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ORIGINAL CONTRIBUTION

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Wettability of proteins adsorbed on silica glasses treated with fluorosurfactants

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Abstract Generally, the apolar/polar surface is probed by water-wetting, which is measured using a method such as the sessile liquid drop method. However, when one tries to measure the wetting of a surface where biological macromolecules are adsorbed, there is the problem of a change in conformation due to drying the surface; hence, using this method in situ information cannot be obtained. We have developed a new method that can be used to measure the wettability of the adsorbed protein surface without drying. This method, the dropping time method, which is based on measuring the dropping time of a film of liquid along a protein-covered surface when this surface is instantaneously vertically removed from the protein solution. The adsorption behavior of four proteins (albumin, lysozyme, β-lactoglobulin, ovalbumin) on the surface of silica glass that has been treated with various fluorosurfactants is studied using this method. At a high concentration of protein, the surfaces of adsorbed proteins of any kind are fairly hydrophilic on glass treated with all fluorosurfactants. At a lower concentration of protein, the hydrophilicity of the protein layer depends on the kind of fluorosurfactant and also on the protein adsorption process. The apolar glass surface becomes more hydrophilic with increasing dipping time in the protein solution. On the other hand, the hydrophilic glass surface shows a complex change in the hydrophilicity with elapsed time after dipping it into a solution of albumin or lysozyme, i.e., the hydrophilicity decreases in the early stage of the adsorption and then increases with proceeding adsorption.

Key words Contact angle · Fluorosurfactant · Hydrophilicity · Protein adsorption · Wetting

Introduction

Protein adsorption on a solid surface is an important problem in various fields such as medicine, dentistry, the development of new biocompatible artificial materials, and food science [1–5]. It is important to investigate the wetting of a protein layer that is adsorbed on a solid surface in an aqueous environment. The apolar/polar character is an important factor for further adhesion or adsorption of another biological component on the layer [6, 7]. For instance, the initial stage of dental plaque formation is the adsorption of salivary proteins on the tooth surface followed by attachment of oral bacteria on the adsorbed protein surface [8–10].

Usually, the apolar/polar character can be assessed by monitoring the surface wetting measured by the sessile liquid drop method (SDM) [11], the sessile air bubble method [12], and the tilted plate method; however, these methods may induce a change in the conformation of the protein layer and give misleading information about the wetting in situ. The largest problem with the SDM is that the sample plates should be dried before measurement. The drying process may cause a conformational change in the proteins. In the

sessile air bubble method, protein molecules adsorbed at the air/liquid interface become a serious problem. In the tilted plate method, the evaporation of water will occur in the contact water line region. The new method that we developed can be used for the study of the wetting of a protein layer on a solid in an aqueous solution [13].

In this paper, we report the study of the influence of the pretreatment of glass plates by surfactants on the wetting of adsorbed proteins, using this new method. We used various kinds of fluorosurfactants, i.e., cationic, anionic, nonionic, and amphoteric ones. These surfactants yield glass surfaces of varying degree of hydrophilic nature. The main purpose of this work is to clarify the effect of the surfactant-induced hydrophilicity of the subphase plate on the adsorption behavior of proteins and on the wettability of the surface of adsorbed protein layers.

Basic concept of a dynamic method for wetting

A sample plate is steeped in protein solution in a vessel. After immersion of the plate, we suddenly raise it to some distance above the air/solution interface. In this situation, there is a film of liquid attached to the plate, which moves down toward the air/solution interface (Fig. 1). The velocity of the liquid-film motion is determined by three forces: the gravitational force (f_g), the viscous force (f_h), and the force (f_i) due to the interfacial tension at a boundary line of the three phases; air, liquid, and solid plate. For a film of width l, height h, and thickness dy, the three forces are expressed by the following equations.

The gravitational force is

$$f_g = lh\,\mathrm{d}y\,\rho g \quad , \tag{1}$$

where y is a coordinate axis perpendicular to the plate surface, ρ is the density of the liquid film and g is the acceleration due to gravity.

The hydrodynamic force is

$$f_h = \mu(\mathrm{d}^2v/\mathrm{d}y^2)lh\,\mathrm{d}y \quad , \tag{2}$$

where μ is the viscosity coefficient and v is the velocity along the gravitational force line.

The force exerted by the interfacial tension is

$$f_i = \gamma_1 \cos\theta l\,\mathrm{d}y/D^* \quad , \tag{3}$$

where γ_1 is the surface tension at the liquid/air interface, D^* is the virtual thickness of the liquid film, which is the thickness where $\mathrm{d}v/\mathrm{d}y = 0$, and θ is the contact angle between the liquid film and the plate surface. This equation is derived from the Young–Laplace equation [14]; the pressure difference between the air and the solution can be written as $\Delta p = \gamma_1/(D^*/\cos\theta)$.

In the stationary state, the three forces are balanced: $f_g - (f_i + f_h) = 0$ and, hence,

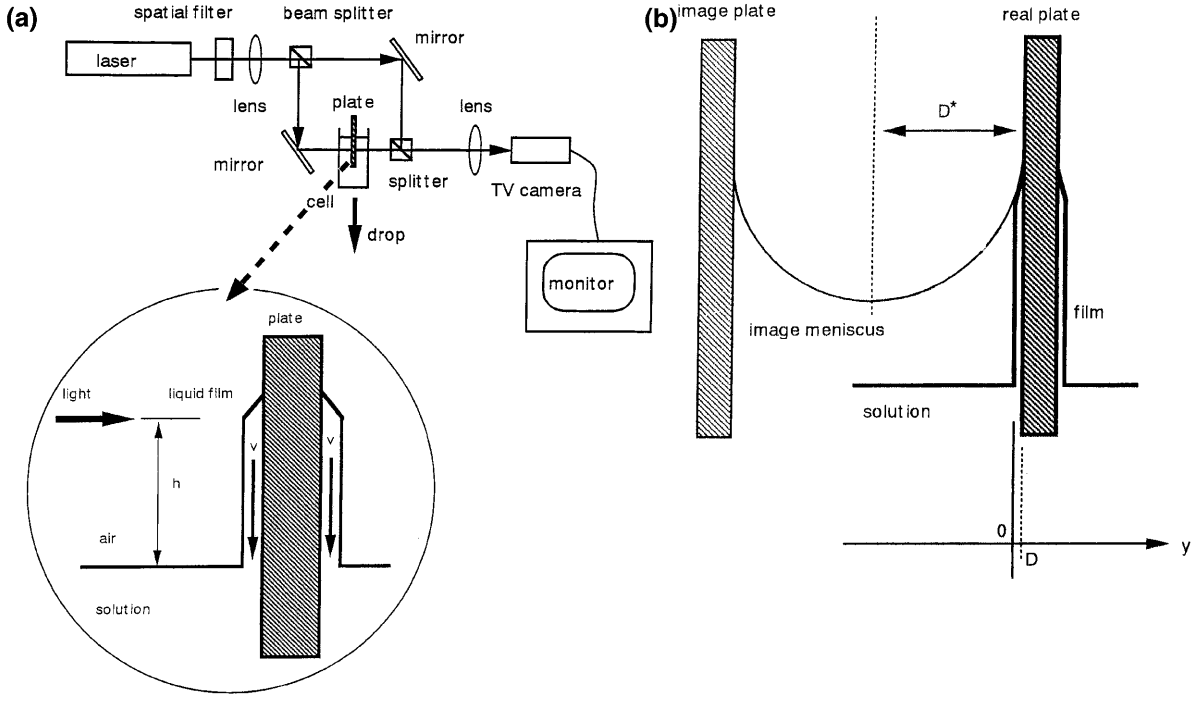

Fig. 1 **a** An interferometer set-up for measuring the motion of a liquid film on a solid surface and the schematic representation of a liquid film dropping along the plate surface. **b** Some geometrical quantities in the basic concept of the dropping time method (DTM)

$$\mu lh(\mathrm{d}^2v/\mathrm{d}y^2)\mathrm{d}y = lh\rho g\,\mathrm{d}y - \gamma_1 \cos\theta l\,\mathrm{d}y/D^* \quad . \tag{4}$$

This equation is integrated with respect to y using the boundary condition $\mathrm{d}v/\mathrm{d}y = k_m$ at $y = 0$ (surface of the film). We obtain the resultant relation

$$\mathrm{d}v = (\rho g/\mu)y\,\mathrm{d}y - (\gamma_1 \cos\theta/\mu D^* h)y\,\mathrm{d}y + k_m\,\mathrm{d}y \quad . \tag{5}$$

This equation is integrated again with respect to y using the boundary condition that at the solid surface (i.e., $y = D$)$v = v_d$, the velocity at the surface of the plate.

The following equation is obtained:

$$v = (y^2 - D^2)\rho g/2\mu - (y^2 - D^2)\gamma_1 \cos\theta/2\mu D^* h + (y - D)k_m + v_d \quad . \tag{6}$$

It is impossible to determine v_d directly, but the average velocity of the movement of the liquid film can be determined from the volume change of the film above the solution surface. To do so, we assume that the configuration (thickness, contact angle, etc.) of the film is constant. The volume change of the liquid film ($\mathrm{d}V/\mathrm{d}t$) is obtained by integration of $vl\,\mathrm{d}y$ from $y = 0$ to $y = D$. Next, the height change ($\mathrm{d}h/\mathrm{d}t$) of the film is calculated using the relation $\mathrm{d}V = lD\,\mathrm{d}h$. The resultant expression is

$$\mathrm{d}h/\mathrm{d}t = -(D^2\rho g/3\mu + k_m D/2 - v_d) + (\gamma_1 \cos\theta D^2/3\mu h D^*) \quad . \tag{7}$$

At any fixed position, hi, we obtain the relation

$$(\mathrm{d}h/\mathrm{d}t)_{hi} = -A + B\cos\theta \quad , \tag{8}$$

where A and B are constants. This approximation holds if the film configuration remains unaltered during movement toward the surface level of the solution. Under most experimental conditions the protein concentration in solution is so small that it does not significantly affect the viscosity and the density of the liquid phase. Thus, it can be derived from Eq. (8) that the time (Δt) needed for the top of the film to move between two positions separated by a distance (Δh) is given by

$$\Delta t/\Delta h = -1/A + (B/A)\cos\theta(\Delta t/\Delta h) \quad . \tag{9}$$

Based on this equation, the contact angle of wetting can be obtained by measuring the time interval for the film moving down between two fixed positions.

Experimental

Materials

We used human serum albumin (HSA; Sigma Chemical Co., A-7638), hen's egg lysozyme (LSZ; Sigma Chemical Co., L-6876), bovine milk β-lactoglobulin (LAG; Sigma Chemical Co., L-0130) and hen's egg ovalbumin (OVA; Sigma Chemical Co., A-2512) as sample proteins. Two concentrations of protein aqueous solution were utilized: 0.01 g/l (below adsorption saturation) and 1 g/l (adsorption saturation).

As surface-treatment agents, fluorocarbon surfactants DS-102 (anionic surfactant), DS-202 (cationic surfactant), DS-301 (amphoteric surfactant), and DS-401 (nonionic surfactant) were employed. These are products of Daikin Co., Japan.

As a solid surface we used cover glasses for an optical microscope, 24 × 32 mm (Matsunami Co., Japan). A hydrophilic glass plate was prepared by washing it with a detergent solution (R.B.S.-25, Junsei Chemical Co., Japan) under ultrasonic vibration. The glasses were treated with each surfactant by dipping them into surfactant aqueous solutions (1 g/l) for more than 15 min and the excess surfactant molecules were washed off with pure running water.

Methods

To detect the position of the top of the liquid film on the solid plate, an optical interferometer was utilized [13]. In one of the light beams of the Mach–Zehnder interferometer (Fig. 1), we mounted the vessel filled with the protein solution. After immersion of the plate for 1, 5, 10, 30, or 60 min, the vessel was suddenly dropped by some distance. (To avoid the influence of the adsorbed proteins at the air/water interface, it is necessary to sweep away the adsorbed proteins at the air/water interface before dropping the plate.) As a light source a He-Ne laser (543.5 nm; Melles Griot) was used. The beam was cleaned and expanded by passing it through a spatial filter and a collimator lens. The light beam was split into two by a prism splitter. One light beam passed through the protein film normal to the surface of the plate and was mixed with the other light beam at the second splitting prism. By using a microscope lens and a TV camera (Hitachi Electric Co., Japan and Panasonic Co., Japan), the resulting interferograms were recorded on video tapes. All small optical components were purchased from Sigma Optical Co. (Japan).

The films were formed on both sides of the sample plates and the interferograms were the averaged signals of both films. The time of the liquid film movement from the edge of the interferogram between two points that were 2 mm apart was measured. We call this the "dropping time", and this method to determine the contact angle is called the dropping time method (DTM).

Contact-angle measurements were also undertaken for dried samples using the usual SDM [15]. A SDM measurement was as follows. Distilled water (1 μl) was dropped using a micropipette from 3 mm above the glass surface. Immediately, we recorded the width and the height of the sessile liquid drop using a charge-coupled-device camera connected to a computer. Then, the contact angle was calculated using a computer program.

Results and discussion

The contact angles (SDM) of water droplets on the surfactant-treated glass plates using various kinds of fluorosurfactants are shown in Fig. 2a: the data of three trials are shown. Cationic (DS-202) and amphoteric (DS-301) surfactants make the glass surface rather hydrophobic, but an anionic surfactant (DS-102) has almost no effect. The effect of a nonionic surfactant (DS-401) is intermediate. The dropping time for a pure water film on each glass plate corresponding to the ones in Fig. 2a is shown in Fig. 2b. The dropping times (Fig. 2b) correspond fairly well with the hydrophilicity as determined using the SDM (Fig. 2a), i.e. the dropping time increases with a decrease in hydrophobicity (or, for that matter, contact angle).

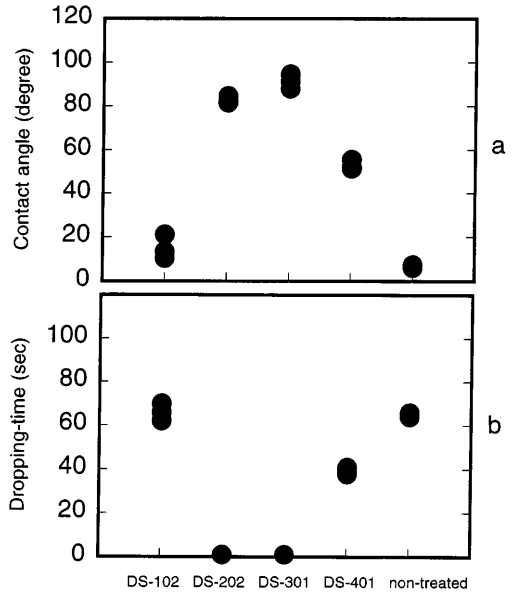

Fig. 2 **a** Contact angles of a water drop for silica glasses treated with various kinds of fluorosurfactants using the sessile liquid drop method. **b** Dropping times measured using the DTM in water for silica glasses treated with various kinds of fluorosurfactants. Three trials are shown

Dropping times versus the dipping times in each protein solution for the surfaces treated with four kinds of surfactants are shown in Table 1.

At a high protein concentration (1 g/l), the surfaces of an adsorbed protein layer of any kind on the glass treated with any fluorosurfactant are fairly hydrophilic; however, when we measured the contact angle using the SDM, the surface of adsorbed HSA on the glass pretreated with any surfactant has a hydrophobic nature (Table 2). This clearly shows that drying in air for SDM measurements causes a change in protein conformation, i.e., the hydrophobic amino acid residues come out to the surface. Only in the case of the OVA solution on the DS-202-treated glass surface is a dipping-time dependency of the dropping time clearly observed: the dropping time gradually increases with increasing dipping time (Fig. 3). Keshavarz and Nakai [16] reported that the surface of OVA molecules is very hydrophilic. It is conceivable that the hydrophilic amino acid residues are dominant on the OVA surface; hence, the OVA surface has a weak interaction with the hydrophobic DS-202 surface. The DTM measurements provide the information that the surfaces of all the test proteins dipped in the 1 g/l protein solution are hydrophilic. This result implies that the water-soluble proteins have a hydrophilic surface under conditions of adsorption saturation at any kind of surface.

At a lower protein concentration (0.01 g/l) the hydrophobic glass surfaces treated with DS-202 or DS-301 become more hydrophilic with increasing dipping time. These data show that the DTM can be used to monitor the adsorption process of proteins. One can observe the saturation behavior in the dropping time or hydrophilicity around 60 min after dipping the plate in the protein solution. Except for LSZ, the dropping time

Table 1 Dropping times of fluorosurfactant-treated glasses in protein aqueous solutions of human serum albumin (*HSA*), lysozyme (*LSZ*), β-lactoglobulin (*LAG*), and ovalbumin (*OVA*) measured using the dropping time method. The values in the table are averages of three trials

	Dipping time (min)	Dropping time (s)			
		DS-202	DS-301	DS-401	Untreated
HSA (1 g/l)	0	0	0	43	64
	1	51	66	63	59
	5	65	66	60	60
	10	57	64	62	60
	30	59	72	63	61
	60	66	66	58	61
HSA (0.01 g/l)	0	0	0	43	64
	1	4	26	40	39
	5	5	28	33	11
	10	11	37	40	15
	30	20	40	42	41
	60	22	44	44	42
LSZ (1 g/l)	0	0	0	43	64
	1	44	53	55	56
	5	47	53	52	54
	10	51	54	54	45
	30	51	55	55	49
	60	49	56	54	44
LSZ (0.01 g/l)	0	0	0	43	64
	1	7	0	49	17
	5	17	1	55	34
	10	21	5	47	47
	30	48	48	48	51
	60	53	51	47	45
LAG (1 g/l)	0	0	0	43	64
	1	48	48	39	57
	5	51	51	43	68
	10	54	54	43	68
	30	57	53	52	70
	60	55	54	44	65
LAG (0.01 g/l)	0	0	0	43	64
	1	6	22	43	51
	5	10	30	42	40
	10	11	34	43	37
	30	14	44	38	35
	60	35	33	39	31
OVA (1 g/l)	0	0	0	43	64
	1	9	42	50	52
	5	14	41	45	52
	10	31	53	47	53
	30	47	56	48	51
	60	58	52	50	54
OVA (0.01 g/l)	0	0	0	43	64
	1	4	27	42	46
	5	7	30	49	41
	10	7	34	49	46
	30	11	37	44	48
	60	18	38	53	46

Table 2 Contact angles of fluorosurfactant-treated glasses drying after adsorbing (HSA), (1 g/l) measured using the sessile liquid drop method. The values in the table are averages of three trials

Dipping time (s)	Contact angle (degree)			
	DS-202	DS-301	DS-401	Untreated
1	84	71	55	68
5	80	76	46	72
10	81	74	64	66
30	88	84	76	74
60	86	76	72	79

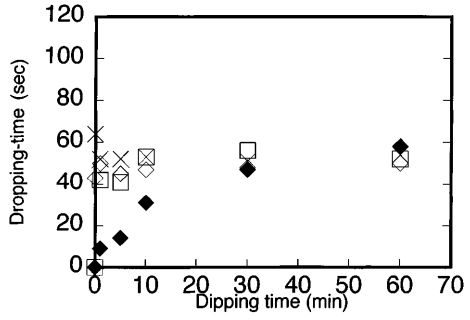

Fig. 3 Dropping times of fluorosurfactant-treated glasses in ovalbumin (*OVA*) (1 g/l) aqueous solutions. (◆) DS-202, (□) DS-301, (◇) DS-401, (×) untreated. Each datum is the average of three trials

in the case of DS-202-treated glass is shorter than that of DS-301-treated glass. This means that there is a difference in the amount of protein adsorbed or in the adsorption state for each protein between the DS-202-treated glass and the DS-301-treated glass. The dropping time is between about 40 and 50 s for the DS-401-treated glass for all proteins. The difference in saturation value may correspond to the difference in the nature of the surface after each treatment.

As for the hydrophilicity, there is a modern definition of it by van Oss [17]; however, in the field of amino acids and protein chemistry, it has not been utilized very much and there are not so many data for these biomolecules for us to utilize. Therefore, we considered our data on the basis of the traditional method in biological fields [18].

Many investigators have studied the hydrophilicity of protein molecules based on the polarity of the constituent amino acids [19–21]. A comprehensive study was reported by Bigelow [21]. He calculated the average hydrophobicity of proteins by summing the hydrophobicity of the constituent amino acids; however, this does not represent the hydrophobicity of the surface of the protein. Keshavarz and Nakai [16] reported that no significant relationship was observed between the hydrophobicity measured by hydrophobic affinity chromatography and the average hydrophobicity determined by Bigelow. They reported that the hydrophobicity for typical proteins has the following order: bovine serum albumin (BSA) > LSZ > LAG > OVA. Figures 4 and 5 show that neither Bigelow's data nor Keshavarz and Nakai's data correlate with our dropping-time data. These results show that the data obtained by the different methods give different kinds of information on the hydrophobic nature of proteins. Hydrophobic affinity chromatography gives information on the strength of the hydrophobic interaction between proteins and resin gels; therefore, the interaction between the hydrophobic surface of the resin and the hydrophobic domain of the proteins is the main factor for the hydrophobicity parameter (retention coefficient). On the other hand, the DTM gives the hydrophobic nature of the surface of the layer of adsorbed protein molecules.

At a lower protein concentration (0.01 g/l), in the case of the untreated glass (hydrophilic surface), the dropping times for HSA and LSZ show interesting behavior, i.e., they decrease sharply during short dipping times and then increase in the longer-dipping-time

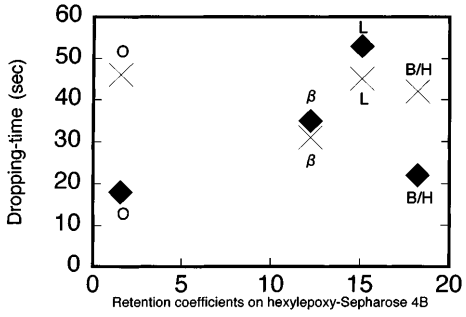

Fig. 4 Correlation between the effective hydrophobicity measured on a hexylepoxy-Sepharose 4B column and the dropping time. (◆) DS-202-treated glass in protein (0.01 g/l) aqueous solutions, (×) untreated glass. (*O*) OVA; (*β*) *β*-lactoglobulin (*LAG*); (*L*) lysozyme (*LSZ*); (*B*/*H*) bovine serum albumin (*BSA*)/human serum albumin (*HSA*)

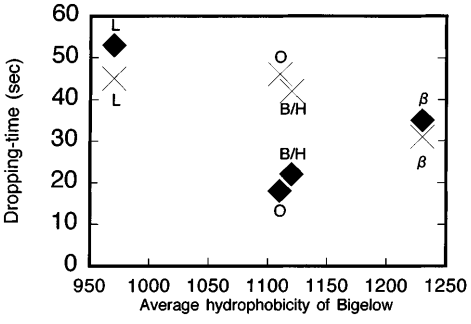

Fig. 5 Correlation between the average hydrophobicity calculated by Bigelow and the dropping time. (◆) DS-202-treated glass in protein (0.01 g/l) aqueous solutions, (×) untreated glass. (*O*) OVA; (*β*) LAG; (*L*) LSZ; (*B*/*H*) BSA/HSA

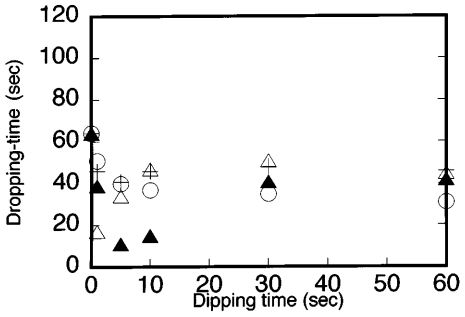

Fig. 6 Dropping times of untreated glasses in aqueous solutions of each protein (0.01 g/l). (▲) HSA, (△) LSZ, (○) LAG, (+) OVA. Each datum is the average of three trials

region (Fig. 6). This shows that for low adsorbed amounts the surface of the protein is primarily hydrophobic, but it changes to hydrophilic in later periods of adsorption. In the case of OVA, this behavior is also observed but is much less pronounced; however, for LAG, such interesting behavior is absent. These data suggest that the protein molecules on the hydrophilic surface can rather easily rearrange their orientation or/and change their conformation. Usually, the hydrophobic side chains are gathered to each other and are located inside the core region of the protein molecules; however, it is reported that hydrophobic side chains also frequently occur on the surfaces of protein molecules [22].

So, the surface of a protein molecule has a mosaic structure composed of hydrophilic and hydrophobic domains. The change in orientation and conformation of the proteins results in a change in the hydrophilicity of the surface of the adsorbed protein layers. Generally, the orientation and/or conformation of the protein molecules on the surface of a sorbent is determined by the interactions not only between a protein molecule and the sorbent but also between protein molecules; i.e., when adsorption of protein proceeds, the number of protein molecules on the surface increases, which increases the mutual interaction among protein molecules. Paulsson and Dejmek [23] reported the change in the surface tension of a BSA solution. They concluded that the change in surface tension did not depend in a simple manner on adsorption time and/or protein concentration. Similar behavior to that previously mentioned is possible in the case of the solid/liquid interface, i.e., the conformation of protein molecules adsorbed on the surface may change in the course of the adsorption process. A protein structure is flexible, for example, secondary structures as such as an α – helix may be destroyed by adsorption. Such a conformational change upon adsorption has been reported by Norde [5] and by Haynes and Norde [24]. On silica, Kondo et al. [25] reported a stronger conformational change in BSA than in OVA. BSA changes its conformation after adsorption. The adsorbed protein rearranges its structure in the low-concentration range; however, in the high-concentration range, interaction between protein molecules compresses the adsorbed layer. In that case, there is not enough vacant area on the surface for the protein molecules to relax and change their conformation; hence, the protein structure will be similar to that in the solution. Similar phenomena may occur in the wetting measurements of the surfaces of adsorbed proteins on the hydrophilic glass reported in this paper.

Conclusions

1. The hydrophilicity of the protein layer adsorbed on the surface of solids can be measured by the DTM.
2. When glass is dipped in a protein solution of high concentration, it is found that the surface of the adsorbed protein is rather hydrophilic. This result deviates from the one obtained by the SDM. The difference originates from the fact that the DTM measures the wetting of the surface of the adsorbed protein without drying it and hence without destruction of the protein structure.
3. At low protein concentration, it is found that the different surfactant treatments and also the different kinds of proteins result in different changes in the hydrophilicity. In the case of an apolar glass, the dropping time increases with increasing dipping time. This time-dependent hydrophilicity corresponds to the time dependency of the amount of protein absorbed; however, this time dependence of wetting is not only due to the amount adsorbed but also to a difference in adsorption conformation. This is clearly observed in the case of the time-dependent wetting of the proteins on the hydrophilic glass surfaces.

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