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A Thermodynamic Study of Albumin Adsorption onto Some Solid Surfaces[1)]

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In order to understand protein adsorption, the thermodynamic parameters were evaluated from the initial slope of the isotherms of human albumin adsorption onto glass, Biomer (a hydrophobic polyurethane), 2-600 (a hydrophilic polyurethane) and silicone surfaces. The Gibbs free energy (ΔG) values of albumin adsorption in 10 mM phosphate buffer at pH 7.35 and at 23 °C were −5.24, −6.46, −4.55, and −7.21 kcal/mol, respectively. These values changed in 1 M NaCl–10 mM phosphate to −3.75, −6.99, −4.57 and −8.21 kcal/mol, respectively. There was a clear influence of salt concentration on the ΔG values of albumin adsorption on glass surfaces in the range of 0—0.5 M NaCl in the phosphate buffer.

Keywords——albumin; adsorption; glass; silicone coated surface; polyurethane; free energy

Drug adsorption on a glass surface and a silicone-coated surface was studied using porous glass as a reference standard for glass containers. Glass surfaces coated with silicone are water-repellant, and therefore it has been supposed that silicone-coated surfaces of pharmaceutical glass containers show decreased adsorption of drugs. However, in practice, silicone-coated surfaces adsorb more insulin, atropine, physostigmine,[2)] diazepam,[3)] and antibodies[4)] than noncoated glass surfaces.[5)] Some protein drugs are stored in glass containers and we have studied protein adsorption on the glass surfaces.[6)] In order to understand protein adsorption on solid surfaces, we report here the values of the parameters ΔG of adsorption of human serum albumin on glass, Biomer (a hydrophobic polyurethane), 2-600 (a hydrophilic polyurethane) and silicone-coated surfaces in 10 mM phosphate buffer (pH 7.35) containing various concentrations of NaCl.

Materials and Methods

Human albumin (Behringwerke AG, Marburg, Germany) was labelled with [^{125}I]iodine according to the literature,[7)] as follows. Albumin (10 mg) was dissolved in 0.1 ml of 2 M glycine at pH 8.8. To this solution, 0.07 ml of ICl and 0.03 ml of 2 M glycine were added. Then 6 μl of Na^{125}I solution was added and the mixture was incubated for 2 min at room temperature, and applied to a Bio-Rad AG1 (100—200 mesh) column, made of a 3 ml syringe, which had previously been equilibrated with 10 mM phosphate buffer at pH 7.35. Protein was eluted with 7 ml of phosphate buffer. Protein concentration was determined by measuring absorbance at 280 nm using a value of $E^{1\%}_{1\,cm}=4.54$ for human albumin. The specific radioactivity of the labelled albumin was 2000—3000 cpm/μg. This solution was used when the protein concentration in adsorption experiments was low (1—50 μg/ml). When protein concentration was 0.1—1 mg/ml, cold human albumin was added to the labelled albumin. No influence of the ratio of labelled to non-labelled albumin in adsorption on solid surfaces was found. The size of human serum albumin is 27 × 27 × 116 Å and the molecule is ellipsoid,[8)] so a value of 27 Å was used as the thickness of the albumin layer adsorbed on surfaces.

The buffer used for the adsorption experiment was 10 mM phosphate buffer, pH 7.35, because albumin had stronger affinity for glass surfaces in phosphate buffer among many buffers tested.[9)] The pH values of solutions containing high concentrations of NaCl were adjusted to pH 7.35 with 1 N NaOH.

As the glass surface, a slide glass (0.9 cm × 3.5 cm) was used (Wheaton Scientific, N.J., U.S.A.). The glass was put in a chromic acid mixture for two days and then thoroughly washed with distilled water. Surfaces of Biomer (Ethicon Inc., Somerville, N.J., U.S.A.) were prepared by coating of the above glass slides with 10% Biomer solution in dimethylacetamide. A glass slide was dipped in 10% Biomer and dried at 80 °C and under vacuum for 1 h. Dimethylacetamide was removed by extraction with water seven times for 4 h and then the slides were dried at 80 °C for 4 h under vacuum. The surface of 2-600, a hydrophilic segmented polyurethane covered with alcoholic OH residues,[10] was prepared by coating of the Biomer-slide with a 10% solution of 2-600 in dimethylformamide. The slide coated with 2-600 solution was dried at 80 °C under vacuum for 4 h. These three surfaces were used after equilibration in buffers for 3 h. The contact angles were 10.7° for glass surfaces, 82.3° for Biomer and 46.2° for 2-600, and these values are in good agreement with the values in the literature.[11] The silicone-coated surfaces were prepared with a 1% Siliclad water solution (Clay Adams, N.J., U.S.A.).

Adsorption experiments were performed in a Plexiglass cell (8.4 cm × 1.7 cm × 8.5 cm) which contained a slide holder to accomodate 6 slides. The apparatus was filled with 75 ml of buffer and then the slide holder having 6 slides was placed in the apparatus under stirring. Protein was introduced by injecting a protein concentrate into the buffer. In this way passage of the slides through the air–solution interface and thus Langmuir–Blodgett film transfer could be avoided. With vigorous stirring the protein concentration was assumed to reach its final value instantaneously.[12] Adsorption was carried out for 3 h with stirring. After adsorption, 0.1 ml of the solution in the apparatus was counted for determination of the specific radioactivity of albumin. The slide holder was removed from the apparatus after dilution of the protein in order to exclude the adsorption of Langmuir–Blodgett films as follows. A part (30 ml) of the solution in the apparatus was discurded and buffer solution was poured into the apparatus under stirring. This change was repeated 8 times more to dilute the protein. All of the solution in the apparatus was discurded. The apparatus was filled with buffer and the slide was washed with the buffer. This was repeated once more and then the slides were put in counting vials. The radioactivity of protein adsorbed on the slides was counted with a Biogamma γ-autowell counter (Beckman, U.S.A.). The amount of protein adsorbed is given as the mean of 6 slides of one experiment.

The Gibbs free energy (ΔG values) of albumin adsorption was determined from the relation between the surface concentration and the protein concentration in the solution.

Results and Discussion

The surface concentrations of human albumin at surfaces of glass, Biomer, and 2-600 were measured at 23 °C in the phosphate buffer and at albumin concentrations from 2 μg/ml to 1 mg/ml. The plateau surface concentrations of the isotherm on the three surfaces were obtained at about 100 μg/ml, and the maximum amounts were similar at 100 μg/ml to 1 mg/ml. These patterns were similar to the results for fibrinogen.[12] The maximum surface concentrations on Biomer, glass and 2-600 were 0.38, 0.15 and 0.05 μg/cm^2, respectively. Hydrophilic 2-600 had less affinity for albumin than did glass. From the measurement of contact angle, glass is more hydrophilic than 2-600. This high value of glass is presumably caused by ionic charges. Figure 1 shows the results at low concentrations of albumin (2—26 μg/ml). The Gibbs free energy values were determined from the initial slopes in Fig. 1 as follows;

$$\Gamma/\delta = (K/\delta)\, C = \exp[-(\Delta G/RT)]C \tag{1}$$

$$\Delta G = -RT \times \ln(K/\delta) \tag{2}$$

In the equations, δ is the thickness of adsorbed albumin (27 Å), K is the slope of the isotherm, C is the concentration of albumin, and Γ is the surface concentration. Those values are summarized in Table I. The ΔG values for the initial adsorption on glass, Biomer, 2-600 and siliconized glass were −5.24, −6.46, −4.55 and −7.21 kcal/mol, respectively. The decrease in free energy for albumin adsorption on the hydrophobic surfaces is thus greater than on the hydrophilic surfaces.

Next, the influence of NaCl concentration on albumin adsorption in phosphate buffer at pH 7.35 was studied. The results at low protein concentration in 1 M NaCl are shown in Fig. 2. The ΔG values were estimated from the initial slope and are summarized in Table I. The ΔG values of adsorption on the glass surface decreased in 1 M NaCl to −3.75 kcal/mol compared

TABLE I. Summary of the dG Values of Albumin Adsorption on Surfaces

Surface	$-dG$ (kcal/mol)	
	10 mM phosphate	1 M NaCl–10 mM phosphate
Glass	5.24	3.75
Biomer	6.46	6.99
2-600	4.55	4.57
Silicone	7.21	8.21

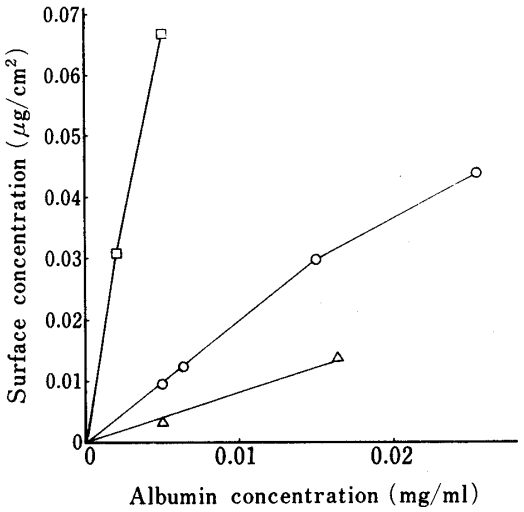

Fig. 1. Adsorption Isotherms of Albumin on Glass (○), Biomer (□) and 2-600 (△) at Low Concentration in 10 mM Phosphate Buffer, at pH 7.35

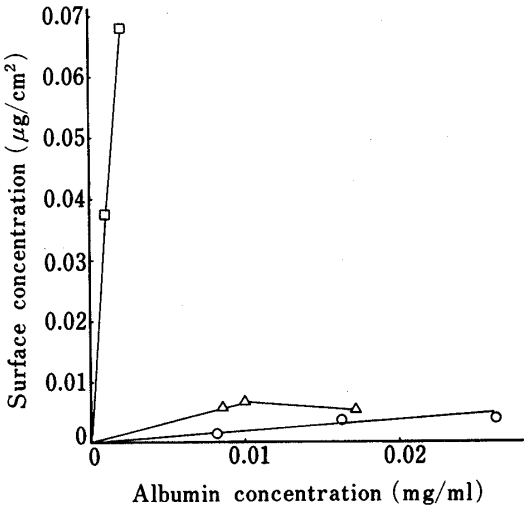

Fig. 2. Adsorption Isotherms of Human Albumin on Glass, Biomer and 2-600 at Low Concentration in 1 M NaCl in the Phosphate Buffer

Symbols are identical to those in Fig. 1.

to the values (−5.24 kcal/mol) in 0 M NaCl solution. This result indicates that the protein adsorption on glass surfaces should be related to electrostatic interactions.[10] The ΔG values of adsorption on Biomer increased in 1 M NaCl to −6.99 kcal/mol. This result suggests that protein adsortion on Biomer depends upon hydrophobic interactions.[10] The small change in ΔG for the 2-600 surface in 1 M NaCl versus salt-free buffer is probably not significant. Electrolyte appears to be without effect on adsorption on this surface. Figure 3 shows the adsorption isotherm on the silicone surface and indicates that protein adsorption on a silicone surface is related to hydrophobic interactions. The ΔG value of the isotherm on the silicone surface changed from −7.21 kcal/mol in salt-free buffer to −8.21 kcal/mol in 1 M NaCl. The slope of albumin adsorption on silicone in 1 M NaCl was the steepest among those on the various surfaces. Thus, the silicone surface has the strongest affinity for albumin at high salt concentration (greatest isotherm slope).

The surface concentration data for glass, Biomer and 2-600 exhibited plateaux between 0.1 and 1.0 mg/ml albumin concentration. The plateau values on Biomer (0.34 $\mu g/cm^2$) and 2-600 (0.05 $\mu g/cm^2$) in 1 M NaCl were similar to those in 10 mM phosphate buffer. Therefore, the plateau surface concentrations on Biomer and 2-600 were not influenced by salt concentration. On the other hand the ΔG values calculated from the initial isotherm slopes for Biomer showed a slight effect of salt. The plateau adsortion on glass in 1 M NaCl was 0.04 $\mu g/cm^2$ compared to 0.15 $\mu g/cm^2$ in 10 mM phosphate buffer. Thus for glass, the

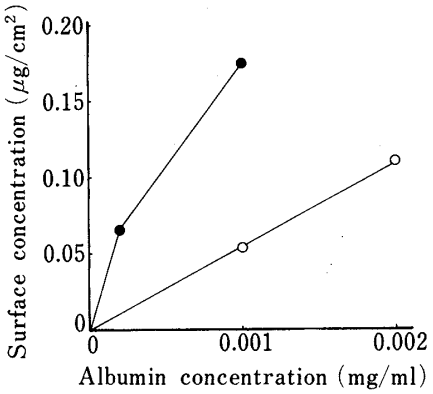

Fig. 3. Adsorption Isotherm of Albumin on a Silicone-Coated Surface at Low Concentration

Open circles, in 10 mM phosphate buffer at pH 7.35; closed circles, in 1 M NaCl in the buffer.

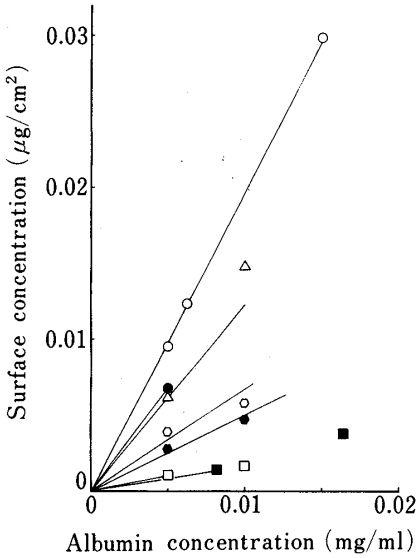

Fig. 4. The Influence of NaCl Concentration on Adsorption of Albumin to Surface

○, 10 mM phosphate buffer at pH 7.35; ●, 0.1 M NaCl in the buffer; △, 0.15 M NaCl in the buffer; ○, 0.2 M NaCl in the buffer; ●, 0.3 M NaCl in the buffer; □, 0.5 M NaCl in the buffer; ■, 1 M NaCl in the buffer.

maximum surface concentration and $-\Delta G$ both decreased in 1 M NaCl.

The effect of salt concentration on the initial slope of albumin adsorption on glass surfaces was studied. Figure 4 shows the relation between the surface concentration and the protein concentration at various salt concentrations. The influence of salt appeared at 0—0.5 M NaCl in 10 mM phosphate. The results showed that no effect of salt at 0.5 M or more NaCl. This character of the anionic glass surface was consistent with that obtained in fibrinogen adsorption.[12] The ΔG value of albumin adsorption on a glass surface in 10 mM phosphate buffer was -5.24 kcal/mol. The value in 0.5 M NaCl solution was -3.89 kcal/mol and this value was similar to that in 1 M NaCl as shown in Table I. The surface concentration in 1 M NaCl was in general about one-tenth of that in 10 mM phosphate, as shown in Figs. 1, 2 and 4, and therefore 90% of the adsorption depends upon ionic bonding and the residual 10% should depend upon other factors. Thus, albumin adsorption on an anionic glass surface must be intimately related to ionic interactions. This may appear paradoxial since both surface and protein are overall negatively charged at neutral pH. However, local regions of positive and negative charge are present on the protein and these can presumably act as independent binding sites.

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References and Notes

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