becomes faster [3]. ABM binding sites are gradually being exposed to a more hydrophilic environment which leads to a reduction of fluorescence intensity. ABM fluorescence is significantly enhanced when it is adsorbed into hydrophobic sites in albumin after N-F transition.

We determined the binding parameters of the probe ABM in case of N-F transition of albumin. Affinity for the probe decreased, but due to a bigger number of binding sites the fluorescence intensity of ABM increased.

Albumin self-fluorescence

During excitation of the human blood serum with 286 nm (emission wavelength 330 nm), practically the only source of

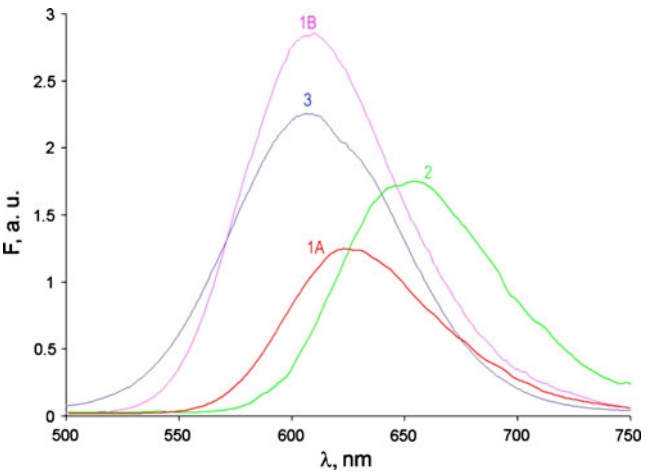

Fig. 3 Spectral characteristics of ABM in human blood plasma: 1A-acidic expansion; 1B-N-F transition; 2-healthy donors, 3-patients with epilepsy

Table 1 Spectral characterization of albumin from chernobyl clean-up workers blood plasma

Groups of patients	Probe ABM		Albumin auto-fluorescence	
	Fluorescence emission max, nm	F, a.u.	Fluorescence emission max., nm	F, a.u.
1A. (acidic expansion)	620–630	1.24+0.07	311–318	2.15±0.04
1B. (N-F transition)	600–620	2.85±0.07	309-311	1.85±0.06
2. Healthy	650	1.74±0.06	330	2.48±0.04
3. Epilepsy	604–614	2.25±0.07	309–311	1.38±0.07
Control	630	2.11 0.06	330	2.96 0.05
$p>0.05$ between groups		1 A-1B, 1A-2		1A-1B, 1A-2
		1A-3, IA-4		1A-3, 1A-4
		1B-2, 1B-3		1B-2, 1B-3, 1B-4, 2-3
		1B-4, 2-3, 2-4		2-4, 3-4

fluorescence is tryptophanyl groups of albumin molecule. Fluorescence of the tryptophane residue has effectively been used to shed light on protein dynamics and protein folding dynamics. A shift of the fluorescence zone to the short wave region (318–309 nm for Group I) is an evidence of hydration of the tryptophanyl region of albumin molecule during the transitions and conformational changes of this protein [2, 4].

A spectral characteristic of tryptophane autofluorescence has resulted in wealth of information on protein (albumin) dynamics. Tryptophane moiety of plasma albumin is located in a conformation labile hydrophobic fold of the structure which is accessible for water. This hydrophobic fold is closed due to N-F transition of the molecule. Under this process the environment near tryptophane residue becomes non-polar [18]. The affinity of binding sites of ABM was halved. There is a strong correlation between an increasing ABM fluorescence intensity with a shift of the fluorescence zone to the short wave region and increasing hydration of tryptophanyl region of albumin molecule (Table 1). Localization of probe ABM is determined by the kind of the binding site, e. a. hydrophobic or hydrophilic. According to literature, two different binding sites were obtained: the highest associative sites (acidic expansion) are located far from the tryptophane residues, and the lowest affinity site (N-F transition) is found at or near tryptophane. These results agree with the data of other authors using bovine serum albumin and probe ANS. Two binding types were found, one located in the midst of the protein interior and the other more external and water accessible [20].

Epilepsy

From the initial group of clean-up workers those with an epilepsy paroxysm were extracted. The ABM spectral characteristics of their blood plasma and results of albumin self-fluorescence give evidence that the so-called N-F transition takes place in this group of clean-up workers. This transition is accompanied by dehydration of the triptophanyl region of albumin molecule. Alterations of the investigated parameters in this group, especially dehydration of triptophanyl region of albumin molecule, are more expressed as compared to the initial group of clean-up workers and may be caused by epilepsy paroxysm.

Taken together, obtained patterns of fluorescence spectra suggest that various qualitative changes are evident in blood plasma albumin of Chernobyl clean-up workers in comparison with previously examined healthy donors or patients having no professional contact with radioactivity [11, 12]. Results of the 1996–1997 screening of peripheral blood mononuclear cell (PBMC) membrane have been described in previous work [21]. The obtained modifications of plasma albumin are associated with the effects of ionizing radiation on biological membranes including alterations of membrane proteins and peroxidation of unsaturated lipids. All these variations of the biophysical properties of membranes result in certain patterns of ABM fluorescence spectra. Dynamics of PBMC fluorescent characteristics in the presence of ABM reveals a progressive trend toward a certain resemblance to those of chronic B-cell lymphoid leukemia [11]. These findings reinforce our understanding that the blood plasma albumin is a significant biological target of radiation. Thus the role of albumin in the expression and course of cell damage after radiation exposure must be considered. It is now widely accepted that proteins play a prime role in most functional characteristics of organism [8].

Results of the present research showed that plasma albumin alterations were induced by ionizing radiation, and tryptophanyl region dehydration is also due to radiation-induced conformational changes in albumin molecules.

According to the results obtained in our previous investigations (ANS anisotropy values, ABM binding with peripheral blood mononuclear cell membrane), alterations in membrane proteins are also more expressed than in lipids in 83% of clean-up workers [21].

It is necessary to note that ABM spectral patterns in cell suspension and blood plasma are associated with the level of immunoglobulins (IgA, IgG, IgM), distribution of lymphocytes among the subpopulations (phenotype), and frequency of several diseases.

There is a strong correlation between the investigated parameters such as spectral characteristics of ABM in blood plasma and albumin auto-fluorescence, on the one hand, and tryptophanyl region dehydration of albumin molecule, on the other.

The 20 years dynamics gives evidence of the increasing incidence of albumin pathologic alterations (growing acidic expansion, appearance of the so-called N-F transition, dehydration of the tryptophanyl region of albumin molecule). The principal cause of the increase of membrane damage was probably the long-live radioisotopes that had penetrated in the bodies of clean-up workers as a permanent radiation source.

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