Adsorption of bovine serum albumin by granulated and fibrous sorbents

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Adsorption of bovine serum albumin by macroporous glasses and fiber sorbents was studied. Differences in the mechanism of protein sorption by sorbents depending on the chemical nature of the surface and the texture of the initial matrix were found. Optimum conditions for protein immobilization on thin-layer sorbents were determined. The reversibility of protein adsorption was studied by the radioactive indicator technique. The extent of reversibility of protein adsorption by thin-layer titanium hydroxide coatings was shown to depend significantly on the texture of the initial matrix.

Key words: adsorption, carbon fiber, macroporous glasses, protein, thin-layer inorganic sorbents, titanium hydroxide.

Concentrating by sorption is widely used in protein chemistry as it makes possible the selective isolation of these substances from complex biochemical systems.[1] However, interaction with the sorbent surface is known to often change the structure of protein molecules and to cause their irreversible sorption.[2] This complicates the use of sorbents in practical protein chemistry. The modification of the chemical nature of the surface, the structure, and the texture of a sorbent can influence protein sorption and conformation. This work deals with study of the sorption of bovine serum albumin (BSA) by macroporous glass and carbon fiber coated with titanium hydroxide and also by the initial silica and carbon matrices. This made it possible to find differences in the character of protein sorption by sorbents with various chemical natures of the surface and textures of the initial matrix.

Experimental

BSA preparation produced by NIIEV (Minsk, Belarus') was used. The purity of the preparation was controlled by disc electrophoresis. Macroporous glass MPS-1000 (NIINGP, Nizhnii Novgorod, Russia) with an average pore size of 1120 Å and a specific surface of 60 $m^2 g^{-1}$ and a carbon fiber bundle (Aktilen, Institute of General and Inorganic Chemistry (IONKh), Minsk, Belarus') with a total pore volume of 0.4 $cm^3 g^{-1}$, a total micropore volume of 0.4 $cm^3 g^{-1}$, and a specific surface of 1000 $m^2 g^{-1}$ were used. Sorbents obtained by coating the initial matrices — MPS-1000 and carbon fiber — with a thin layer of titanium hydroxide[3] were also used. The synthesis conditions made it possible to obtain a porous structure of a thin-layer sorbent for macroporous matrices, which is rather similar to that of the initial matrix. The average pore radius of MPS-1000 is 112 nm, while for MPS-1000 covered with titanium hydroxide this characteristic is 119 nm. At the same time microporous materials change both the fiber structure and the matrix texture during the synthesis. Protein adsorption was studied by the procedure described earlier.[4]

The concentration of BSA was determined according to Lowry procedure[1] and from the difference in absorption at 278

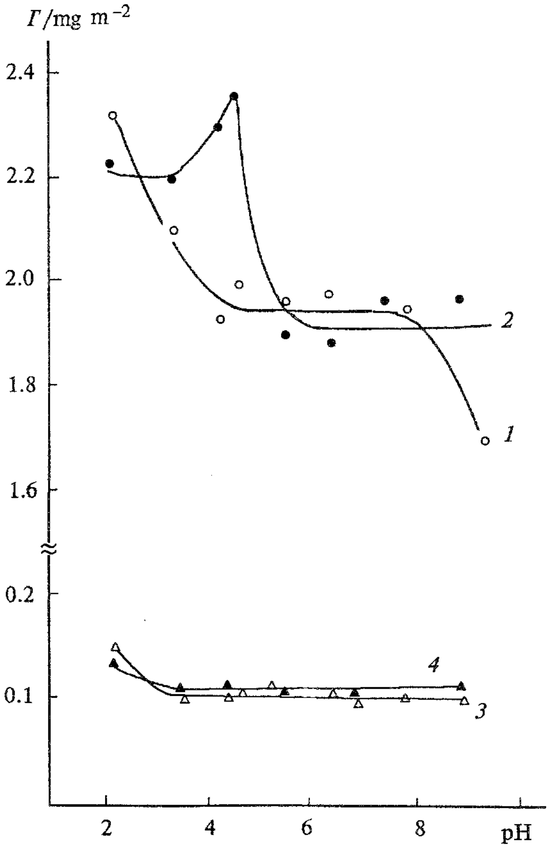

Fig. 1. Dependence of BSA adsorption on pH of the solution for MPS-1000 (*1*), MPS-1000-Ti (*2*), Aktilen (*3*), and Aktilen-Ti (*4*). Analysis of BSA was performed according to Lowry procedure.

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 8, pp. 1586—1589, August, 1995.

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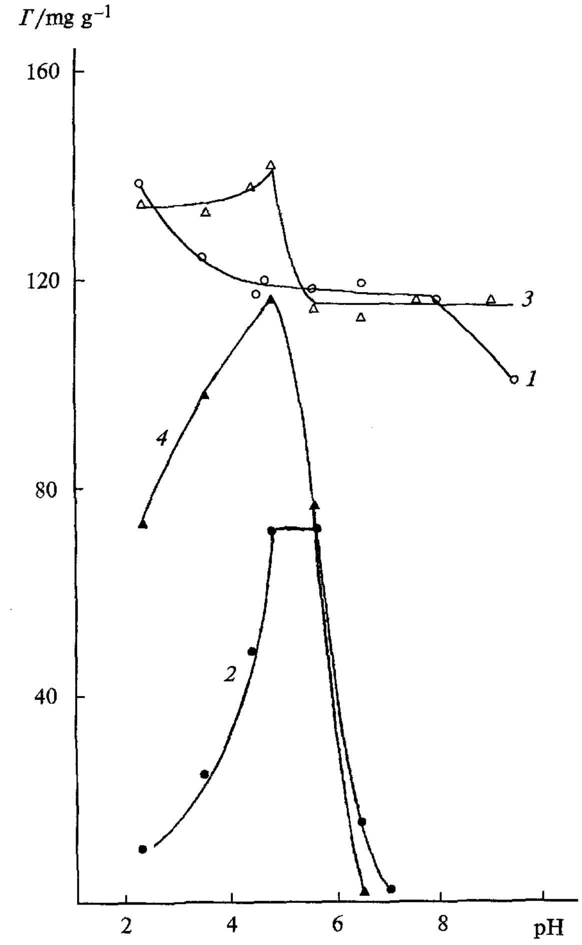

Fig. 2. Dependence of BSA adsorption on pH of the solution for MPS-1000 (*1, 2*) and MPS-1000-Ti (*3, 4*) determined according to Lowry procedure (*1, 3*) and by UV spectroscopy (*2, 4*).

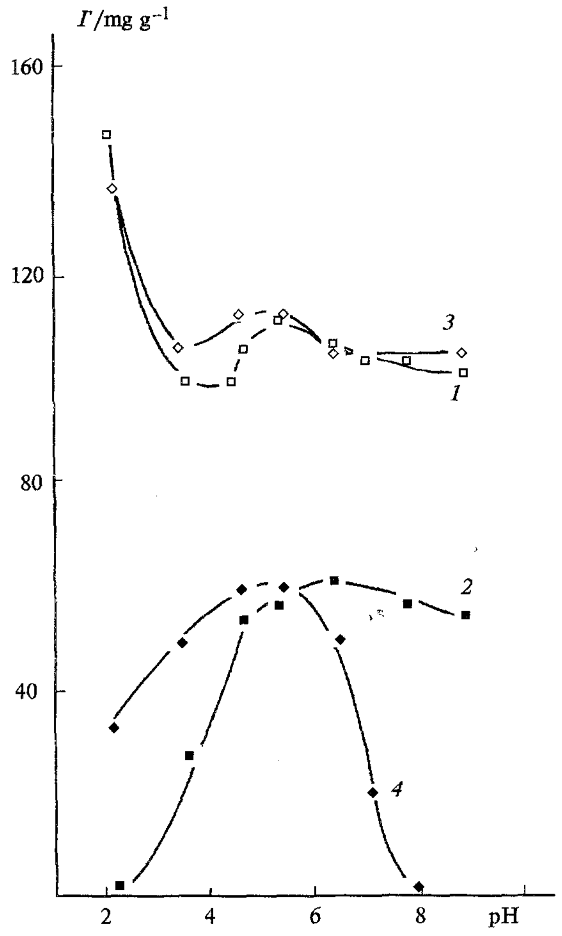

Fig. 3. Dependence of BSA adsorption on pH of the solution for Aktilen (*1, 2*) and Aktilen-Ti (*3, 4*) determined according to Lowry procedure (*1, 3*) and by UV spectroscopy (*2, 4*).

and 330 nm. All measurements were carried out in the universal buffer solution, sodium phosphate—acetate—borate. The reversibility of adsorption was studied using BSA labeled with ^{125}I according to the reported procedure.[5]

Results and Discussion

Figure 1 represents the adsorption of BSA by the sorbents studied as a function of pH. The better adsorption ability of MPS-1000 as compared to that of the carbon fiber is caused by the differences in the porous structure of these materials. The size of Aktilen pores (0.4 nm) hinders the penetration of protein macromolecules in the intraporous space, and the adsorption takes place only on the outer surface of the fiber. This is likely the cause of the absence of any noticeable difference in the amounts of BSA adsorbed by the initial fiber matrix and by that coated with titanium hydroxide. Macroporous sorbents are characterized by some difference in the character of BSA sorption, which is likely connected with the difference in the mechanisms of protein sorption by the initial silica surface and by that covered with titanium hydroxide. The maximum adsorption ability for MPS-1000-Ti is observed in the pH region that accords with the isoelectric point (pI) of BSA.

As can be seen from Figs. 2 and 3, the character of the pH dependence of the BSA sorption varied essentially with the method of deducing the protein concentration. This can be explained by the fact that protein investigation according to Lowry is conformationally independent, whereas in the case of spectrophotometrical method the extinction depends on the protein state.[1,2] BSA denatured (*e.g.*, by heating)[2] has a higher extinction than the native protein. This is caused by the fact that the close surrounding of the protein chromophores in the denatured state differs from that of the native protein, and also by the light scattering by the aggregates of denatured protein. The pronounced denaturation of proteins can take place during their interaction with the surface. The microelectronic photographs of the surfaces with the protein adsorbed show that the protein mol-

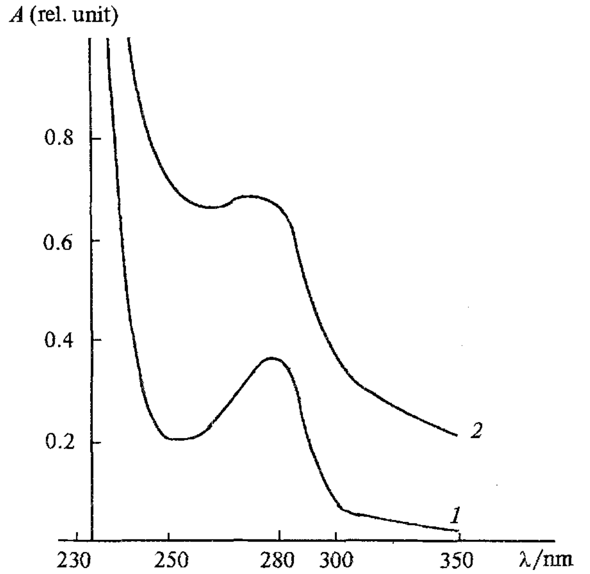

Fig. 4. UV absorption spectra of initial (native) (*1*) and denatured (*2*) protein.

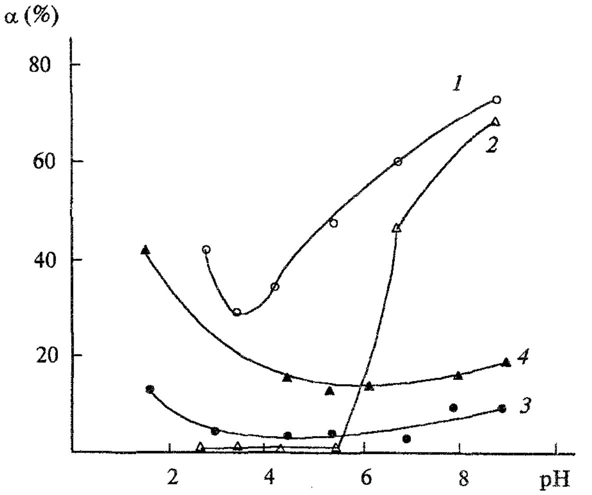

Fig. 5. Dependence of the extent of BSA exchange on the pH of the solution for MPS-1000 (*1*), MPS-1000-Ti (*2*), Aktilen (*3*), and Aktilen-Ti (*4*).

ecules firmly adsorbed are denatured, whereas the molecules adsorbed reversibly have the structures close to those of the native ones[2]. The curves shown on Fig. 4 also prove the dependence of the extinction on the protein state. The difference between the curves *1* and *2* is caused by the presence of the denatured protein in the solution. This is the result of the exchange process involving the BSA molecules being in contact with the sorbent surface. The protein concentration deduced according to Lowry is invariant in this case.

The reversibility of protein adsorption was studied using BSA labeled with ^{125}I. Figure 5 shows that the MPS-1000 surface covered by titanium hydroxide has the maximum irreversibility of BSA adsorption in acidic medium, where the divergence between the sorption values determined according to Lowry and by UV spectroscopy is minimum. It should be mentioned that for the silica surface (see Fig. 5, curve *1*) the exchange process takes place at any pH value. This is caused by differences in the mechanisms of sorption by the silica and titanium hydroxide surfaces. The latter is able to coordinate the protein donor molecules or donor groups due to Ti^{IV} ions.[5] Adsorption in thin-layer titanium hydroxide proceeds with the formation of stable $H_2O—Ti^{IV}_{oct}—NH(Prot)$ complexes, while in the case of the tetrahedral surroundings of silicon hydroxide similar structures are not found. It should also be noted that the reversibility of the sorption by fiber sorbents is dependent on pH decreasing near pI in contrast to the sorption by granulated thin-layer sorbents. The comparison with the thin-layer sorbents on the basis of granulated carbon matrix (activated carbon SKT)[4] allows us to suppose that thin-layer coatings do not stabilize the texture of the carbon fiber material. It can vary during the sorption of protein molecules at the expense of decrease of the cohesion component of intramolecular interactions among individual carbon fibers.

We therefore can conclude that the efficiency of the modification of thin-layer sorbents largely depends on the texture of the matrix, which was coated with titanium hydroxide film. In addition, the data obtained shows the $3 < pH < pI$ interval to be the most preferable for protein immobilization on granulated thin-layer sorbents. The optimum conditions for the immobilization of proteins on thin-layer sorbents on the basis of carbon fibers are appreciably depend on the texture of the fiber material.

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Received November 16, 1994