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RADIOCHEMICAL STUDY OF BIOPOLYMERS SORPTION ON HYDROPHOBIC SURFACES

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The behavior of globular proteins (lysozyme, human serum albumin) and humic acids of coal (Powhumus) in aqueous/oil and aqueous/graphene systems was studied by means of tritium tracer. Tritium labeled biomolecules were obtained by tritium thermal activation method. Adsorption isotherms were obtained by liquid scintillation spectrometry of tritium either in traditional performance or in scintillation phase technique.

INTRODUCTION

The behavior of biomacromolecules in hydrophilic/hydrophobic systems plays an important role in different fields of technology and industry. The interest was extremely increased with the development of nanotechnology. Mechanisms of the processes which occur at the interfaces are under the high influence of both nature of hydrophobic surface and biomolecule itself. There are a number of instrumental methods used for studying the adsorption either at liquid/solid or liquid/liquid interfaces. X-ray photoelectron spectroscopy [1], reflectometry [2], and Raman spectroscopy [3] are most frequently used for the investigation of adsorption on aqueous/solids.

Liquid/liquid interfaces are more challenging for the experimental studying. The most prevalence experiments are conducted by pendant-drop technique [4], Fourier transform infrared spectroscopy [5], total internal reflection fluorescence microscopy [6] or by radiotracer method with ^{14}C-labeled proteins first introduced by D.E. Graham and M.C. Phillips in 1978 [7] and still used for studying proteins adsorption at aqueous/oil interfaces [8]. Thus the universal method for aqueous/solids and aqueous/oil interface has not yet developed.

For this purpose radiotracer assay is rather promising. In our previous researches we have shown that tritium is a perspective tracer in surface experiments [9–13]. Since tritium is a radioactive isotope of hydrogen, it is related to all classes of organic molecules. On the other hand, the advantages of tritium as a tracer would not have become applicable to such a wide extent if it were not relatively easy to label organic molecules with tritium at high specific activities and in versatility that is not obtained with any other isotopes. For interfacial researches tritium-labeled compounds can be obtained by means of tritium thermal activation method [14]. The technique is based on the bombardment of solid target of organic compounds with tritium atoms which forms on tungsten filament at 1500–2000 K. Since tritium can substitute protium in any possible position, double purification is usually conducted. First, treated compound is purified from labile tritium (OH–, COOH–, NH_2–, SH-groups). Then [^3H]-product is released from the mixture of labeled compounds. This labeling technique is applicable to most kinds of organic materials from low molecular weight amino acids [15] to humic substances [16].

Because of unique nuclear-physical properties of the isotope ($T_{1/2}$=12.4 years, E_{max}=18.6 keV) the path length of tritium β-particles reaches few microns in the condensed media. This fact was used when tritium labeled compounds were applied to studying the adsorption of individual surfactants and their mixtures with polymers at aqueous/organic liquid interface [10, 11].

The only way to determine tritium labeled compound is liquid scintillation spectrometry. For sorption experiments with solid/liquid surfaces, it is used in the traditional performance for measuring aqueous solution [17]. In case of the systems of two immiscible liquids, liquid scintillation spectrometry is applied in variant of scintillation phase method [10–13]. It is a direct method of the determination of both bulk concentration of tritium labeled compound and its excess at aqueous/organic scintillator interface. The technique is based on the measuring counting rate of the system of aqueous solution of tritium labeled compound / organic scintillator which is immiscible with water. The counting rate

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results in both distribution of labeled compound in the bulk of scintillator and its concentration at the liquid/liquid interface. The radiochemical theory of the method was previously described in [13].

In this manuscript we have compared the adsorption for biomacromolecules on hydrophobic/hydrophilic interfaces including aqueous/solid and aqueous/organic liquid interfaces. Since recent increased interest in nanoscale particles graphene was used as hydrophobic solids. In case of liquid/liquid experiments scintillators based either on toluene or *p*-xylene were applied. Two globular proteins lysozyme and human serum albumin and coal humic acid were under the test. Based on our previous experimental data the conditions of tritium labeling were chosen according to formation of labeled product with high specific radioactivity on the background of small amount of by-products.

EXPERIMENTAL

Graphene was synthesized in Institute of Elementoorganic Compounds RAS, Moscow. Specific surface 107 m^2/g was determined by BET.

Lysozyme and human serum albumin were purchased from MP Biomedicals and used without further purification. Coal humic acid (CHA-Pow) was a commercially available preparation Powhumus (Humintech GmbH, Germany) desalted using dialysis before the experiments [16].

Tritium label was introduced into the biopolymers by means of tritium thermal activation method [16, 18]. Briefly, 0.8 mL of aqueous solution of compound (1.25 g/L) was distributed on the walls of glass reactor and lyophilized. Then the reactor was connected to the gas tritium devise which includes W-wire in the middle of glass reactor and a cooler. Air was pumped out and the reactor was filled with tritium gas till 1.2 Pa. W-wire was heated with electric current up to 1800 K during 10 s. After the reaction the compound was dissolved in 2 mL of aqueous solvent: 0.4% NaOH for CHA-Pow and phosphate saline buffer (PBS, pH 7.2±0.1) for globular proteins. To purify labeled compound from the labile tritium and labeled by-products 30 days dialysis and size exclusion chromatography were used as it described in [16, 19]. Specific radioactivities of final products were 1.5, 3.2 and 12.8 Ci/g for human serum albumin, lysozyme, and CHA-Pow, respectively.

Adsorption experiments were conducted for aqueous/oil and aqueous/solids interfaces. First were carried out by means of scintillation phase method. To 1 mL of aqueous solution of [^{3}H]-compound (specific radioactivity *ca.* 1.5 μCi/mL) 3 mL of scintillation phase (non-aqueous scintillator based on p-xylene or toluene) was added. The amount of [^{3}H]-compound in both oil phase and interfacial excess were calculated as it was previously described [10].

Sorption experiments on solids were conducted for graphene. To 4–5 mg sample of carbon nanomaterial 0.8 mL of aqueous solution of [^{3}H]-compound (specific radioactivity *ca.* 3 μCi/mL) was added. Ultrasonication of the dispersion was carried out for 20 min using a bath sonicator with rated power of 50 W. Then systems were incubated at room temperature during 24 hrs. 200 μL of suspension was picked out and centrifuged. 100 μL of the solution was filtered throw the syringe filter with 0.2 μm PVDF membrane (Life Science). 10 μL aliquot of filtered solution was picked out for radioactivity measuring. Radioactivity of aqueous solution was measured in scintillation cocktail OptiPhase Hi Safe 3 (PerkinElmer) by means of scintillation spectrometer RackBeta 1215 (Finland).

Protein concentration in aqueous solution was calculated as

$$c_1 = \frac{I}{\varepsilon \cdot V_1}. \qquad (1)$$

Here I is counting rate, V_1 is volume of aliquot, ε is the registration efficiency of tritium β-radiation (for OptiPhase Hi Safe 3 ε = 53±2%).

The value of protein sorption was calculated as

$$\Gamma = \frac{(c_0 - c_1) \cdot V_0}{m \cdot S}, \qquad (2)$$

c_0 is the initial concentration of protein solution, V_0 is the initial volume, m is mass of graphene, S is specific surface of nanomaterial.

RESULTS AND DISCUSSIONS

Tritium labeled compounds are useful in studying their behavior in different systems. Here we have labeled biological macromolecules with tritium to investigate their hydrophobic and surface active properties. Experiments were conducted for aqueous/oil and aqueous/graphene interfaces. Fig. 1 and Fig. 2 show the comparison of adsorptions of lysozyme, human serum albumin and CHA-Pow at aqueous/arene and aqueous/graphene interfaces in semi-logarithmic coordinates. One can see that for all tested compounds the adsorption at liquid/liquid interface is higher than one obtained for aqueous/graphene. It can be explained by the strong interaction between molecules of organic phase either with hydrophobic amino acids residue of proteins or with hydrophobic core of humic acids.

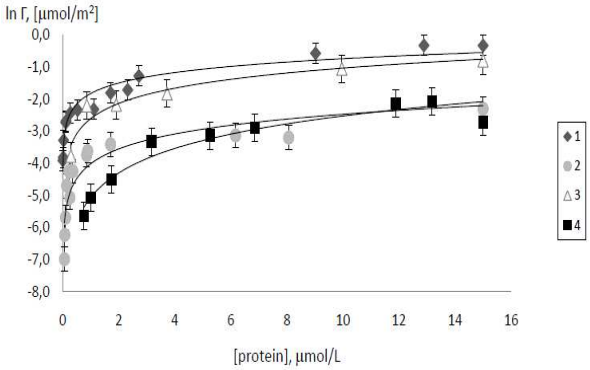

Fig. 1. The comparison of the adsorption of globular proteins at different aqueous/hydrophobic interfaces. *1* – human serum albumin at aqueous/*p*-xylene interface; *2* – human serum albumin at aqueous/graphene interface; *3* – lysozyme at aqueous/*p*-xylene interface and *4* – lysozyme at aqueous/graphene interface

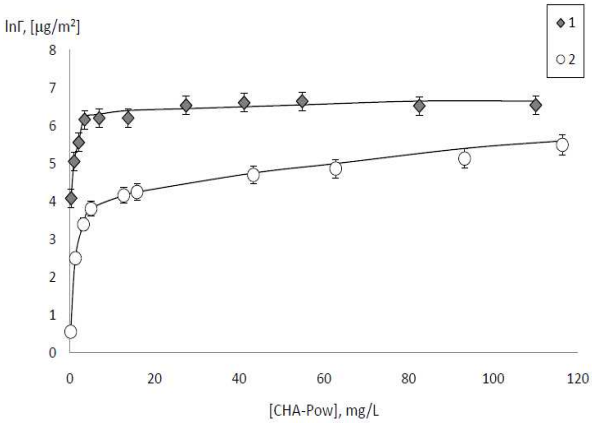

Fig. 2. The adsorption of coal humic acids at aqueous/toluene (*1*) and aqueous / graphene (*2*) interfaces

Such associates possess high surface activity compared with aqueous/air interface [8] and can penetrate into the bulk of organic liquid with the partition coefficients $(1.0\pm0.2)\times10^{-3}$, $(3.2\pm0.6)\times10^{-3}$ and $(3.7\pm0.2)\times10^{-3}$ for lysozyme, albumin, and CHA-Pow, respectively . It has to be emphasized that tritium labeling procedure and purification secured the radioactivity of organic phase provide only by labeled compound not by exchangeable tritium.

As to liquid/liquid interface tritium tracer have unquestionable advantage compared with methods based on measuring interfacial tension which lies in the fact that it is applicable in both monolayer region and higher concentration limited by the formation of stable emulsion of water in the organic phase in presence of surfactant [10]. The formation of stable associates with biomolecules was confirmed with the experiment when organic phase without scintillation additives saturated with albumin that was subjected to vacuum evaporation and then it was dissolved in water. We have observed the 2.5×10^{4} nm emulsion by means of photon correlation spectrometry that allowed suggest that associates of proteins with molecules of organic phase do not destruct even under vacuum while organic phase itself is volatile liquid.

Not only interaction with hydrophobic interface but also the nature of sorbate itself is of significance in the adsorption mechanism. Fig. 3 shows the comparison of adsorption isotherms of globular proteins on graphene surface. While albumin adsorption was subjected to Langmuir model in monolayer region, adsorption of lysozyme linearly increased in whole concentration range ($r^2 = 0.97$). Henry constants calculated from the liner part of the isotherms were 4×10^{-2} and 1×10^{-2} mol/m^2/mol/L for albumin and lysozyme, respectively.

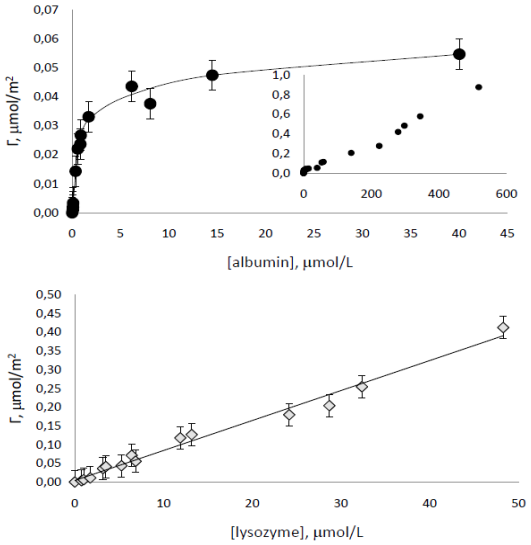

Fig. 3. Comparison of adsorption of human serum albumin (*top*) and lysozyme (*bottom*) on aqueous/graphene interface

The data obtained for albumin were compared with one obtained for carbon single-walls nanotubes in [2]. In cited paper, adsorption of bovine serum albumin was tested by reflectometry at different pH. At pH 7 the authors observed a plateau with the value of maximum adsorption 2.3±0.2 mg/m^2 at concentration 0.5 mg/mL (7.24×10^{-3} mmol/L) and did not discuss future behavior. In case of graphene we also observe first plateau at concentration range from 1.71×10^{-3} to 7×10^{-2} mmol/L. The value of adsorption corresponds to the formation of monolayer of this protein.

One can see that the values of lysozyme adsorption in ten times higher than for albumin at the same concentration range. The difference in adsorption

mechanism of albumin and lysozyme can also be explained by peculiarities of the physical properties of tested globular proteins. Since lysozyme is rather small and structural stable molecule, it probably preserves the possibility to interact with molecules in the bulk of aqueous phase, when adsorbed on graphene surface. Human serum albumin is a molecule of large size and non-stable structure. When adsorbed on hydrophobic surface, it loses the interaction possibility with proteins both in aqueous phase and adsorbed on graphene surface. One can see that at high concentrations of albumin adsorption corresponds to formation of polylayers but this adsorption is a reversible process. Desorption at monolayer region for albumin and in case of lysozyme was less than 2% even if 10 order excess of sodium dodecylsulfate was added. When albumin polylayers were formed, desorption started in pure buffer.

Furthermore, samples of initial graphene and of that modified by proteins were analyzed by photon correlation spectrometry after purification from free protein that was performed by centrifugation of the system then supernatant was carefully collected followed by the addition of pure PBS and further ultrasonication. The purification procedure was controlled by radioactivity measuring and it was continued until radioactivity of supernatant archived a background value.

It was found that graphene modified by lysozyme was identical with the initial. In both cases, average size of the particles was *ca*. 1.3×10^4 nm on a background of high polydespersity. In case of albumin modification (Fig. 4), the suspension contains particles with average size 556 nm (88% intensity).

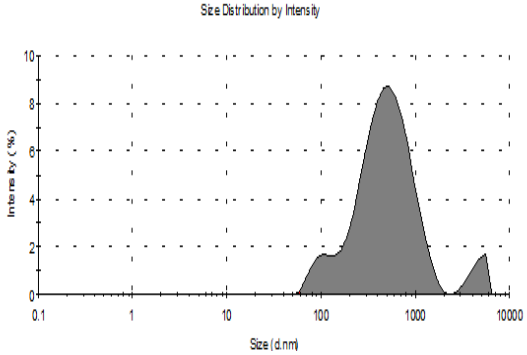

Fig. 4. Particle size distribution of graphene-albumin associates determined by photon correlation spectrometry

CONCLUSIONS

In present research we have shown that adsorption of biomacromolecules at hydrophobic/hydrophilic interfaces included aqueous/solids and aqueous/oil can be studied by radiochemical assay where tritium is used as a tracer. The adsorption process is hydrophobic interaction controlled and its mechanism is under high influence of the structural peculiarities and stability of sorbate molecules.

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REFERENCES

1. *Chen R.J., Choi H.Ch., Bangsaruntip S. et al.* An investigation of the mechanisms of electronic sensing of protein adsorption on carbon nanotube devices // J. Am. Chem. Soc. – 2004. – V. 126, N 5. – P. 1563–1568.
2. *Valenti L.E., Fiorito P.A, García C.D., Giacomelli C.E.* The adsorption–desorption process of bovine serum albumin on carbon nanotubes // J. Colloid Interface Sci. – 2007. – V. 307. – P. 349–356.
3. *Karajanagi S.S., Yang H., Asuri P. et al.* Protein–assisted solubilization of single–walled carbon nanotubes // Langmuir. – 2006. – V. 22. – P. 1392–1395.
4. *Freer E.M., Yim K.S., Fuller G.G., Radke C.J.* Interfacial rheology of globular and flexible proteins at the hexadecane/water interface: comparison of shear and dilatation deformation // J. Phys. Chem. B. – 2004. – V. 108. – P. 3835–3844.
5. *Adams S., Higgins A.M., Jones R.A.L.* Surface-mediated folding and misfolding of proteins at lipid/water interfaces // Langmuir. – 2002. – V. 18. – P. 4854–4861.
6. *Gajraj A., Ofoli R.Y.* Quantitative technique for investigating macromolecular adsorption and interactions at the liquid-liquid interface // Langmuir. – 2000. – V. 16. – P. 4279–4285.
7. *Graham D.E., Phillips M.C.J.* Proteins at liquid interfaces // J. Colloid Interface Sci. – 1979. – V. 70. – P. 403–414.

8. *Sengupta T., Razumovsky L, Damodaran S.* Energetics of protein-interface interactions and its effect on protein adsorption // Langmuir. – 1999. – V. 15. – P. 6991–7001.
9. *Lukashina E.V., Badun G.A., Chulichkov A.L.* Atomic tritium as an instrument for study of protein behavior at the air–water interface // Biomol. Eng. – 2007. – V. 24, N 1. – P. 125–129.
10. *Badun G.A., Soboleva O.A., Chernysheva M.G.* Surfactant adsorption at the water-p-xylene interface as studied by the scintillation phase method // Mendeleev Commun. – 2007. – V. 17, N 6. – P. 357–358.
11. Chernysheva M.G., Soboleva O.A., Badun G.A. Adsorption of surfactant mixture at water – p-xylene interface as studied by scintillation phase technique // Mendeleev Commun. – 2008. – V. 18, N 6. – P. 345–346.
12. *Chernysheva M.G., Tyasto Z.A., Badun G.A.* Scintillation phase method: A new approach for studying surfactant behavior at liquid/liquid interface // J. Radioanal. Nucl. Chem. – 2009. – V. 280, N 2. – P. 303–306.
13. *Badun G.A., Chernysheva M.G., Tyasto Z.A., Fedoseev V.M.* Liquid scintillation spectrometry of tritium in the investigation of compound adsorption at a water/nonpolar liquid interface // Moscow Univ. Chem. Bull. – 2009. – V. 64, N 5. – P. 282–288.
14. *Shishkov A.V., Filatov E.S., Simonov E.F. et al.* Preparation of tritium-labeled biologically active compounds // Dokl. Akad. Nauk SSSR. – 1976. – V. 228. – P. 1237–1239..
15. *Chernysheva M.G., Badun G.A., Tyasto Z.A. et al.* Nonequilibrium processes in reactions of hot tritium atoms with cooled solid targets. Influence of the atomizer temperature on formation of labeled substances // Radiochemistry. – 2007. – V. 49, N 2. – P. 186 – 189.
16. *Badun G.A., Chernysheva M.G., Tyasto Z.A. et al.* A new technique for tritium labeling of humic substances // Radiochim. Acta. – 2010. – V. 98, N 3. – P. 161–166.
17. *Severin A.V., Badun G.A., Tyasto Z.A.* Radionuclide sorption diagnostics of nanoagglomerates and textures based on them // Radiochemistry. – 2009. – V. 51, N 1. – P. 55–58.
18. *Badun G.A., Chernysheva M.G.* How to increase labeling efficiency by tritium thermal activation method? // VIII Finnish-Russian Symp. on Radiochemistry (4–5 Sept. 2009, Turku, Finland). – P. 96–97.
19. *Chernysheva M., Badun G.* In vitro study of proteins surface activity by tritium probe // 16 Radiochemical Conf. (18–23 Apr. 2010, Marianske Lazne, Czech Republic). – P. 217.

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Вивчення сорбції біополімерів на гідрофобних поверхнях за допомогою радіохімічних методів

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Метод радіоактивних індикаторів був застосований для дослідження поведінки глобулярних білків (лізоцим, сироватковий альбумін людини) та гумінових кислот вугілля (Powhumus) в системах вода/олія та вода/графен. Мічені тритієм біополімери були одержані методом термічної активації тритію. Ізотерми адсорбції біологічних макромолекул на межі поділу вода/графен і на міжфазній границі вода/олія були отримані за допомогою відповідно рідинно-сцинтиляційної спектрометрії тритію в традиційному варіанті та методом сцинтилюючої фази.

Изучение сорбции биополимеров на гидрофобных поверхностях с помощью радиохимических методов

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Метод радиоактивных индикаторов был применен для исследования поведения глобулярных белков (лизоцим, сывороточный альбумин человека) и гуминовых кислот угля (Powhumus) в системах вода/масло и вода/графен. Используемые меченые тритием биополимеры были получены методом термической активации трития. Изотермы адсорбции биологических макромолекул на поверхности раздела вода/графен и межфазной границе вода/масло были найдены с помощью жидкостной сцинтилляционной спектрометрии трития в традиционном варианте и в варианте метода сцинтиллирующей фазы соответственно.