Conformational changes of bovine serum albumin upon adsorption on nano-sized thermosensitive magnetic particles

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Introduction

Surface modified magnetic nanocomposites have promising applications in biotechnology; due to its novel physical chemical properties owing to their extremely small size and large specific surface areas[1, 2]. The surface of the magnetic nanoparticles is modified by using stimuli responsive polymeric materials (changes in temperature, pH, ionic strength etc). Stimuli responsive polymer microspheres have many versatile applications in biomedical and biotechnological field such as drug delivery, immobilization of enzyme, cell separation especially, separation and purification of protein[3-7].

Adsorption of protein on solid surfaces induces structural changes which may affect the entire molecule. The study of conformational behavior of protein on solid surfaces is necessary for better understanding of the adsorption mechanism. However, the adsorption of protein on polymer latexes is generally rapid and irreversible process with respect to desorption, and the adsorption depends on hydrophobic and electrostatic interaction as well as external conditions such as pH, temperature, ionic strength and nature of buffer. The protein denaturation may occur under the influence of hydrophobic interaction. The conformational changes of the protein occurs either due to the physicochemical properties of the latex surface or due to the intrinsic properties of the protein[7, 8].

Conformation changes of protein can be characterized and monitored by using variety of different methods such as circular dichroism (CD), differential scanning calorimeter (DSC) and fluorescence spectroscopy. Among those CD spectroscopy is commonly used method for studying protein conformation. It shows the global measure of the secondary structural features present in a molecule. Mcmillin and Walton[9] suggested that this methodology is useful in the investigation of the interaction of protein with polymeric latex. Jansson[10] studied adsorption of protein on silica nanoparticles and the influence of particles curvature on proteins secondary structure using CD. However, so far no work was published on structural change of protein when adsorbed and desorbed from thermosensitive magnetic nanoparticles.

The soft protein bovine serum albumin (BSA) undergoes conformational changes very quickly so it is a good model protein to check the structural changes. In this experiment we studied the conformational change of BSA due to adsorption and desorption by nanosized thermosensitive magnetic particles. Monosodium hydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), sodium dodicyl sulphate (SDS) and Tween 80 was used as Desorption agent. The efficiencies of these desorbents for BSA / particle system were compared.

Experimental

Magnetic fluids were prepared by chemical co-precipitation method under inert environment. A complete precipitation of Fe_30_4 was achieved under alkaline condition and the molar ratio was

maintained at Fe^{2+}: Fe^{3+} = 1:2. In a typical synthesis to obtain 1 g of Fe_30_4 precipitate, 0.86 g of $FeCl_2$. $4H_2O$ and 2.35 g of $FeCl_3$. $6H_2O$ were dissolved under N_2 atmosphere in 40 ml of deaerated Mili-Q water with vigorous stirring at a speed of 1000 rpm[11]. As solution heated to 80°C, 100 mg of thiodiglycolic acid (TDGA) was added, followed by 5 ml NH_4OH. Further, TDGA was added to the suspension in five 0.2 g amounts over 5 minutes. The experiment was continued for 30 minutes at 80°C. The stable water based suspension was then cooled at room temperature and washed using Mili-Q water. The precipitates were isolated from the solvent by magnetic decantation. 4-vinylaniline was used as secondary surfactant. 1 g of the fresh precipitate obtained from the previous preparation was combined with 20 ml mili-Q water and the mixture was heated to about 25°C under vigorous stirring. About 0.2 ml of 4-vinylanile was added drop wise using a syringe. Bilayer coated magnetic particles were first cleaned to eliminate any free electrolyte and adsorbed surfactants. One g of the seed magnetic particles were purged with nitrogen for thirty minutes at a temperature of 70°C , then a mixture of NIPAM (0.3g), MBA (0.02g), KPS (0.005g) was added. Polymerization was carried out for six hours. The polymerization conversion was gravimetrically determined and the final particles were washed at least three times before adsorption.

Adsorptions of BSA (bovine serum albumin) on thermosensitive magnetic particles were carried out by mixing 5 ml of BSA solution of certain concentration and 137 mg of wet solid magnetic particles. The mixture was incubated at 40°C in 10 mM phosphate buffer solutions at 4.7 pH. After 2 hours of incubation the protein adsorption was determined from the supernatant analysis by UV at a wavelength of 280 nm. The solid content of the wet magnetic particles were measured to be 15.59%. Desorption experiments were performed as follows. First, protein adsorbed thermosensitive magnetic particles at 40°C were separated from the medium and redispersed in 0.5M Na_2HPO_4 (pH 9.35), 10 mM phosphate buffer, 0.05M SDS solution and 1×10^{-3} M Tween 80 solution. Then desorption was carried out at 20°C for 17 hours under constant shaking. Finally, the desorbed amount of protein was determined by measuring the free protein concentration in the supernatant.

Circular dichroism (CD) spectroscopy (Jacob J810 spectrometer) was used to measure the conformation change of desorbed BSA with respect to native BSA. Solutions of the native and desorbed BSA were diluted in the range of 0.05-0.1 mg ml^{-1} and scanned over the wavelength range 200-260 nm, using 5 mm quartz cylindrical cell. The secondary structures of native and desorbed BSA were evaluated by comparing the α-helix content, corresponding to the ellipticity of the bands at 208 nm.

Results and Discussions

Adsorption isotherm

Typical adsorption isotherms obtained at 40°C are shown in Figure 1. The experimental results indicate that temperature dependent adsorption of BSA on thermosensitive magnetic particles is mainly attributed to the change in the properties of the particles' surfaces. Although the shapes of the isotherms were Langmuir, the isotherms did not show the initial rapid increase, so the Langmuir isotherm failed the experimental data. Therefore, all isotherms were fitted to the Langmuir-Freundlich isotherms (Eq 1) using nonlinear regression method[12].

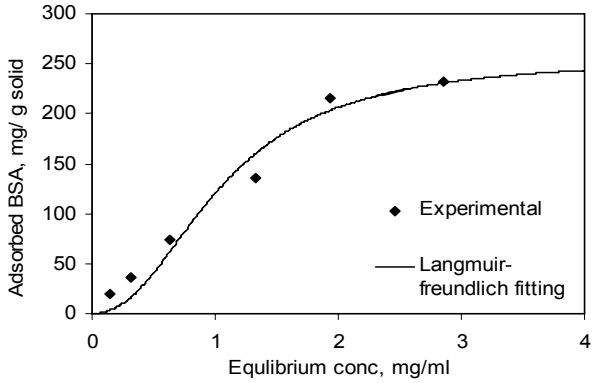

Figure1: Adsorption isotherm of BSA on thermosensitive magnetic particles

(pH 4.7 and .01M ionic strength)

$$C_s = C_m \frac{KC_b^{1/n}}{1+KC_b^{1/n}} \quad (1)$$

Where, C_b (mg/ml) and C_s (mg/ g solid) are BSA concentration in the aqueous solution and the absorbed BSA on the solid at equilibrium, respectively. C_m in the maximum adsorption amount, K is the adsorption constant and n is the exponential factor. The values of n were relatively constant between 0.4 and 0.5, and hence n could be fixed to its average value of 0.45.

Desorption of BSA from thermosensitive nanomagentic particles

The Desorption of BSA from thermosensitive nanomagnetic particles were carried out by 0.5M Na_2HPO_4 (pH 9.35), buffer of same pH (pH 4.7), 0.05M SDS solution and 1×10^{-3} M Tween 80 solution. The adsorbed and desorbed quantities of BSA at equilibrium for feed concentration of 0.503 and 1.0075 mg/ml are shown in Table1.

Table 1: Desorption results of BSA from nanomagnetic particles

Desorption agent	Initial BSA concentration (mg/ ml)	Adsorbed BSA (mg/ g solid)	Desorbed BSA (mg/ g solid)	Desorption percentage (%)
0.5M Na_2HPO_4 (pH 9.35	0.503	36.164	26.497	73.27
	1.01	88.42	64.37	72.80
0.01M NaH_2PO_4 (pH 4.7)	1.01	73.31	15.906	21.69
0.05 M SDS	1.01	92.46	60.85	65.81
1×10^{-3} Tween 80	1.01	89.69	32.08	35.36

From Table 1 we can observe that no significant desorption was measured with the buffer of same pH and Tween 80. On the other hand, high desorption was measured by alkaline solvent (pH 9.35) and SDS solution. Desorption efficiency was about 73 and 65 percent respectively.

Circular dichroism (CD) measurements

The CD spectra of native BSA and desorbed BSA by 0.5M Na_2HPO_4 (pH 9.35), buffer of same pH (pH 4.7), 0.05M SDS solution and 1×10^{-3} M Tween 80 solution are shown in Figure 2. From the figure we can observe that there are two extreme valleys at 208 and 222nm for native bovine serum albumin. However, the CD spectrums of desorbed protein by Na_2HPO_4 and SDS show extreme valleys at 208 and 222nm but at a lower helical structure. That means there is a structural change in the desorbed proteins. On the other hand desorbed BSA by NaH_2PO_4 and Tween 80 show a very different spectrum from the native one. This leads to the conclusion that these desorption agents' caused a large conformational change in BSA.

Protein has different level of structures-such as primary, secondary, tertiary and quaternary structures. α – helix is one of the elements of secondary structure therefore the quantitative analysis of the structural change of BSA could be evaluated by the content of α –helix content preserved. Moreover, the CD spectrum data provide quantitative information about the change in the content in secondary structure. The α –helix content is estimated from the molar ellipticity at 208 nm $[\theta]_{208}$ according to the following equation.

$$\%\ \text{of}\ \alpha\text{-helix} = \frac{[\theta]_{208} - 4000}{33000 - 4000} \times 100\% \tag{9}$$

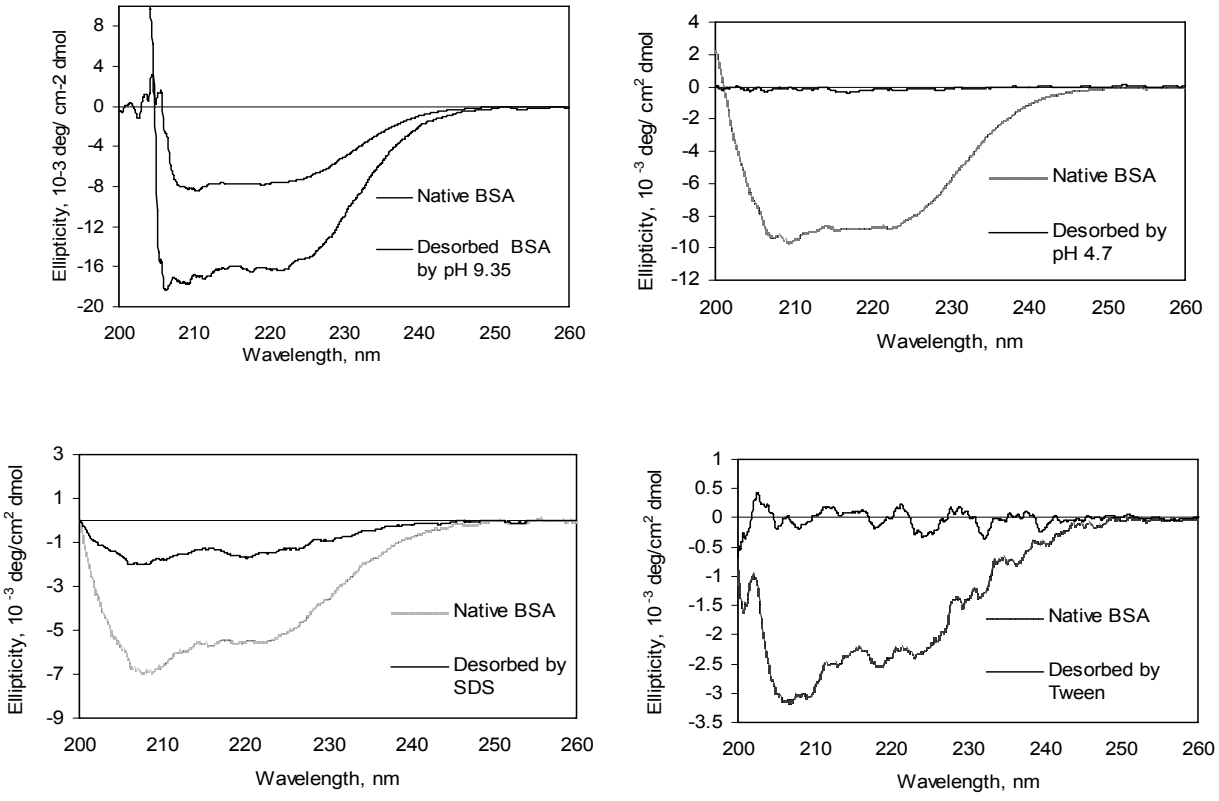

Figure2 Comparism of CD spectra of native BSA and desorbed BSA by different Desorption agent

Table 2: The estimated percentage of α – helix contents from circular dichroism spectrum

% of α – helix							
Native BSA				Desorbed BSA			
Na_2HPO_4 pH 9.35	NaH_2PO_4 pH 4.7	SDS	Tween 80	Na_2HPO_4 pH 9.35	NaH_2PO_4 pH 4.7	SDS	Tween 80
46	17.93	10	4	14	-	7.24	-

Table 2 shows that the native BSA at pH 9.35 has 46% α – helix which is very close to the literature value 48% between pH 8 to 10 [13]. On the other hand, native BSA at pH 4.7, SDS and Tween 80 the percentage of α –helix is very low that means there are a significant structural change in the protein. The desorbed protein by pH 9.35 has only 14% α –helix. This is because soft proteins tend to undergo larger changes in secondary structure upon adsorption on solid surfaces. Moreover, in the case of denaturation by heat, proteins usually completely lose secondary structures. The phenomena could be explained as when the temperature increases the hydrophobicity of the thermosensitive polymer increases so the bonding with the surface and adsorption becomes heavily entropic in nature to cause denaturation of surface. The results show that the use of proper desorption environment can make less structural changes.

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