OPTICAL PROPERTIES OF DETONATION NANODIAMOND COMPLEXES WITH GLOBULAR PROTEINS IN WATER SOLUTION

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Abstract

It has been studied the changing of spectral characteristics of water-soluble 10 nm detonation nanodiamonds (DND) upon their complexation with a globular blood protein: bovine hemoglobin (Hb), human serum albumin (HSA) and rabbit immunoglobulin G (IgG) by fluorescence and absorption spectroscopy. The structure of the protein globule and its concentration in aqueous solution can significantly affect the complexation with DND ($2 \cdot 10^{-3}$ g/l) in the process of ultrasonic treatment (42 kHz). In all cases, it was observed when adding the DND the increasing of intensity of own luminescence of proteins (λ_{em} = 330-346 nm) and bathochromic shift of protein luminescence bands. The magnitude of the shift of the maximum of protein luminescence bands at a protein concentration of 10^{-11} M was 5, 16, 18 nm, at the concentration 10^{-5} M - 7, 9, 2 nm for Hb, HSA and IgG, respectively. The aggregates' size of DND was evaluated by AFM method in monocomponent film on the glass substrate. The results are of interest for investigations of the dynamic interaction of DND with biomacromolecules in vivo.

Keywords: Detonation nanodiamonds, hemoglobin, albumin, immunoglobulin G, luminescence

1. INTRODUCTION

Recently there has been a steady increase of interest in the use of nanoparticles in biology and medicine. One of the most promising objects are ultradisperse detonation nanodiamonds (DND) [1-4]. From all carbon nanoparticles, DND have the lowest toxicity and are used, for example, to create biosensors, treat tumors, and create biocompatible implants. For use in biomedicine, DNDs are of greatest importance, which in complexes with biomacromolecules are able to form stable colloidal solutions in an aqueous medium.

DND possess a number of unique physical and physicochemical characteristics, including pronounced chemical stability, a high degree of biocompatibility, a developed surface and, as a result, high sorption capacity, the presence of polar oxygen-containing chemical groups on the surface, which ensure their water solubility. Furthermore, oxygen-containing groups providing effective binding with amino groups of protein [5]. The properties of DND complexes with biomacromolecules can vary significantly depending on the intrinsic characteristics of both DND and biomacromolecules. The structural diversity of biomacromolecules and the complexity of the environment in biological systems determine the weak investigation the difficulty of determining physical and physical-chemical mechanisms of this direction.

The interaction of DND with biomacromolecules comes not only to adsorption, but leads to the destruction of aggregates of DND, with the formation of aggregates of DND-bio objects [1]. Thus, DND are an effective carrier of immobilized biomacromolecules due to thermodynamically more advantageous binding to biomacromolecules compared to the interaction of DND with each other.

In the present work spectral changes in aqueous solutions of the DND's interaction with three different globular proteins: Human serum albumin (HSA), hemoglobin (Hb) and rabbit immunoglobulin (IgG) were studied by fluorescence and absorption spectroscopy methods.

2. MATERIALS AND METHODS

Lyophilized human serum albumin (HSA), bovine hemoglobin (Hb), and rabbit immunoglobulin (IgG) (Sigma-Aldrich Co.) and 10nmsized - water-soluble detonation nanodiamonds (Adamas Nanotechnologies) with an initial concentration of 1 g / l suspended in distilled water were used. The protein solutions were prepared in distilled water with a NaCl content of 0.1 mol / l. Solutions of proteins with concentrations of 10^{-5} and 10^{-11} M were prepared by successive dilution, then the same amount of DND was added in all cases, so that their concentration were 4.10^{-3} g / l. Thus in all the investigated solutions of mixtures of proteins and DND, concentrations of DND and NaCl were constant, only the concentration of proteins altered. Solutions of DND mixtures with proteins were ultrasonically processed on a Branson 1510 device (USA), absorption and fluorescence spectra were recorded with the spectrometers Shimadzu (Japan) UV-1800 and RF-5300pc.

3. RESULTS AND DISCUSSION

Human serum albumin, immunoglobulin and hemoglobin are the main and most studied globular blood proteins. The molecular weights of HSA and Hb are close (approximately 67,000 and 65,000 g / mol), the molecular weight of the immunoglobulin G exceeds this value by more than two times (about 150,000 g / mol). The structures of HSA and Hb can be considered relatively monolithic, whereas the structure of IgG is a set of three domains that are movable relative to each other [9]. The structures of serum albumin [10] and hemoglobin [11] can be regarded in some approximation as particles of a protein nature with a diameter of approximately 8 and 6 nm, respectively; the IgG molecule can be represented as a cylindrical formation 24 nm in length and transverse sizes from 1.9 to 5, 7 nm.

The ultraviolet luminescence (UL) of proteins is determined mainly by the presence of tryptophan residues [12], whose content in the molecule can differ by more than tenfold: from one tryptophan residue per one molecule of serum albumin, to about nineteen per molecule of IgG [13]. Nevertheless, a significant difference in the content of tryptophan slightly affects the overall intensity of luminescence of protein solutions of identical molar concentrations. In our case (**Table 1**), the intensity of intrinsic UL of proteins differs by no more than 20%, and this difference is practically independent of dilution (without adding of DND). We note a noticeable hypsochromic shift of its own UL of proteins at 13, 3 and 16 nm upon dilution of the solution for Hb, HSA and IgG (Table 1). It is interesting that a decrease in the concentration by several orders leads to an insignificant drop in the intensity of UL, by no more than 25%, and the values of the decrease in the intensity of UL are practically the same for all the proteins studied.

Complexation of used proteins with DND leads to no less significant changes in the position of the UL bands and their intensity. We note that the largest spectral shifts were observed at low protein concentrations (10^{-11} M): for HSA - 16 nm, for IgG - 18 nm. However, in the case of Hb, the shift in the maximum of the UL protein band upon addition of DND at low protein concentrations (10^{-11} M) was about 5 nm, which is 2 nm less than the shift in the case of a protein concentration of 10^{-5} M (**Table 1**), which is possible due to the influence of the porphyrin group in the hemoglobin. We also note that in the case of low concentrations of Hb, the UL has the biggest ignition (about 3 times). It is noted that the structure of the protein during complexation of DND undergoes appreciable changes without changing the qualitatively general properties of the protein globule [1].

Globular proteins act as detergents and reduce the degree of aggregation of nanostructures [14], while the presence of porphyrin photoactive groups in their composition can lead to a change in the energy transfer efficiency in the "protein-nanostructure" complex and to significant changes in the photoluminescence intensity

when the structure of the complex is changed [15]. The formation of DND-protein complexes may lead to the formation of several types of complexes, differing in the ratio of the components [16], which may make analysis of the spectral characteristics difficult. The basic forces involved in the formation of DND complexes with proteins are of an electrostatic nature [16], therefore, the ratio of the charge of the surface of proteins and DND determines the specificity of their interactions. In [16], it was shown that the size of the DND-HSA aggregates in the solution varies significantly depending on the pH of the medium. This can also affect the spectral characteristics of the complexes.

Table 1 Spectral properties of hemoglobin (Hb), human serum albumin (HSA), rabbit Immunoglobulin (IgG) and its mixtures with detonation nanodiamonds (DND): λmax of ultraviolet luminescence band, its intensity (LI) and the value of change of this characteristics

	Protein without DND		**Protein-DND solution**		**The effect of DND adding**	
					Bathochromic shift, nm	
					Enhancing of LI, a.u. (times)	
[Protein], mol/l	**10^{-5}**	**10^{-11}**	**10^{-5}**	**10^{-11}**	**Δ, 10^{-5} M**	**Δ, 10^{-11} M**
Hb, λ_{max}, nm	342.7	329.5	349.5	334.2	6.8	4.7
Hb, LI, a.u.	2.19	1.67	2.63	5.14	0.44 (1.2)	3.47 (3)
HSA, λmax, nm	337.4	334	346.2	350.3	9	16
HSA, LI, a.u.	2.41	1.85	2.55	2.6	0.14 (1.05)	0.75 (1.4)
IgG, λmax, nm	346.2	330	347.6	347.6	1.5	17.6
IgG, LI, a.u.	2.67	2.02	3.74	3.21	1.07 (1.4)	1.19 (1.6)

4. CONCLUSION

Investigations of globular blood proteins (serum albumin, immunoglobulin and hemoglobin) in aqueous solutions in the formation of complexes with detonation nanodiamonds showed significant differences in the spectral characteristics of complexes depending on the protein concentration and the nature of the protein molecule at the same detonation nanodiamond content. Specificity of changes in the spectral properties of complexes with different types of globular proteins can serve as a basis for the development of methods for their qualitative and, possibly, quantitative analysis.

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