Albumin Characteristics in Patients with Type 2 Diabetes Mellitus

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Abstract

Albumin is one of the most generously represented proteins in the human blood plasma. Therefore it is important to follow and assesss transportation function of albumin in clinic researches. Disturbances in structural/functional properties of albumin play an important role in the pathogenesis of various diseases (e.g. diabetes mellitus). Changes in albumin transformation can serve as a diagnostic and prognostic criterion in pathologies. ABM (3-aminobenzanthrone derivative developed at Daugavpils University, (Latvia) has been previously shown as a potential biomarker for determination of the immune state of patients with different pathologies. The aim of this study was to determine and compare several aspects of plasma albumin alterations in patients with diabetes mellitus and healthy donors. The following parameters were examined: 1) the spectral characteristics of ABM in blood plasma; 2) "effective" (EA) albumin concentration in blood plasma; 3) quantitative parameters of albumin auto- fluorescence, characterizing tryptophanyl region of molecule and AGE (advanced glycation end products). Qualitatively different albumin modifications were obtained in diabetics and healthy humans. The different modifications of albumin are associated with different binding sites for probe, differing in affinity, quantum yield, and degrees of polarization. In diabetics the the albumin auto-fluorescence spectra has two emission maxima: 1) 332-333nm corresponding to the data observed in healthy persons (intrinsic fluorescence) and 2) shifted to a long wave region by 10-20 nm (340-350 nm), that corresponds to advanced glycation end products. Rigidity (dehydration) of the tryptophanyl regionis more expressed in diabetics as compared to control group. Diabetes-induced complications may be mediated by different pathways in men and women. ABM is a sensitive probe of albumin alterations, and can be used to elucidate the changes in protein systems. Significant differences in albumin dynamics exist between diabetics and control group.

Keywords: Albumin; Fluorescent Probe; Diabetes Mellitus

Introduction

Disturbances in structural/functional properties of albumin play an important role in the pathogenesis of Various diseases (e.g. diabetes mellitus) [1-4]. These diseases are accompanied by conformational changes in albumin while its concentration often remains unchanged.

Changes in albumin transformation can serve as diagnostic and prognostic criterion in different pathologies.

Diabetes mellitus is one of the most crippling diseases man has ever seen, and its prevalence has risen dramatically over the past two decades. Understanding the molecular properties of the diabetic progression is a big challenge in systems-the biology era.

Albumin is one of the most generously represented proteins in the human blood plasma. Therefore it is important to follow and assess transportation function of albumin in clinic research [1,2]. It has been assumed that physicochemical properties of molecules are extremely important for normal body functions, and their disturbances may cause diseases. The data indicate that metabolitic alterations, including sugar metabolites are associated with a higher risk of diabetes mellitus. Advanced glycation end products (AGEs) are known to be involved in the pathogenesis of diseases [5]. Many issues regarding structure of cell membrane, physical properties related to albumin structure (especially in molecule tryptophan area), functional role ligand and transportation of their oxidation products with various pathologies remain unclear.Fluorescent probes (derivatives of benzanthrone) are very sensitive to minor changes in albumin conformation, and physicochemical properties play an important role in the analysis of these parameters and their association with physiological state of organism [3,4,6]. Spectral characteristics of probes (derivatives of benzanthrones) satisfy all the requirements for ideal fluorescent tracers. Bright fluorescence, high extinction coefficient, photo -, thermo- and chemical stability, and reduced background signal makes them attractive as biomaging agents [6].

ABM, one representative of aminobenzanthrones, exhibits high affinity for model and biological membranes. Probe was synthesized at Daugavpils University, Latvia [7]. The synthesis, properties and research results to determine the immune state of patients with different pathologies have been summarised in our earlier study [3,4]. The data on conformational changes in albumin molecule under pathological conditions (e.g. Type 2 diabetes mellitus, gastrointestinal cancer etc.) and ionizing radiation have been investigated in our previous works [3,4,8-10]. It has been assumed that physicochemical properties of albumin are extremely important for normal body functions and their disturbances may cause disease. The aim of this study was to determine several aspects of plasma albumin alterations (binding sites properties) in the patients with Type 2 diabetes mellitus. AGEs were characterized with respect to the extent of side chain modifications and the fibrillar state (using probe ABM).

Materials and Methods

Study subjects

The study subjects were patients diagnosed with Type 2 diabetes mellitus, aged from 35 to 80 years and followed up for diabetic retinopathy, nephropathy or neuropathy (groups 1, 2, 3; 42 patients).

Characteristics of these groups were mentioned in Results and Discussion. Individuals who had been under treatment for acute infection, active autoimmune diseases and malignant tumours were excluded. The control group (17 donors, male) consisted of healthy individuals of corresponding age.

Blood Collection

The Ethics Committee of Rīga Stradiņš University approved the protocol of the present study design. Peripheral blood samples for plasma albumin studies were usually taken at 8–10 a.m. The venous blood was collected in vacuum tubes containing heparin 30 IU per tube.

Effective Albumin Content in Blood Plasma

Effective albumin concentration is a signal of "healthy" albumin in blood plasma, measured by the fluorescent method (in this case using probe ABM). Fluorescence intensity is in proportion to the number of free, unoccupied binding sites of plasma albumin (equivalent of "healthy" albumin in blood plasma) [1,2].

Albumin auto-fluorescence

In albumin auto-fluorescence investigations fluorescence spectra were measured at an excitation wavelenght 286 nm. The human plasma albumin fluorescence is dominated by tryptophanyl (330 nm) [1,2,11].

Sample Preparation and Fluorescence Measurements

Investigations were performed using the ABM fluorescent probe, developed at Daugavpils University, Latvia. In the current study, blood plasma (200-fold diluted) incubated without probe was used as a patient's personal "blank" for each experiment. ABM (resulting concentration in sample=19.6 nmol/ml) was added to 1 ml aliquot of each patient's blood plasma at the temperature of 18-20ºC and the mixture was then

allowed to set for about 5 min. The time interval between plasma isolation and fluorescence measurement was held constant for all samples (i.e, 3 hrs). Resulting fluorescence parameters were then registered by the Spectrofluo JY3 spectrofluorimeter (ISA Jobin Yvon Instruments S.A., Longjumeau, France) at an excitation wavelength of 470 nm and an emission wavelength of 520-700 nm. To register luminescence, every sample was placed in a cuvette ($1\times10\times40$ mm^3) fixed at the angle of 30° to the excitation light beam. Fluorescence intensity was then recorded and reported in terms of arbitrary units (au). The final intensity value for each patient's sample was calculated taking into account a corresponding personal "blank"; this approach thereby eliminated any potential contributions from any autofluorescing constituents in the plasma sample.

Statistical Analysis

Statistical differences among groups having different spectral characteristics were determined using the Student's t-test and Mann-Whitney U-test. Correlative relationships between spectral charasteristics of the ABM and the measured albumin auto-fluorescence parametrs were determined as outlined by Duncan [12].

Results

The spectral characteristics of ABM in in blood plasma, plasma auto-fluorescence data are shown in (Table 1).

Characteristics of Groups of Patients

We observed patients with Type 2 diabetes mellitus (groups 1, 2, 3), aged from 35 to 80 years and followed up for diabetic retinopathy, nefropathy or neuropathy (groups 1.2.3; 42 patients).
Group 1: Patients with Type 2 diabetes mellitus (male and female)
Group 2: Patients with Type 2 diabetes mellitus (male)
Group 3: Patients with type 2 diabetes mellitus (female)
Group 4: Control (healthy individuals of corresponding age

ABM Binding with Plasma Albumin

The emission maximum of ABM in blood plasma for healthy donors is 650 nm; fluorescence intensity F=2.11 a.u. The florescence zone in type 2 diabetes mellitus patients is shifted by 32-48 nm (603-618 nm) to a short wave region of spectra and fluorescence intensity in observed patents group's 1-3 decreases by 23%, 42%, and 14% as compared to mean control value (Table 1). It is interesting to note that most significant changes occur in male group as compared to female and control groups.

Effective Concentration of Albumin

The Type 2 diabetes mellitus was also accompanied by a decrease in EA concentration: 39 (male group), 59 g/l (female group). In the donor group, it is 68 g/l (Table 1).

N	Groups of patients	ABM spectral characteristics in plasma		Plasma auto-fluorescence				Albumin binding properties
		A max (nm)	F, a.u.	A max (nm)	F, au.	A max (nm)	F, a.u.	EA, g/l
1	Patients with diabetes mellitus (male and female)	603-618	1.62±0.09	332-333	2.05±0.07	340-348	1.95±0.08	52.2 ±2.6
2	Patients with diabetes mellitus (male)	607-614	1.22±0.04	332-333	2.14±0.13	342-348	2.11±0.13	39.3±2.0
3	Patients with diabetes mellitus (female)	603-618	1.82±0.08	332-333	2.01±0.09	340- 346	1.88±0.09	58.6±2.8
4	Control (healthy donors)	650	2.11±0.06	330	2,96±0,05	-	-	68.0±3.4
	p<0.05 (between Groups)		1-2; 2-3; 1-4; 2-4		1-4; 2-4; 4-Mar	-	-	1-2; 1-4; 2-3; 2-4

Table 1: Alterations of albumin structural and functional properties in patients with type 2 diabetes mellitus
Note: F (pl)-fluorescence intensity of ABM in blood plasma; EA -"effective" albumin concentration

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Albumin Auto-Fluorescence

The human plasma albumin fluorescence is dominated by tryptophanyl (exc/em 286nm/330 nm). In patients with Type 2 diabetes mellitus the fluorescence spectra (exc. 286 nm) has two emission maxima: 1) 332–333 nm corresponding to the data observed in healthy persons (intrinsic fluorescence) and 2) shifted to a long wave region by 10–20 nm (340–350 nm), that corresponds to advanced glycation end products (AGE) (Table1).

Discussion

Diabetes mellitus is a metabolic disorder that may affect not only all membrane structures, but also blood albumin. Albumin is subjected to many modifications, including glycation and oxidation, which occurs physiologically in low intensity, however are significantly increased in various pathological conditions (e.g. diabetes mellitus) [13]. Many issues regarding structure of albumin, physical properties related to albumin structure (especially in molecule tryptophan area), functional role, ligand and transportation of their oxidation products with various pathologies remain unclear. Variuos 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 posttranslation glycosylation of normal protein chains.

Nonezymatic glycosylation of albumin occurs in diabetes, and fluorescent methods were used to determine the effect of these products upon intramolecular movement in HSA (human serum albumin). Low levels of glycosylation products significantly reduced and left shifted tryphanyl fluorescence and inhibited penetration of probe. The most known pathways of diabetic complications involve oxidative stress. These studies suggest that albumin molecule of the diabetic patients plasma is modified by the chronic hypoxic conditions provoked mainly by hyperglycemia and oxidative stress [5]. Hyperglycemia in diabetic patients induces many pathological states, especially in disturbances like disorders in oxidative/antioxidative balance. The advantage of reactive oxygen species (ROS) over antioxidants is a cause of oxidative stress and creates an oxidative molecular damage of proteins, etc [5,14]. The reason for examining albumin is that this protein is practically the only source of ABM binding in blood plasma with a very high selectivity and subsequent fluorescence [8-10]. In Type 2 diabetes mellitus patients (groups 1-3) the fluorescence spectra of ABM in blood plazma is shifted to a short wave region, and fluorescence intensity decreases as compared to control value. The levels of pathological and pharmacological metabolites (fatty acids, antioxidants, plasma levels of lipidperoxidation products, etc.) balance differs in patients groups comparable to controls and hence their correlation to seizures pathophysiology and their degree. According to the data the plasma levels of measured parameters of oxidative protein damage showed significant changes in diabetic patients compared with the control group [11].

In albumin auto-fluorescence spectra two emission maxima were obtained at an excitation wave lenght at 286 nm. Fluorescence maximum at 333 nm wave length is associated by intrinsic tryptophanyl fluorescence, but at 340–350 nm – with glucation end products. However, fluorescence detection in plasma with AGE still bears some difficulties, arising from absorbance characteristics of often colored samples and quenching effects. The influence of quenching effects can be partially circumvented by using properly diluted samples. Questions of more detailed detection of AGE products need closer investigation. The data indicate that metabolic alterations, including sugar metabolites amino acids, and choline containing phospholipids, are associated early on a higher risk Type 2 diabetes melitus. According to literatura data the glycolysation products are known to be involved in the pathogenesis of several diseases.

Rigidity (dehydration) of the trypthophanyl region is more expressed in group 1-3 as compared to the control group. Fluorescence intensity of ABM plasma albumin in these groups is halved more significantly as compared to the control group. Tryptophanyl residue is located in a conformationally labile hydrophobic fold of the structure which is accessible for water. The hydrophobic fold is closed due to albumin transformation and under this process the environment of tryptophanyl becomes more rigid (dehydrated) [1,2]. The additional "binding shifts" seen in plasma samples of diabetes mellitus patients could be partly due to decreased binding of albumin because of conformational changes in it. These conformation alterations of albumin can provide the basis for the development of various pathologies and complications.

Another interesting tool to follow the formation of glycolysation products is the use of intrinsic fluorescence (excitation/emission wavelengths 280/350 nm), namely the tryptophanyl and tyrosine fluorescence. Tryptophanyl fluorescence data correlate very well with the data from Thioflavin T [15] and ABM [3,4, 8-10] assay, here

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conformational changes inside the proteins have already been demonstrated. Compared to classical amyloid marker Thioflavin T ABM displays: greater extent of fluorescence increase, higher affinity to fibrilar structures, weaker binding to the native protein, larger Stokes shift [6]. The decrease in intrinsic protein fluorescence was found to be correlated to the fibrillar state data of albumin. This can be expected since both parameters are sensitive to changes in the protein structure. The decrease in tryptophanyl fluorescence was obtained when comparing AGE-modified proteins with those from tryptophanyl. Therefore, detection of decreasing tryptophanyl fluorescence could be very useful for sensitive detection of very weakly modified proteins and or weakly fluorescent modifications.

A large number of autoimmune diseases (e.g. diabetes mellitus) obtaine gender differences [16]. Studies have shown that in Type 2 diabetes mellitus, men have a higher mean level of urinary albumin excretion rate compared to women. Observations of the study demonstrated that the difference vanished after adjusting age and HDL (high density lipoproteins) levels. HDL plays an important role in the progression to albuminuria because men have lower plasma HDL levels they have a higher risk of progression to albuminuria [17]. In previous experiments was obtained that ABM fluorescence is in inverse correlation with membrane (liposomes) microviscosity-content of HDL [18].

The probe ABM as biomarkers of modified albumin could also help much fuller identify and understand the state of albumin binding sites in different pathologies and open the way to elucidating the nature of changes in the albumin molecule in pathology.

Fluorescent method reveals the "effective" concentration of albumin (equivalent to 'healthy' albumin in blood plasma). In contrast to total albumin concentration, effective albumin concentration very sensitive to pathology. This index depends not only on albumin concentration, but also its molecular state. The present study revealed significant changes in ABM fluorescence associated with plasma (re: albumin) of the patients of the observed groups seems to correlate with ABM fluorescence intensity. Changes in probe fluorescence turned out to be a typical albumin response to pathological processes. Changes in albumin are nonspecific and accompany many diseases. The shift of probe fluorescence maximum to a short wave region may be also an evidence of hydration of tryphtophanyl region of albumin molecule [1]. According the literature data- the plasma proteins dynamic significantly differentiated from

time to time during the diabetic progression. 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 [19]. Plasma albumin level was also correlated with most traditional risk factors and hemostatic variables.

It is known that there are gender differences in the progression of albuminurea and cardiovascular diseases. Furthermore the prevalence of ischemic heart disease was significantly higher in men with albuminurea compared to men without albuminurea [16]. An abnormally elevated rate of albumin excretion reflects cardiovascular damage that is more severe in Type 2 diabetics [20]. There are conflicting reports concerning the effect of gender on rate of urinary albumin excretition in patients with diabetes. Hashim et al. demonstrated that urinary albumin excretion is associated with insulin resistance and cardiovascular risk factors in patients with diabetes that is more pronounced in women [21]. Fasting blood AGE influence albuminurea in men (see Table1, group 2). Probe ABM in male and female (groups 2 and 3) reveals different spectral shifts in binding parameters. Such shifts in binding parameters would be in agreement with the observations of other authors [11] who noted that albumin molecules contained different binding sites (i.e., Classes) that differed in affinity, quantum yield, and degrees of polarization, i.e., higher mobility of a bound probe and increased accessibility by water, for ABM and various other probes.

Results clarify the heterogenous nature of albumin molecules and reveal qualitative different its conformation in observed groups of patients. Despite the 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. It is interestingly to note that all investigated parameters (ABM fluorescence intensity, "effective" albumin concentration, dehydration of tryptophanyl region of molecule etc.) was more pronounced in diabetics groups (groups 1-3) as compared with control. The elevated levels of nodified albumin could be related to the severity of disease.

The present study reveals significant changes in albumin modifications in diabetic patients and healthy donors. Taken together, qualitatively different albumin modifications were obtained in diabetics (see Table 1). The different modifications of albumin is associated with

different binding sites for probe ABM that differed in affinity, quantum yield, and degrees of polarization, i.e. higher mobility of a bound probe and increased accessibility by water for probe. Analysis of ABM binding and fluorescence showed that low level of effective albumin blood plasma is due to structural changes in binding centers of albumin molecule, probably, associated with changes in body functions. We suggest that diabetes –induced complications may be mediated by different pathways in men and women. This is an interesting topic for future prospective studies.

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