J Therm Anal Calorim (2010) 100:987–991
DOI 10.1007/s10973-009-0604-4

DSC and spectroscopic investigation of human serum albumin adsorbed onto silica nanoparticles functionalized by amino groups

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Received: 1 June 2009 / Accepted: 11 November 2009 / Published online: 2 December 2009
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Abstract Human serum albumin (HSA) adsorbed onto silica nanoparticles modified by 3-aminopropyltriethoxysilane (APTES) and polyethyleneimine (PEI) was investigated by differential scanning calorimetry, IR spectroscopy, and photon correlation spectroscopy. The structural alterations of the protein molecules induced from adsorption process were estimated on the basis of temperatures of denaturation transition (T_d) of the protein in free (native) and adsorbed form. It was found that adsorption of the protein onto the APTES-modified silica nanoparticles results in an increase in the temperature of denaturation transition from 42 to 47.4 °C. HSA adsorbed onto the PEI-modified silica nanoparticles unfolds extensively.

Keywords DSC · Human serum albumin · Modified silica nanoparticles temperature of denaturation transition · Structural alterations

Introduction

One of the most important areas of modern nanotechnology is the development of nanoscale drug delivery systems. Such systems provide easy drug penetration through cell membrane, targeted and dosed delivery of the drug resulting in improvement of therapeutic index, and reduction of side effects of the drug [1–3]. Various inorganic, organic, and hybrid materials have been proposed as drug carriers. Much attention is paid to silica nanocarriers [4–7]. This is due to specific properties of the nanoscale silica providing its functionality in biological applications (toxicological and biological inertness, mechanical photochemical, thermal stability, possibility of variation of the particle size, modification of their surface, etc.). This study is aimed to the development of nanocarrier on the basis of silica for drug of protein nature. As the drug is expensive, human serum albumin (HSA) serves as a model compound of the drug.

Upon adsorption onto nanocarrier surface, the protein drug should not undergo significant structural alterations. Otherwise, the drug may lose its pharmacological activity. It is known that the adsorption-induced changes in protein structure depend on both physical and chemical properties of the protein and sorbent surface. The curvature of the nanoparticles influences the degree of conformational change induced in the secondary structure [8, 9]. Particles with 15-nm diameter cause a 6-fold higher change in secondary structure of human carbonic anhydrase than 6-nm particles [8]. A greater loss of α-helicity was observed for the lysozyme adsorbed onto larger nanoparticles than on smaller ones [9]. A larger number of particle–protein contacts take place, which result in larger perturbations of the protein's secondary structure upon interaction. Nanoparticle shape has also been found to influence protein activity [10, 11]. The previously reported results [11] show that electrocatalytic activity of cytochrome c adsorbed onto gold nanoparticles is different for the particles of different shape.

Various studies have shown that larger structural changes are induced upon adsorption onto hydrophobic sorbent surfaces than to hydrophilic surfaces [12–16]. Studies concerning protein adsorption onto hydrophilic surfaces emphases on crucial role of electrostatic interactions between the protein and sorbent surface. Strong electrostatic attraction between opposite charged protein molecule

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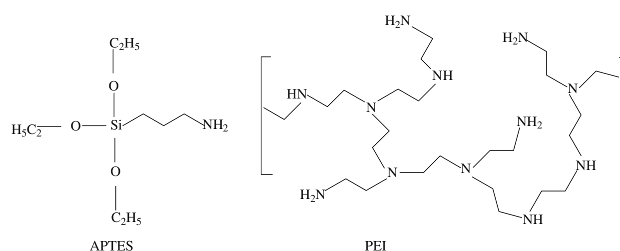
and solid surface often leads to more extensive structural changes in the protein structure [17, 18]. Molecular dynamics simulations also predict that the shape of cytochrome c molecules is less spherical on the hydrophilic surface due to polar interactions between the protein and the surface [19]. However, as has been mentioned above, conformational changes also depend on the intrinsic properties of the protein. With respect to interactions with hydrophilic surfaces, proteins are determined as structurally stable, "hard," and labile, "soft" [20]. HSA is a "soft" protein [20, 21], which undergoes conformational changes more easy upon adsorption due to larger flexibility of its molecules in comparison with "hard" proteins. All these results indicate that the changes in protein structure depend on the adsorbing surface and the protein.

A powerful tool for performing thermodynamic investigations of adsorption-induced changes in protein structure is differential scanning calorimetry (DSC) [16, 18, 22–25]. As has been indicated in the study [25], the basic idea of the DSC studies is that thermal denaturation will not be observed if the protein has been denatured upon adsorption/desorption. Therefore, the adsorbed protein is considered totally or partly denatured if the expected melting transition of the protein is not observed or reduced. It is assumed that thermal denaturation of the adsorbed molecules leads to similar denatured state as in solution. One of the parameters characterizing adsorption-induced conformational changes of protein molecule is temperature of denaturation transition (T_d). It is defined as the temperature at which a local maximum occurs in the DSC curve. Interactions between protein molecule and sorbent surface may result in a shift of the T_d. That is why a monitoring the denaturation temperature of a protein in the adsorbed state gives information on protein–surface interactions and the influence of adsorption on the protein's conformational stability.

According to the literature data, the adsorption-induced structural changes lead to both an decrease [18, 22] and an increase [16, 23] in the denaturation temperature of protein as compared to that of the protein in solution. A higher T_d for adsorbed protein implies that the adsorption-induced perturbed state has a higher thermal stability than the native state.

Taking into account the above- indicated facts, in this study, silica nanoparticles functionalized by amino groups were investigated as nanocarriers for the HSA. The modified silica nanoparticles were prepared by the sol–gel technique. (3-Aminopropyl)-triethoxysilane (APTES) and polyethyleneimine (PEI) serve as modifying agents. APTES contains only primary amino groups. PEI is a branched chain polymer possessing primary, secondary, and tertiary amines in the ratio of 35:35:30 [24]:



Thermal denaturation of HSA adsorbed onto the APTES- and PEI-modified silica nanoparticles was studied by DSC method to determine whether the protein undergoes significant structural alterations upon adsorption onto the modified silica nanocarriers.

Experimental

Materials

Tetraethoxysilane (TEOS, high purity grade, Russia), PEI (Aldrich, Mw = 25,000), APTES (Aldrich, 99%), ethanol (96% wt), and HSA (Sigma, 97–99%) were used without further purification. Sodium hydrogen phosphate and sodium dihydrogen phosphate (Sigma) were used to prepare buffer solutions on the basis of doubly distilled deionized water (pH = 7.4).

Synthesis

The modified silica nanoparticles were synthesized by sol–gel method in ethanol–water medium.

The amino groups were introduced in the silica network by adding the agents (APTES and PEI) during the TEOS hydrolysis (TEOS:agent = 3:1 v/v). The mixture was stirred at room temperature for 24 h. White suspension was dried in warm air flow for several days. The product was white powder.

Adsorption of HSA onto the APTES- and PEI-modified silica was carried out in buffer solution (pH = 7.4). Suspension of the modified silica particles in the buffer was mixed with a protein solution in buffer. The mixture was gently stirred for 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 under the same conditions.

Methods of investigation

DSC measurements were performed using DSC 204 F1 (Netzsch). The aluminum sample container was filled with 5–7 mg of the obtained powder. An empty aluminum

container serves as the reference sample. The container was allowed to stabilize at room temperature for 2 h in the DSC instrument prior to the temperature initiation of the scanning experiment over the temperature range from 20 to 160 °C in argon atmosphere. The heating rate was 10 °C/min. The DSC curves were corrected for a baseline obtained for two empty aluminum containers under the same conditions. The DSC measurements of each sample are repeated thrice.

Identification of the obtained powders was carried out by IR spectroscopy. IR spectra were recorded using an Avatar 360 FT-IR ESP spectrometer at room temperature. The spectra were recorded in the range of 400–4,000 cm^{-1}. The samples were examined as KBr disks.

Photon Correlation Spectroscopy (PCS) measurements were carried out using a Photocor instrument (He–Ne laser, 360–680 nm). Computation of the particle size distribution (PSD) and *R* (average hydrodynamic radius, i.e., the particle radius plus the double shear-layer thickness) was performed assuming that the particles were roughly spherical. The measurements were done at 24.0 ± 0.1 °C and at a scattering angle of 90°.

Results and discussion

IR spectroscopic investigation

IR spectra of the APTES- and PEI-modified silica powders are presented in Fig. 1. The IR spectrum of unmodified silica obtained by sol–gel method is also presented here. Comparison of the spectra shows appearance of one new peak at 1,552 cm^{-1} for the APTES-modified silica and two new peaks at 2,854 and 1,558 cm^{-1} for the PEI-modified

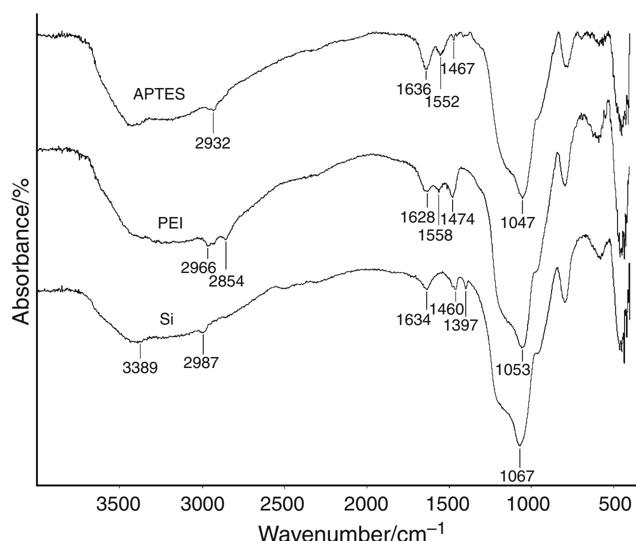


Fig. 1 IR spectra of APTES-, PEI-modified and unmodified silica

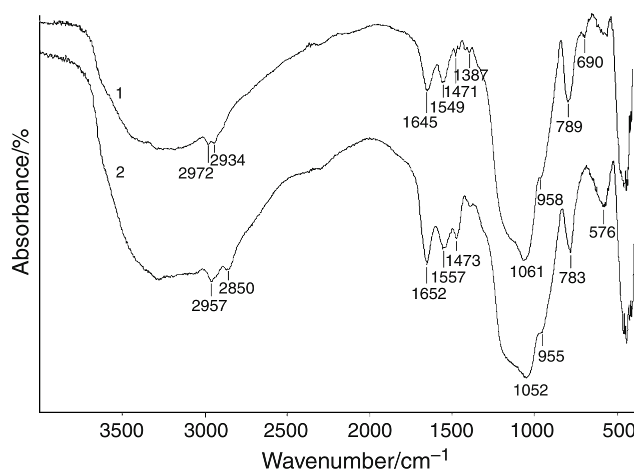


Fig. 2 IR spectra of APTES-modified (*1*) and PEI-modified (*2*) silica after adsorption of HSA

silica. According to the literature data [18], the peak at 2,854 cm^{-1} is assigned to symmetrical stretching vibrations (ν_s) of CH_2 groups. The peaks at 1,552 and 1,558 cm^{-1} are due to deformation vibrations of N–H bond. These results testify about successful modification of the silica. Appearance of the amide I band (1,700–1,600 cm^{-1}) and the amide II band (1,510–1,570 cm^{-1}) [25, 26] in the spectra of the modified silica powders after adsorption of HSA indicates on binding of the protein to the silica surface (see Fig. 2).

PCS results

The average size of the modified silica particles after adsorption of HSA as well as their size distribution was determined by proton correlation spectroscopy. As an example, the data on the APTES-modified silica with adsorbed HSA are presented in Fig. 3. The formed particles are polydisperse with an average radius of 66.6 ± 0.6 nm. For the HSA adsorbed onto PEI-modified silica, the particle average radius is 47.2 ± 0.5 nm.

DSC investigation

Figure 4 shows DSC curves for the APTES- and PEI-modified silica powders after adsorption of HSA. The DSC curves testify about dehydration process of the samples upon heating which attains its maximum at 95.5 °C for the sample with the APTES-modified silica and at 87.4 °C for the PEI-modified silica. Against the dehydration process, the endothermic peak at 47.4 °C is observed on the DSC curve of the sample with the APTES-modified silica. Our DSC measurements indicate that this transition is reversible. The estimated enthalpy value of the transition is 11 J/g. The peak at 47.4 °C cannot be assigned to the APTES-modified silica nanoparticles themselves. As can be seen from Fig. 5, no

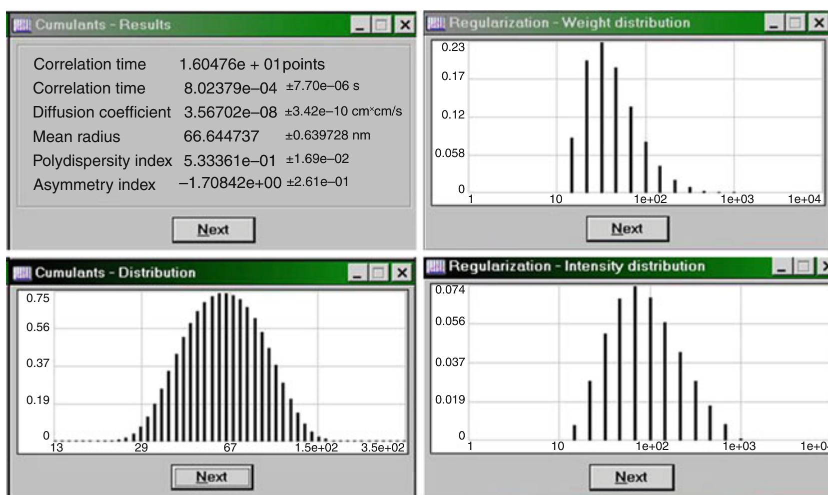


Fig. 3 PCS results of APTES-modified silica after adsorption of HSA

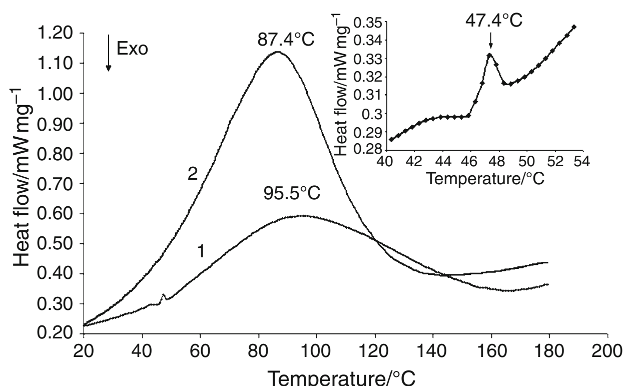


Fig. 4 DSC curves of APTES-modified (*1*) and PEI-modified (*2*) silica powders after adsorption of HSA

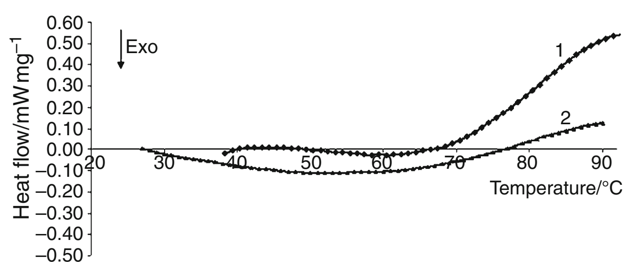


Fig. 5 DSC curves of APTES-modified (*1*) and PEI-modified (*2*) silica powders before adsorption of HSA

endothermic or exothermic transitions over given temperature range are observed for the modified silica powders. The literature data [27–29] indicate that the thermal unfolding of free HSA is a multistep process. According to the results reported in [27], one of the steps of HSA unfolding in buffer solution (pH = 7) was registered at 42 °C by DSC, circular dichroism, and UV spectroscopy. The conformational changes of HSA due to the rise of temperature from 40 to 45 °C are only accompanied by a minor conversion of α-helix to other structures. The fluorescence studies showed that raising the temperature to <50 °C forces to move apart domains I and II of acrylodan-labeled HSA molecule during this stage resulting in some conformational changes in HSA domains structures [28]. Possibly the peak at 47.4 °C on the DSC curve for the APTES-modified silica sample after HSA adsorption is assigned to analogous structural alterations of the adsorbed HSA onto the nanoparticles. Adsorption of the protein onto the APTES-modified silica nanoparticles results in an increase in the temperature of denaturation transition from 42 to 47.4 °C. The T_d shift shows that the protein adsorption promotes heat stabilization of the protein structure, in the other words, increases its resistance to the indicated conformational transition. It should be noted that no analogous transition peak was detected for HSA adsorbed onto the PEI-modified silica nanoparticles. It is likely that the above-mentioned structural alterations has already realized upon adsorption. This finding is not associated with the particle size (see PCS data). This may be due to the protein interactions with the PEI-modified silica surface. As has been mentioned above, PEI is a branched chain polymer which contains a high density of amino groups. The larger amount of amines per gram of the PEI-modified nanoparticles in comparison with the APTES-modified ones results in an extensive interactions of the protein with the polymer-modified surface. The strong multipoint interactions force to unfold the protein molecules allowing internal regions to form additional contacts with the particle surface. Absence of other inherent to HSA transitions in the DSC thermograms of the APTES- and PEI-modified silica nanoparticles may be explained by structural alterations of the protein that had already been released upon adsorption as result of the protein interactions with the substrates.

Conclusions

The obtained results showed that interactions of HSA with numerous binding centers of the PEI-modified surface upon adsorption lead to the loss of the protein native structure. Adsorption of the protein onto the APTES-modified silica nanoparticles results in an increase in the temperature of denaturation transition from 42 to 47.4 °C. Thus, the protein adsorption promotes heat stabilization of the protein structure and increases its resistance to the indicated conformational transition. The APTES-modified silica nanoparticles may be considered as potential nanocarrier for protein drug.

Acknowledgments This study was supported by a grant from Russian Foundation of Basic Research 09-03-97513.

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