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Calorimetric study of adsorption of human serum albumin onto silica powders

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ABSTRACT

Silica materials with different surface functionalities were synthesized by sol–gel method. The isothermal enthalpies of adsorption of human serum albumin ($\Delta_{ads}H^{HSA}$) onto the silica surfaces from buffer solution (pH = 7.4) were measured at 298.15 K. The values of $\Delta_{ads}H^{HSA}$ were found to be endothermic. Possible contributions to $\Delta_{ads}H^{HSA}$ are discussed.

1. Introduction

Protein adsorption onto solid adsorbents plays an important role in a wide variety of applications in medicine, pharmacology, biotechnology, and cosmetic industry. The practical importance of the problem is related to the fact that protein adsorption is the first step in the acute biological response to materials that dictates biocompatibility, and hence utility in medical-device applications. However this phenomenon can induce undesirable effects, e.g., fouling of contact lenses. Whether the protein adsorption is desirable or not, knowledge of mechanisms which govern the adsorption is required to control the interactions between proteins and the surfaces. For solution of this problem it is important to know what amount of protein is able to adsorb on given adsorbent and what is the energy of interaction between the protein and the adsorbent surface. It is well known that the adsorption energy depends on various factors: specific and nonspecific interactions between the protein and the surface, hydration state of the adsorbent and protein surfaces, conformational changes of the protein, temperature, etc. Quantitative information about heat effect of protein adsorption can be obtained by calorimetric method. The sign and magnitude of the adsorption enthalpy are governed by a competition between the above mentioned processes. It has been found that adsorption of human plasma albumin (HPA) and milk R-lactalbumin on colloidal AgI [1] as well as α-amylase and lysozyme on some hydrophobic adsorbents [2] is accompanied by exothermic effects. In many cases, the process is endothermic. This was observed for adsorption of native and hydrophobized human IgG onto silica [3], BSA and lysozyme on hydroxyapatites [4] even if the protein molecules and the surface were opposite.

In this work, the adsorption of human serum albumin (HSA) onto silica materials with different surface functionalities was investigated by calorimetric method. Human serum albumin was chosen because it is the most abundant protein in human plasma and because it has been well characterized in crystal state and in solution [5–8].

The silica materials were synthesized by sol–gel method using tetraethoxysilane (TEOS) as precoursor. Polyethyleneimine (PEI), 3-(aminoporopyl) triethoxysilane (APTES), methyltriethoxysilane (MTEOS) serve as modifiers:

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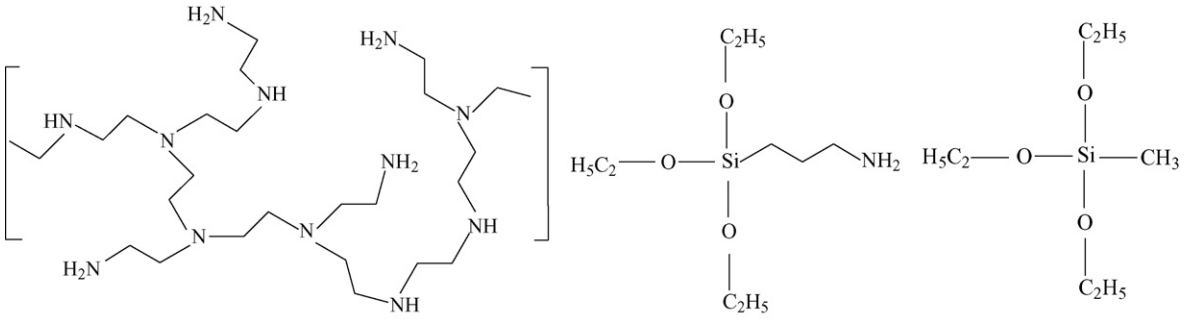

PEI APTES MTEOS

Introduction of different chemical functionalities into silica network creates chemical patterns on surfaces that can favor or reduce in interactions between surfaces and the protein. The aim of this work to obtain enthalpy change during the protein adsorption on silica surfaces functionalized by various chemical groups and to gain insight into the binding mechanism of the protein adsorption onto silica materials.

2. Experimental

2.1. Materials

Tetraethoxysilane (TEOS, high purity grade, Russia), polyethyleneimine (Aldrich, Mw = 25,000), (3-aminopropyl) triethoxysilane (Aldrich, 99%), methyltriethoxysilane (MTEOS) (Acros, 98+%), ethanol (96 wt%), diethylamine (Merck, >90%), human serum albumin (HSA) (Fluka, >95%) were used without further purification. Sodium hydrogen phosphate and sodium dihydrogen phosphate (Russia, analytical grade) were used to prepare buffer solutions on the basis of doubly distilled deionized water (pH = 7.4).

2.2. Syntheses

2.2.1. Sol–gel synthesis of unmodified silica powder

Tetraethoxysilane (TEOS) (10 ml) was dissolved by water–ethanol mixture and then diethylamine (2 ml) was added. The molar ratio of TEOS to water was 2.5. The resulting mixture was stirred for 24 h at room temperature. The obtained product was washed with distilled water and centrifuged four times. The powder was dried at 80 °C for several days.

2.2.2. Sol–gel synthesis of PEI-, APTES- and methyl-modified silica powders

To a solution of tetraethoxysilane (TEOS) (10 ml) in ethanol, an aqueous solution of polyethyleneimine (PEI) (3 ml/10 ml) was added. The APTES- and methyl-modified silica products were obtained by co-hydrolysis of TEOS and APTES and MTEOS, respectively. The hydrolysate composition was 3: 1 (v/v) (TEOS:APTES or MTEOS). Then the procedure was as described above.

2.2.3. Adsorption of human serum albumin onto the silica materials

Adsorption of HSA onto the silica materials was carried out in buffer solution (pH = 7.4). Suspension of the silica particles in the buffer was mixed with a protein solution in buffer. The mixture was gently stirred during several hours. Then the mixture was dried in warm air flow (~30 °C) to remove the solvent. The obtained powder was washed three times with the buffer to remove free protein and dried in the same conditions.

2.2.4. Brunauer–Emmett–Teller (BET) surface area measurements

The surface area and porosity determinations of the silica materials were carried out using Micromeritics ASAP 2010 analyzer. The amount of N2 gas adsorbed at various partial pressures ($0 < P/P_0 < 1.0$) served to determine the BET surface areas.

2.3. Methods of investigation

2.3.1. Calorimetric measurements

The adsorption enthalpies of human serum albumin were measured with an isoperibol calorimeter at 298.15 [9]. The calorimetric cell was filled with the buffer solution (pH = 7.4) or suspension of silica powder (0.2 g) in the buffer solution. The amount of sample of the protein in glass ampoule was ≅0.040 g. The ampoule breaking-heat effect was negligible. The uncertainty in the experimentally measured heat effects was estimated to be less than 2%.

The enthalpy of solution of the protein in suspension of silica powder in buffer can be considered as

$$\Delta_{sol}H^{HSA}_{buf+p} = \Delta_{sol}H^{HSA}_{buf} + \Delta_{ads}H^{HSA} \cdot \alpha, \quad (1)$$

$$\alpha = q'/q$$

where $\Delta_{sol}H^{HSA}_{buf}$ and $\Delta_{sol}H^{HSA}_{buf+p}$ are the enthalpies of solution of HSA in buffer and in suspension of silica powder in buffer (per gram of dissolved protein), respectively; $\Delta_{ads}H^{HSA}$ is the enthalpy of HSA adsorption onto silica powder (per gram of adsorbed protein); q and q' are the amounts of dissolved and adsorbed protein, respectively. The amounts of the adsorbed protein were determined by UV absorption method. The values of $\Delta_{sol}H^{HSA}_{buf+p}$ and $\Delta_{ads}H^{HSA}$ for the studied systems as well as α values are presented in Table 1.

2.3.2. UV-absorption measurements

The amount of the adsorbed protein was evaluated by measuring the bulk protein concentration before and after adsorption using a UV absorption band at 278 nm. The UV absorption was measured with a spectrophotometer Agilent 8453. To determine the protein concentration after adsorption, the suspension after calorimetric measurement was centrifuged at 10,000 rpm for 15 min and the supernatant was analyzed by UV absorption.

2.3.3. IR spectroscopy

Identification of the obtained silica materials was carried out by IR spectroscopy. IR spectra were recorded using a Avatar 360 FT-IR ESP spectrometer at room temperature. The spectra were recorded in the range of 4000–400 cm^{-1}. The samples were examined as KBr disks.

Table 1
Enthalpies of solution of HAS (J/g of the dissolved protein) in suspensions of silica powders in the buffer and enthalpies of adsorption of HSA (J/g of the adsorbed protein) onto the silica materials in buffer.

Sample	Unmodified silica	Methyl-modified silica	APTES-modified silica	PEI-modified silica
$\Delta_{sol}H^{HSA}_{buf+p}$	−35.3.0 ± 0.3	−36.4 ± 0.3	−36.9 ± 0.3	−37.9 ± 0.3
$\Delta_{ads}H^{HSA}$	47.0 ± 2.7	36.6 ± 2.5	34.3 ± 2.6	26.0 ± 2.5

2.3.4. Elemental analysis

Samples of the synthesized silica materials were investigated by elemental analysis for carbon, hydrogen, nitrogen and oxygen content.

3. Results and discussion

Fig. 1 shows IR spectra of the synthesized powders. As it can be seen from the figure, new peaks at 2966, 2854, 1558 and 1474 cm^{-1} appear in the spectrum of the PEI-modified silica in comparison with the unmodified silica. In the spectrum of the APTES-modified silica new peaks at 2932, 1553 and 1467 cm^{-1} are observed. According to literature data [10,11], the peaks in 3000–2800 cm^{-1} region can be assigned to symmetrical and asymmetrical C–H stretching modes (ν_s и ν_{as}) of CH_2 and CH_3 groups. The peaks at 1474 cm^{-1} and 1467 cm^{-1} are associated with asymmetrical C–H deformation vibrations (δ_{as}) of the alkyl groups. New peaks at 1558 and 1553 cm^{-1} are assigned to deformation vibrations of amino groups [10,12]. These results testify qualitatively about the modification of the silica materials. The IR spectrum of the methyl-modified silica powder indicates on introduction of CH_3 groups in the silica network. Pronounced peaks at 2972 and 1410 cm^{-1} are associated with asymmetrical and symmetrical C–H stretching vibration of CH_2 and CH_3 groups. A strong peak at 1275 cm^{-1} can be attributed to symmetric C–H bending vibrations of methyl groups [13,14]. The appearance of additional peaks at 1124 cm^{-1} in the Si–O–Si stretching region (1200–1000 cm^{-1}) indicates that the organic groups have been introduced into the silica network via nonhydrolyzable Si–C covalent bonds [14].

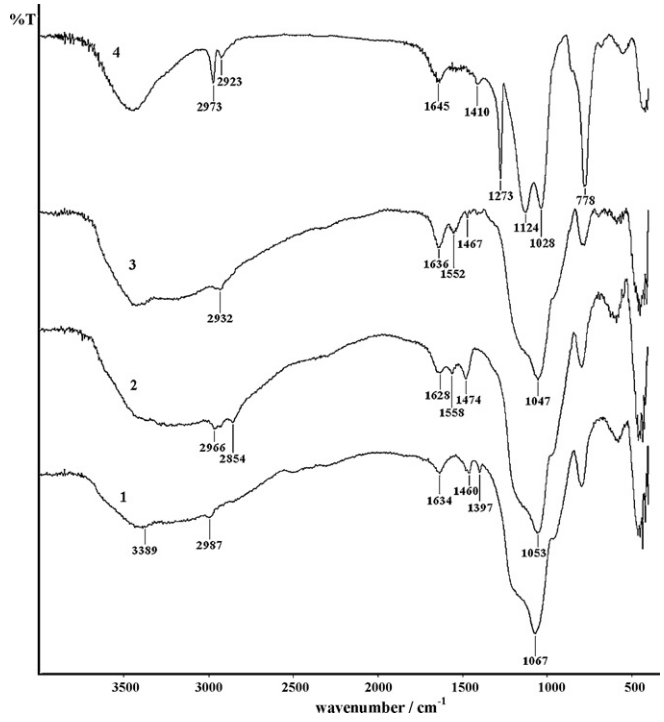

Fig. 1. IR spectra of the powders of unmodified silica (1), PEI-modified silica (2), APTES-modified silica (3), methyl-modified silica (4).

In the calorimetric experiment the solution process of the protein sample is conjugated with partial adsorption of protein onto the silica surfaces. As can be seen from the data presented in Table 1, the values of enthalpies of solution of HSA in suspensions of the silica powders in buffer are exothermic. The enthalpy of solution of the protein sample in buffer ($\Delta_{sol}H^{HSA}_{buf}$) is $-41.6 \pm 0.3\,J\,g^{-1}$. Addition of the silica powders results in decrease of the exothermicity of the process. The highest exothermic effect is observed in the suspension of PEI-modified silica and the lowest one in the suspension of APTES-modified silica particles.

Adsorption of protein at solid interfaces is controlled by the properties of the surface (the most important are charge and hydrophobicity) as well as by the properties of the protein itself (amino acid composition, structure, conformational stability, charge, the extent of polarity and hydrophobicity, etc.). These properties have influence on hydration (solvation) states of the interface and protein. Consequently, the chemical modification of the silica surfaces should have an effect on the energy of interactions between the protein and the surfaces [15].

As has been mentioned above, the adsorption enthalpies are the sums of heats generated by several subprocesses such as: dehydration (desolvation) of the protein and silica surfaces and rearrangements of the solvent structure, specific and nonspecific interactions between protein and silica surfaces, rearrangements within the protein structure. The heat effects of HSA adsorption onto the silica surfaces have been measured in buffer solution at pH = 7.4. At these conditions the protein molecules are negatively charged (pI_{HSA} = 4.7–4.9 [16,17]). The surface amino groups of APTES- and PEI-modified silica materials have positive charge due to their protonation. The adsorption of HSA onto the indicated silica materials could be driven by an electrostatic attractive force, accompanying negative (exothermic) $\Delta_{ads}H^{HSA}$ values. However all the $\Delta_{ads}H^{HSA}$ values presented in Table 1 are endothermic, that is, the protein adsorption is entropy driven ($\Delta_{ads}S > 0$).

Unfavorable enthalpic contribution to $\Delta_{ads}H^{HSA}$ may be associated with changes in hydration sate of the sorbents and protein surfaces upon adsorption. Various studies indicate that dehydration can strongly contribute to the overall enthalpy of adsorption [3,18,19]. The contribution of dehydration originates from removal of water molecules surrounding surfaces of the sorbent and protein molecules and their rearrangement.

Structural changes of protein molecules upon adsorption also may lead to endothermic contribution to $\Delta_{ads}H^{HSA}$ values. The enthalpy change in the protein unfolding process is, usually, large and endothermic due to losses of favorable intramolecular interactions (e.g., van der Waals and hydrogen bonds). According to literature data [20], the contribution of structural changes of bovine milk α-lactalbumin on negatively charged polystyrene (at repulsive interaction between the surface and protein) is 215 kJ/mol.

In addition lateral repulsive interactions between negatively charged protein molecules in monolayer are also accompanied by endothermic effect.

The indicated contributions have influence to a different degree on $\Delta_{ads}H^{HSA}$ values presented in Table 1.

The highest endothermic effect is observed at adsorption of the protein onto unmodified silica material. The unmodified silica sur-

Table 2
Specific surface areas ($m^2\,g^{-1}$)of the synthesized silica materials and enthalpies of HSA adsorption ($J\,m^{-2}$) onto the silica materials.

Sample	Unmodified silica	Methyl-modified silica	APTES-modified silica	PEI-modified silica
S BET	108	811	85	12
$\Delta_{ads}H^{HSA}$	2.17	0.22	2.02	10.83

face is negatively charged at pH-7.4 [21,22]. Thus, the protein is adsorbed under conditions of electrostatic repulsion. The electrostatic repulsive interactions between negatively charged protein and the silica surface may give the substantial endothermic contribution to $\Delta_{ads}H^{HSA}$. HSA is "soft" protein that can change easy its conformation upon adsorption onto hydrophilic surfaces [23]. A higher flexibility in the protein structure may lead to an optimization of the interactions between the proteins and sorbent surface even under unfavorable electrostatic conditions. It is likely that the mentioned above enthalpy change due to the protein unfolding process gives significant unfavorable contribution.

The adsorption of HSA onto PEI-modified silica material is accompanied by the least endothermic effect. Obviously, this is due to electrostatic interactions between protonated amino groups of the silica material and negatively charged HSA molecules. It should be noted that the adsorption onto PEI-modified silica powder is accompanied by a smaller endothermic effect in comparison with APTES-modified silica powder. This may be due to more intensive or stronger interaction between the protein and the PEI-modified silica particles. PEI is a branched polymer containing primary, secondary and tertiary amino groups in its chain. PEI-modified silica material contains a larger quantity of amino groups per gram than APTES-modified silica. This fact is confirmed by calculation on the basis of the data obtained by elemental analysis method. The quantity of amino groups per gram is equivalent to a content of nitrogen per gram and was calculated as

$$N = \frac{\text{content of nitrogen}\,(\%)}{14} \times \frac{1}{100\%}$$

According to elemental analysis data, the contents of nitrogen in APTES- and PEI-modified silica powders are 3.95% and 8.94%, respectively. Thus, the quantities of amino groups per gram are 2.82 and 6.39 mmol/g of APTES- and PEI-modified silica powders, respectively. The larger quantity of binding centers onto PEI-modified silica surface may lead to stronger interaction between the surface and HSA.

Replacement of the surface hydroxyl groups by methyl groups leads to significant decrease of endothermicity of the protein adsorption. It is likely that van der Waals attractive interactions between the hydrophobic surface and hydrophobic patches of the protein molecules play an important role upon the adsorption. HSA is considered to be a hydrophobic protein [23,24] and hence, it is reasonable to think that hydrophobic interactions make substantial contribution to the overall enthalpy of adsorption onto the hydrophobic surface.

Thus, the endothermic effects of HSA adsorption decrease in the following order: unmodified silica > methyl-modified silica > APTES-modified silica > PEI-modified silica.

However, it should be noted that the introduction of different chemical functionalities has influence on surface physical properties of the studied silica materials (specific surface area, pore volume and size as well as pore size distribution). In order to eliminate the influence this factor on the enthalpies of adsorption, their values were calculated in $J\,m^{-2}$. The specific surface areas of the silica materials were measured by N_2 adsorption/desorption method. These values as well as the enthalpies of adsorption (in $J\,m^{-2}$) are presented in Table 2. As can be seen from Table 2, the least endothermic effect is observed at adsorption of HSA onto methyl-modified silica and the largest one onto PEI-modified silica. Thus, according to the data presented in Table 2, HSA exhibits a greater "enthalpic affinity" to hydrophobic surfaces than to hydrophilic ones.

4. Conclusion

The enthalpies of adsorption of human serum albumin onto the synthesized silica materials with different surface functionalities were measured with an isoperibol calorimeter at 298.15. The obtained results showed that the protein adsorption is entropy driven. The unfavorable enthalpy changes result from competition of different subprocesses which occur upon the protein adsorption. The enthalpy of adsorption of HSA ($J\,m^{-2}$) onto hydrophobic silica is the least endothermic.

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