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Effect of pore size of silica for the adsorption of proteins

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Abstract The effect of pore size for the adsorption of bovine serum albumin (BSA) and bovine serum fibrinogen (FB) in 0.9 % sodium chloride solutions was studied on non-modified silica gels, porous silica glasses and aerosil 300, and these modified with amine and vinyl groups. The adsorption was not reversible. Surface coatings did not influence the amount adsorbed of BSA in sodium chloride solution. The adsorbed amounts of BSA and FB depended upon the porosity of these adsorbents and were largest at pore diameters of 30 and 70 nm respectively.

INTRODUCTION

Adsorption phenomenon of proteins on solid surface of various kinds of adsorbents such as active carbon and metal oxides is and will be widely used for the technology of separation, purification and identification of these materials. This tendency will be accelerated through the study of the mechanism of adsorption interaction between these biopolymers and solid surface. Therefore, further advanced basic studies are demanded for the applications such as medicine, agriculture and food technology.

Although this area of research have been studied to a considerable extent.(ref.1-10), there has been not enough research on the adsorption of proteins on metal oxides compared with that on polymers and active carbons. There are many chemical and physical aspects of adsorption interaction between solid surface and proteins which should be taken into account to control the adsorption. For instance, some studies have been made on the influence of porosity for the adsorption of macromolecules on porous adsorbents such porous glasses-polystylene (ref.8) and active carbons-oligosaccharides and -protein systems.(ref. 9,10). However, porosity dependence of the adsorption of proteins on well-characterized porous oxides still remains to be studied. In this investigation, the adsorption behavior was studied on bovine serum albumin (BSA), which is one of the most popularly studied materials (ref. 4), and bovine serum fibrinogen (FB) on non-porous and porous silicas having various controlled pore diameters such as silica gels, porous silica glasses and aerosils 300. As the result, the adsorbed amount of these proteins is found to depend upon the pore diameters of these porous silicas as is described below.

MATERIALS

Silica Adsorbents Silica gels (designated as S1-S11) were synthesized by the decomposition of sodium silicate and silicon tetraethoxide and hydrothermal treatment under controlled temperature and pH of either hydrogels or xerogels thus obtained in order to control the pore sizes. Porous silica glasses (PG1 and PG2) were made by annealing and acid etching of sodium borosilicate glasses of various chemical compositions. Aerosil 300 (A) were used as received from Japan Aerosil Co.

Table 1 N_2 BET specific surface areas, A_s m^2g^{-1}, pore volumes, V_p cm^3g^{-1}, and pore diameters by mercury porosimetry, D_p nm, of silica gels, porous glasses and aerosil 300 denoted as **S**, **PG** and **A** respectively.

sample	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	PG1	PG2	A
A_s	609	326	224	171	96	67	78	41	56	51	36	29	33	282
V_p	0.77	0.97	1.03	0.97	1.23	1.08	1.06	1.11	1.18	1.12	1.06	0.85	1.26	
D_p	4.0	9.2	12.6	15.1	26.9	43.8	51.7	54.5	62.8	71.1	95.7	104	172	

The BET nitrogen specific surface areas, A_s m^2/g, pore volumes, V_p cm^3/g both by ordinary volumetric method, and pore diameters measured by mercury porosimetry, D_p nm, of these materials are shown in table 1. Looking from some of the pore size distribution curves in Fig. 1, the pore size of each material seems to be quite uniform. The points of zero charge (pzc) of these samples measured by hydrogen ion titration were about 3.0. The surface of these materials were modified with 3-aminopropyltriethoxysilane and vinyltriethoxysilane in toluene solution at 50°C for 24 h in order to make basic and hydrophobic surfaces.

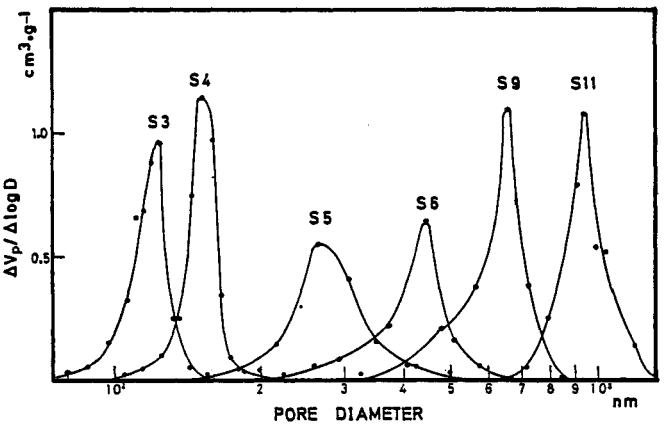

Fig. 1 Pore size distribution curves of silica adsorbents S3, S5, S9 and S11 listed in Table 1 measured by mercury porosimetry.

IR spectroscopic investigation of these materials showed that non-modified samples S1-11 contain various kind of silanol groups in the order of inner OH groups inside closed pores at 3670 cm^{-1} > interparticle hydrogen-bonded OH groups at about 3500 cm^{-1} > surface free OH groups at 3740 cm^{-1} and surface hydrogen-bonded OH groups at about 3500 cm^{-1} as the result of enhanced hydrothermal treatment as is shown in the spectrum of silica gels (full line) in Fig. 2. (ref. 11) Porous silica glasses PG1 and PG2 have free OH groups as well as a fraction of hydrogen-bonded OH groups. Aerosil 300 have mainly free OH groups with a small amount of interparticle hydrogen-bonded OH groups in case of loose packing. The majority of the surface free hydroxyl groups of original materials, which are generally about 3 free silanol groups per nm^2,(ref. 11) were substituted to the surface modifying agents mentioned above and this band has nearly disappeared according to the IR spectrum (broken line) in Fig. 2. Pzc of the sample treated with amino coupling agent was about 8.0. The pretreatment conditions of all these samples were 150°C and 100° in vacuo for the sample with adsorbed protein both under vacuum for 2 h.

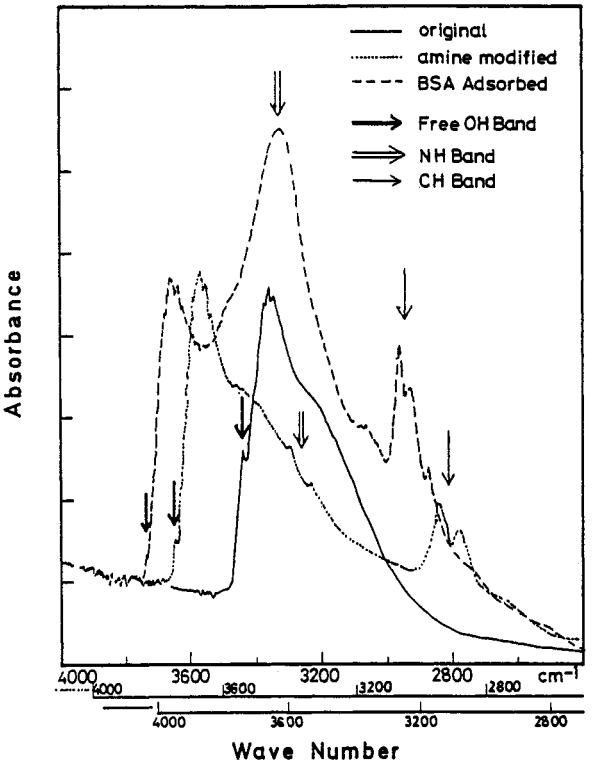

Fig. 2 IR spectra of silica adsorbents without surface modification (full line), modified with amino groups (dotted line) and that after BSA adsorption (broken line).

Bovine Serum Albumin (BSA) 99 % pure BSA of Sigma Co. as received was dissolved in pure water or 0.9 % sodium chloride solution at pH adjusted to 7.0. In both cases, the distribution of its molecular weight was very uniform according to the ultracentrifuge measurement. The molecular weight of BSA measured by this method and gel-filtration chromatogram was 68,000 in pure water and 0.9 % sodium chloride solutions and this value agrees with these in literature.(ref. 4) The molecule has an ellipsoid shape of 4 x 12 nm in a folded form. Its isoelectric point is about 4.7.

About 200 mg of the above-mentioned adsorbents after drying at 150°C were immersed in 5 ml of BSA solutions containing 0.9 % sodium chloride and gases inside pores were replaced to solution under reduced pressure. These suspensions were stirred mildly for 24-96 h at 15°C. Supernatant liquids were removed from the suspensions by centrifuge at 10^4g and were used for quantitative analysis of BSA. The pH of the liquid became approximately 0.2-0.8 lower after equilibrium than before in case of

pure silica samples. The adsorbed amounts of BSA were determined from the intensity changes of 278 nm UV absorption band of BSA in the liquids before and after adsorption equilibrium. Calibration curves for concentration determination was obtained from the standard solution of BSA being commercially available. The desorbed amount of BSA was measured in the same conditions as that of adsorption by immersing samples after removing the solution from the external surface of these samples in equilibrium. No change of turbidity and the shape of UV absorption bands were observed during experiment.

Bovine Serum Fibrinogen (FB) 75 % pure FB was obtained from Katayama Chem. Co. which contained about 25 wt % of citric acid and sodium chloride. Since the stability of this material against coagulation is much lower than that of BSA, the maximum concentration of solution was 0.04 mol/l and all the procedures were made below 4°C. This material was purified by Laki's method (ref. 12) as follows. FB was dissolved in phosphate buffer solution and this solution was obtained after centrifuge. Ammonium sulfate was added to this solution in order to salt out FB from this solution. The residues after mild centrifuge were dissolved in 0.9 sodium chloride solution of pH 7.0 adjusted with ammonia. This solution was dialyzed with continuous flow of 0.9 % NaCl solution for 17 h. The solution after dialysis contained 0.9 % NaCl and its pH was 7.0. The molecular weight of FB was 330.000, and it is considered to have nearly a thin dumbbell shape of maximum size 6.5 x 47.5 nm in a folded form. The isoelectric point was about 5.5.

The same procedure with that of BSA was undertaken for the adsorption of FB at 4°C. Its pH after adsorption was about 6.8-6.2. UV absorption band at 280 nm was used to determine the concentration of FB. The absorbance or extinction coefficient of FB in the solution was 11.0 at the FB concentration of 1 mg cm^{-3} which was obtained gravimetrically.

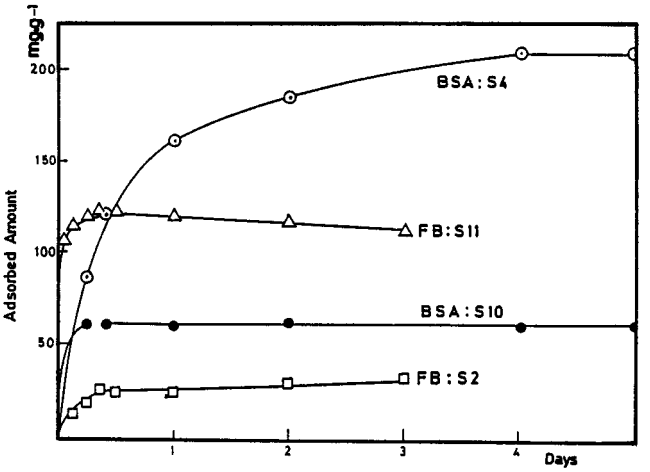

Fig. 3 Adsorbed amount of BSA and FB dissolved in 0.9 % NaCl solution at 288 and 277 K respectively as a function of immersion time.

RESULTS AND DISCUSSION

The rates of adsorption of BSA and FB were relatively rapid in spite of large molecular sizes compared with these of small molecules and ions as are shown in Fig. 3. Generally, adsorption is faster for the samples with smaller and much larger pore sizes relative to the sizes of molecules than these with medium pore sizes. This may perhaps be because of the lack of diffusion or the easy diffusion of these molecules into small and very large pores respectively. Any how, adsorption equilibria of BSA and FB were reached after about 48 and 24 h respectively. The adsorption of FB was faster than that of BSA in spite of the larger molecular weight of the former, perhaps because of very small cross-section area along the longer molecular axis. 2 full days were usually taken for adsorption equilibrium of both materials in this experiment.

Figure 4 shows some of the adsorption isotherms of BSA and FB in 0.9 % NaCl solution at 15° and 4°C respectively on silica gels without surface modification, expressed in the weight of adsorbates per unit surface area of the adsorbents. The shapes of these curves belong to that of type I of ordinary solid/liquid interface adsorption. The de-

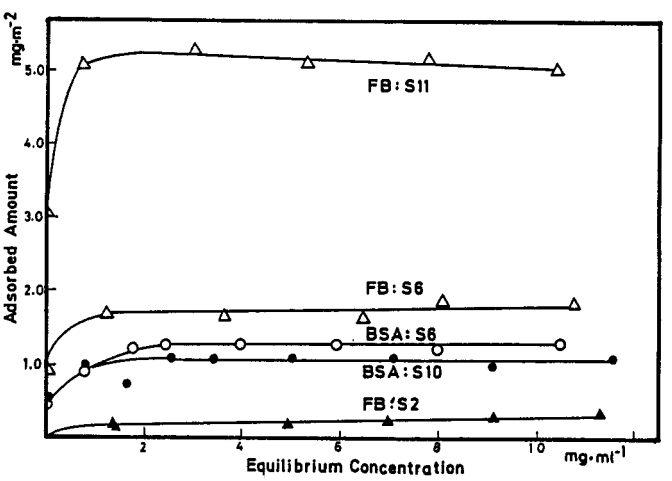

Fig. 4 Adsorption isotherms of BSA and FB dissolved in 0.9 % NaCl solution.

sorbed amount of BSA under the same condition with that of adsorption was almost equal to the amount of BSA which remained in the immersion solution inside pores without adsorbed. Therefore, the adsorption of BSA seems to be almost irreversible. However, about 80 % of BSA adsorbed on silica surface was desorbed from a dilute hydrochloric acid solution of pH 2.

There is a significant difference of the adsorbed amount of BSA and FB per unit area depending upon the pore size of the adsorbent. Fig. 5 illustrates the relation between the pore diameter of these adsorbents without surface modification and the amount of adsorbed BSA and FB per unit weight in 0.9 % NaCl solution. The adsorbed amounts of these materials were very small for the materials with small pores. This result is easily interpreted as that these molecules were difficult to enter into pores smaller than their molecular sizes. Adsorbed amount increased rapidly as the pore size became larger. There are clear maxima of adsorbed amounts of BSA and FB at the pore diameter of about 10 and 70 nm respectively. The adsorbed amounts then drop quickly. These unexpectedly sharp maxima are due to the combined effect of pore size and surface area, which may be interesting from the practical purposes.

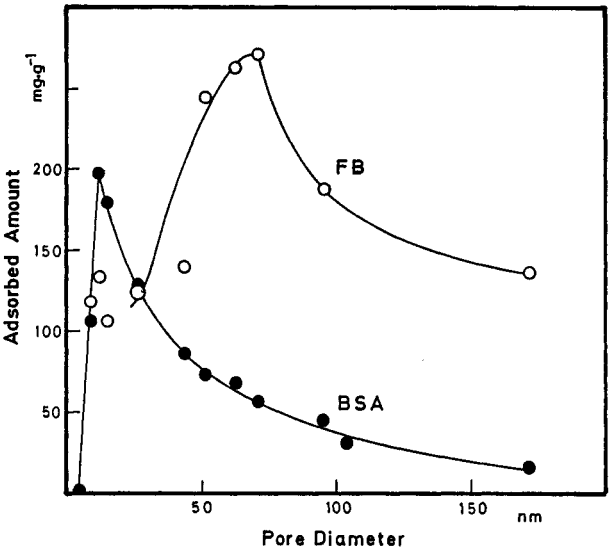

Fig. 5 Adsorbed amount of BSA and FB in 0.9 % NaCl solution per unit weight as a function of pore diameter of silica adsorbents.

It may be more reasonable to express the adsorbed amount per unit surface area rather than in unit weight in order to look into the influence of pore size, as is illustrated in Fig. 6. One can clearly observe the existence of adsorption maxima at about 30 and 70 nm for BSA and FB respectively, although these maxima are very broad. The pore sizes of maximum adsorption is about 3 or 4 times larger than the sizes of these molecules. There may be three possible interpretations for this result as follows. (1) The pore potential for the adsorption would be largest in this size relation like in micropore filling of small molecules. (2) Molecules would be in an extended or unfolded form in the solution (ref. 13), which is less probable. (3) A few molecules would be aggregated in the solution, which is also less probable. (4) The effective size of the pores for the diffusion of molecules would be much smaller owing to the electrostatic repulsion in case of nonmodified surface but this is not true for modified surfaces in this study. The reduced isotherms by taking the amount of saturation adsorption as unity showed that the initial rises of the curves is steepest at around the pore sizes of maximum adsorption. This result may suggest the biggest energy of adsorption in this pore size and support the mechanism of micropore filling. (ref. 14) A similar phenomenon was observed in the polystyrene-latex-vycol porous glass system (ref. 9), in which an adsorption maximum was found at a pore size a few times larger the size of molecules.

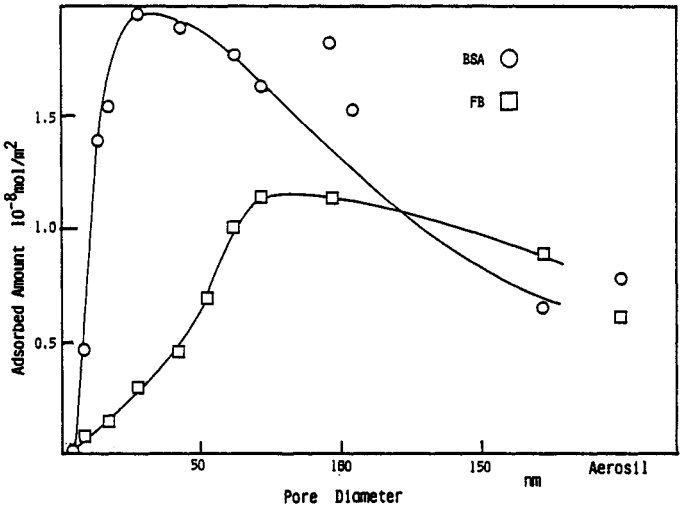

Fig. 6 Adsorbed amount of BSA and FB in 0.9 % NaCl solution per unit surface area as a function of pore diameter of nonmodified silica adsorbents.

The area of silica surface occupied by one protein molecule can be obtained from the type I isotherms of non-porous aerosils or highly macroporous silica beyond these of maximum adsorption, which has a large pore diameter compared with the sizes of molecules and may not have significant influence of pore potential for the

adsorption. Thses values are about 280 and 134 nm^2 per molecule for BSA and FB respectively. The value for BSA is more than 5 times larger than the largest (side on) cross-section area of BSA molecule of a folded form in electrolyte solution, 48 nm^2. The free OH band has almost disappeared in the spectrum shown by broken line in Fig. 2 after BSA adsorption. Hence, most of the surface free OH groups might have interacted with BSA molecules, although this was taken after evacuation of BSA-adsorbed material at 378 K and thus adsorbed BSA molecules would have changed their structure. This disappearance of free OH band may suggest that adsorbed BSA molecules might assume a widely expanded form on silica surface. This inference may account for the fact that the molecular configuration of desorbed BSA is somewhat different from that before adsorption. (ref. 4)

The adsorbed amount of BSA at the adsorption maximum for BSA is about 3 times bigger than the monolayer coverage mentioned above and its pore diameter of about 30-40 nm is 3 to 4 times larger than the size of BSA molecule. This would suggests a similar micropore filling behavior to that seen in micropores-nitrogen systems. The same is true for FB adsorption. BSA molecules in the sodium chloride solution may assume a folded form as is usually believed. It is interesting to see that almost the same results with that mentioned above were observed for both amine- and vinyl-modified surfaces. Since both BSA and non-modified silica surface have negative electric charge, in contrast to that of amine-modified surface which has positive charge, the influence of electrostatic interaction is negligible in this solution. Also hydrophilic interaction at the interface would not be significant, looking from the adsorption on vinyl-modified surface. Therefore, the energy of adsorption of these molecules would mainly consists of hydrophobic interaction of van der Waals type.

On the contrary, the adsorption behavior of BSA in pure water solution is very different from that in sodium chloride solution. Maximum pore diameter shifted to about 50 nm and this shift suggests that BSA molecules may assume an unfolded or expanded form in pure water solution. Maximum adsorbed amounts were in the order of amine-modified > non-modified > vinyl modified surfaces, which suggests the existence of chemical and electrostatic interaction at the interface perhaps because of the absence of electrolytes.

The case of FB is quite different. The pore diameter for the maximum adsorption is about 70 nm, which reflects the larger size of FB molecule than that of BSA. The adsorption cross-section area is about 134 nm^2 per molecule which is in between the smallest (end on) area, 33 nm^2 and the largest (side on) area 300 nm^2 of a FB molecule. This value is difficult to explain, but may be either because the molecule would have been decomposed to smaller fragments on the surface, or the molecule would orient itself on the surface in between an side-on and end-on forms.

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