BIOPHYSICS AND BIOCHEMISTRY

Effects of Fatty Acids on Human Serum Albumin Binding Centers

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Albumin is a carrier of nonesterified long-chain fatty acids and many other ligands. The status of its binding centers was studied for various proportions of nonesterified long-chain fatty acids and albumin as exemplified by palmitic acid. The status of the binding center was tested by recording K-35 probe fluorescence decay in the subnanosecond band. This method showed the work of three types of centers. Palmitic acid enhanced binding activity of all centers, though to a different degree: if the palmitic acid/albumin proportion increased to 2-3, the probe binding to type 1 centers (located in the drug center I region) increased 1.5 times, while binding to type 3 centers increased more than 3-fold. Modification of these centers by nonesterified long-chain fatty acids was similar in the isolated human albumin preparation and in diluted blood serum. Hence, K-35 probe showed the actual status of various albumin centers, their binding capacity depending to a different measure on the fatty acid charge of albumin.

Key Words: *human serum albumin; binding centers; fatty acid impact; fluorescent probe; first episode of schizophrenia*

Blood albumin is the carrier of many low-molecular metabolites, including long-chain nonesterified fatty acids (NEFA; palmitic, oleic, *etc.*). Due to this, albumin is involved in detoxification, oxidation, and other processes [6,14]. If albumin functions are disordered, metabolites accumulate in the blood reaching toxic concentrations and causing endogenous intoxication [4].

Nonesterified fatty acids are essential for albumin binding capacity [5,9,14]. This characteristic of albumin molecule presumably modulates the drug pharmacokinetics and efficiency of toxin elimination by hemoperfusion. However, these effects and moreover, impact for clinical practice are little studied. The difficulties of the problem consist in the intricacy of relationships and mutual effects of binding centers in the albumin molecule. Different metabolites bind to different centers; the number of NEFA in albumin varies greatly and these molecules occupy different positions in albumin and differently modulate the strength of metabolite bonds with albumin. There are no methods allowing tracing the status of different centers in the plasma; new modern physical methods should be developed for this purpose.

Measurements of albumin-bound NEFA are an interesting problem, particularly because fatty acid transport is associated with pathological processes. Correlations between NEFA concentrations with the

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development of diabetes and cardiovascular diseases [10,11,13] and other diseases [12] have been described. For example, high plasma level of NEFA is a sudden death risk factor in arrhythmia in middle-aged individuals without cardiac symptoms [8].

The aim of our study was to find a new methodological approach to evaluation of NEFA effect on albumin binding activity directly in the plasma or blood without isolation of albumin fraction or other plasma processing. Fluorescent probe K-35 was used. Previous studies showed that after adding to diluted plasma/sera, K-35 bound to the so-called drug centers in albumin molecule [2,3,7]. The intensity of the probe fluorescence increased by tens times under these conditions. It depended on plasma concentration of albumin, status of its binding centers, and virtually did not depend on the presence of other plasma components. The fluorescence intensity depended (among other things) on fatty acid charge of albumin [1]. This fact suggested an approach to evaluation of the NEFA/albumin proportion directly in the plasma, which was the aim of our study.

MATERIALS AND METHODS

Lyophilized human serum albumin (HSA, Sigma; cat. No. A 1887) containing no fatty acids was dissolved in buffer (0.137 M NaCl and 0.01 M sodium phosphate, pH 7.4). Palmitic acid (Sigma) solution in ethanol and then fluorescent probe N-(carboxyphenyl)imide-4-(dimethylamino)naphthalic acid (K-35) [2,3,7] were added to albumin solution or to diluted serum during stirring. The final concentration of ethanol in albumin sample was no higher than 0.3%.

Venous blood serum was obtained from healthy volunteers and patients with the first episode of schizophrenia hospitalized at the First Psychotic Episode Clinic of Moscow Institute of Psychiatry before drug therapy. The patients gave written consent to the study. The study was carried out in accordance with the Helsinki Declaration.

Decay of albumin-bound K-35 probe fluorescence after excitation by pulse photodiode at λ=455 nm on a Pico-Quant device at Physical Institute. The kinetics of fluorescence decay after excitatory pulse was recorded at λ=530 nm perpendicularly to the excitatory beam in the horizontal plane in the photon-counting mode. The decay curves measured so were presented as a sum of exponents with different A_i amplitudes and time constants t_i:

$$F(t)=A_1\times\exp(-t/\tau_1)+A_2\times\exp(-t/\tau_2)+A_3\times\exp(-t/\tau_3)+\ldots$$

The optimal set of A_i and τ_i parameters was selected by χ^2 test. Expansion of the decay curve into exponents can introduce errors in each of these parameters, and therefore, the mean square error of expansion for each point calculated by χ^2 test according to recommendations [6] was presented in the figures.

RESULTS

K-35 probe virtually did not fluoresce in water, while in the presence of albumin fluorescence intensity F increased by two orders of magnitude. Fluorescence of HSA-bound K-35 probe was an intricate mixture of components, as different molecules of K-35 were located in different binding sites of HSA [3,7]. Three components made the main contribution to fluorescence. The kinetics of fluorescence decay after pulsed excitation of K-35 molecules looked as a sum of three exponents with different A_1 amplitudes and τ_1 time constants. These three exponents corresponded to location of the probe molecules in binding centers of three types [3,7]. For NEFA-free HSA, the τ_1, τ_2, and τ_3 were 9.3±0.6, 3.6±0.3, and 1.0±0.1 nsec, respectively. The A_i amplitudes corresponded to the number of the probe molecules located in different centers. Due to this, K-35 probe showed the filling of centers of each type separately during changes in HSA molecule. This property of K-35 was its advantage in comparison with other stains used for HSA studies.

Addition of NEFA (palmitic acid) to HSA resulted in an increase of F intensity, reaching the peak (1.6 times) at NEFA/HSA molar ratio of 2-3 [1]. Registration of fluorescence decay kinetics on an HSA preparation free from NEFA (which were removed by special processing) and after addition of palmitic acid showed a significant increase of A_1, A_2, A_3 amplitudes (Fig. 1, *a*), while the time constants τ_1, τ_2, τ_3 changed negligibly. Hence, the increase of F was due to increase of K-35 binding to HSA. The binding increase was characteristic of centers of all three types, but most of all for type 3 (more than 3-fold at NEFA/HSA proportion of 2-3; Fig. 1, *a*). The sum $F=A_1\tau_1+A_2\tau_2+A_3\tau_3$ under these conditions increased 1.6±0.3 times, which confirmed the previous data [1]. Hence, the A_1 amplitudes and the amplitude proportion depended on the NEFA/HSA proportion.

The serum usually contains 0.5-1.5 molecules of NEFA per HSA molecule. In order to compare the results of model experiments with the actual status of the serum, we recorded the kinetics of K-35 fluorescence decay after the probe addition to diluted serum from healthy volunteers and patients with the first episode of schizophrenia. The serum time constants were the same as in isolated HSA. As for the amplitudes — as expected, addition of palmitic acid to the serum with albumin containing NEFA led to reduction of A_1 and A_2 and further increase of A_3. An example of typical normal serum is presented in Fig. 2. Hence, the behavior

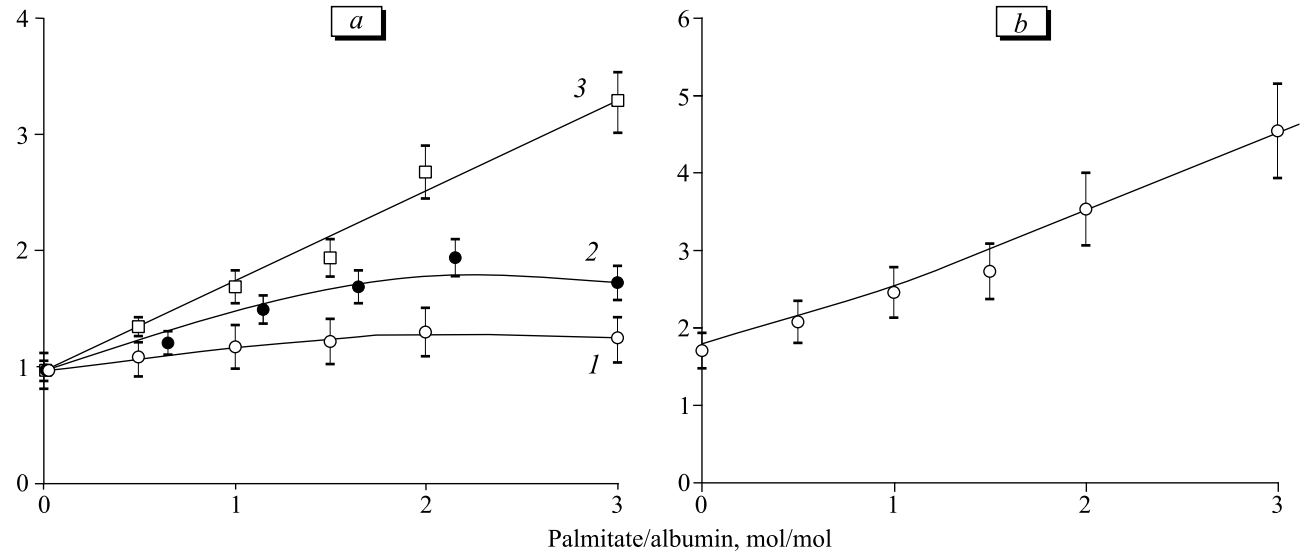

Fig. 1. Changes in the amplitudes *A* (*a*) and amplitude proportion (*b*) in the fluorescence decay components of HSA-bound K-35 probe in response to palmitic acid addition. The concentrations of HSA and K-35: 16 μM. Here and in Fig. 2: the mean quadratic deviation is shown in each experimental point. A_{1no}, A_{2no}, and A_{3no}: amplitudes without palmitic acid. 1) A_1/A_{1no}, 2) A_2/A_{2no}; 3) A_3/A_{3no}.

of amplitudes in response to palmitic acid addition depended on the NEFA initially present in the serum.

Comparative analysis of A_i and τ_i values in diluted serum specimens from 24 patients with the first episode of schizophrenia was carried out. The A_3/A_1 ratio varied in the patients, but the range of variations more or less corresponded to the range after addition of palmitic acid to HSA in 0-3 molar proportion (Fig. 1, *b*). This once more indicated similarity of the serum status in comparison with the model experiment with isolated HSA after addition of palmitic acid.

Hence, fatty acids in physiological concentrations (that is, at NEFA/HSA ratio of 0-(2-3) modulated the HSA K-35-binding centers of all three types and enhanced their binding activity. Type 1 centers reacted less intensely to the fatty acid than type 2 and particularly type 3 centers, their binding activity increasing more than 3-fold. Presumably, the degree of fatty acid charge of HSA was essential for the drug pharmacokinetics and extracorporeal elimination of toxic ligands transported by albumin.

According to previous findings, the first three molecules of fatty acids in HSA crystals occupied sites in subdomain IIA (near drug center 1), IIIA (near drug center 2) and IIIB [15]. Our data indicate that of the K-35 probe binding centers, type 3 centers are the most sensitive to palmitate. Presumably, they were the closest to the fatty acid denoted in the crystal [15] by No. 2 (scheme of albumin molecule was presented previously [7,15]) or were located in domains IIIA or IIIB. However, it is difficult yet to solve this problem, as the location of the fatty acids in the HSA molecule in a real solution can differ from their known location in a crystal.

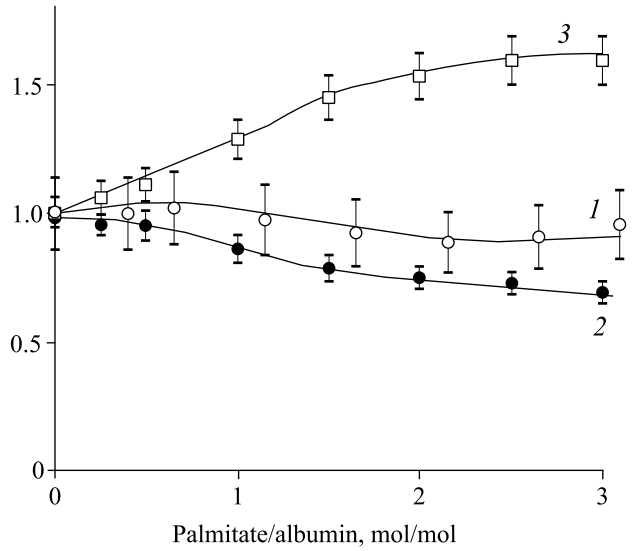

Fig. 2. Time course of amplitudes *A* of three fluorescence components of HSA-bound K-35 in normal diluted serum in response to palmitic acid addition. The serum was diluted to the final concentration of albumin 40 μM; K-35 concentration: 20 μM.

Our results suggest that registration of K-35 fluorescence decay in diluted serum opens new vistas in evaluation of albumin charge by fatty acids and its actual adsorption capacity in the blood. These results can serve the base for development of methods for prediction and evaluation of the efficiency of drug therapy in patients with endogenous mental diseases.

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