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ANESTHETIC-PROTEIN INTERACTION

RANDOM VERSUS HELIX POLYLYSINE MONOLAYERS AND INTERACTION WITH 1-ALKANOLS

AKIRA SHIBATA *, YUKIO SUEZAKI **, HIROSHI KAMAYA and ISSAKU UEDA ***

Anesthesia Service, Veterans Administration Medical Center, Salt Lake City, UT 84148, and Department of Anesthesia, University of Utah College of Medicine, Salt Lake City, UT 84132 (U.S.A.)

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Penetration of 1-alkanols into monolayers of hydrophobic polypeptides, poly(ε-benzyloxycarbonyl-L-lysine) and poly(ε-benzyloxycarbonyl-DL-lysine), was compared with their adsorption on the air/water interface in the absence of monolayers. The polypeptide prepared from L-lysine is generally considered to be in the α-helical form whereas DL-copolymer polypeptide contains random-coiled portions due to the structural incompatibility between the two isomers. The free energy of adsorption of 1-alkanols on the air/water interface at dilute concentrations was -0.68 kcal · mol^{-1} per methylene group and 0.15 kcal · mol^{-1} for the hydroxyl group at 25°C. In the close-packed state, the surface area occupied by each molecule of 1-alkanols of varying carbon chain-lengths showed nearly a constant value of about 27.2 Å^2, indicating perpendicular orientation of the alkanol molecules at the interface. About 75% of the water surface was covered by 1-butanol in this close-packed state. The mode of adsorption of 1-alkanols on the vacant air/water interface followed the Gibbs surface excess while the mode on the polypeptide membranes followed the Langmuir adsorption isotherm, indicating that the latter is characterized by the presence of a finite number of binding sites. The free energies of adsorption of 1-alkanols on the L-polymer monolayers were more negative than those on the vacant air/water interface and less negative than those on the DL-copolymer monolayers. Thus, the affinity of 1-alkanols to the interface was in the order of vacant air/water interface < L-polymer < DL-copolymer. The difference between the air/water interface and L-polymer was about 0.54 kcal · mol^{-1} and that between L-polymer and DL-copolymer was 0.17 kcal · mol^{-1} at 25°C: the adsorption of 1-alkanols to the DL-copolymer was favored compared to the L-polymer. The polar moieties of the backbone of the DL-copolymer may be exposed to the aqueous phase at the disordered portion. Dipole interaction between this portion and 1-alkanol molecules may account for the enhanced adsorption of the alkanols to the DL-copolymer.

Introduction

It has been a matter of debate whether lipid or protein is the site of anesthetic action. Although there appears to be a general consensus that the final target of anesthetics may be membrane proteins, it is often advocated that anesthetics affect

* On leave from Tokushima University, Faculty of Pharmaceutical Sciences, Shomachi, Tokushima 770, Japan.
** On leave from the Physics Laboratory, Saga Medical School, Nabeshima, Saga 840-01, Japan.
*** To whom reprint request should be addressed at VA Medical Center.

Abbreviations: PLL(Z), poly(ε-benzyloxycarbonyl-L-lysine); PDLL(Z), poly(ε-benzyloxycarbonyl-DL-lysine).

membrane proteins only secondarily to their action upon the lipid matrix where the proteins are embedded (see, for instance, Trudell [1]). Against these lipid theories, direct anesthetic effects upon the activities of water-soluble enzymes which lack a lipid bilayer structure have been reported [2–4]. We contend that the action of general anesthetics is nonspecific and directed to the interface between water and all macromolecular structures, regardless of proteins or lipid membranes.

Despite ample reports on the anesthetic interaction with lipid monolayers, studies with protein monolayers are few. The present work is aimed at studying the effect of 1-alkanols on spread monolayers of synthetic hydrophobic polypeptides. When the hydrophilic side-chain of polylysine is protected by hydrophobic moieties, the peptide becomes sparingly soluble into water and can be spread on the air/water interface to form a monolayer. The well-defined polypeptides, poly(ε-benzyloxycarbonyl-L-lysine) and poly(ε-benzyloxycarbonyl-DL-lysine), were used as model macromolecules.

Information about the conformation and stability of polypeptide chains has been accumulated by studies of L- and D-amino acid copolymers [5–7]. The preference of many poly(L-amino acids) to form a right-handed α-helix is attributed to the steric requirements of the L-residue [5–7]. The addition of D-residues to a sequence of L-residues disturbs the conformation of the helical chain, and the DL-copolymer contains random-coiled portions.

If there are differences in the interaction of 1-alkanols with the polypeptide between L-polymer and DL-copolymer, they would represent the role of the structural disorder in the polypeptide for the binding of 1-alkanols. The present report describes the adsorption of 1-alkanols to the vacant air/water interface and the data are compared with those obtained in the presence of the spread monolayers of L-polymer and DL-copolymer of poly(ε-benzyloxycarbonyllysine).

Experimental

Poly(ε-benzyloxycarbonyl-L-lysine), PLL(Z), and poly(ε-benzyloxycarbonyl-DL-lysine), PDLL(Z), were obtained from Sigma. The molecular masses were reported to be $2 \cdot 10^5$ and $1 \cdot 10^4$ daltons, respectively, when measured by viscometry [8]. Ethanol was obtained from IMC Chemicals (Agnew, CA). Alkanols (1-butanol, 1-hexanol and 1-octanol), dichloromethane and *N*,*N*-dimethylformamide were the highest grade available from Fisher (Pittsburg, PA). Water was purified by distillation followed by passage through two mixed-bed ion-exchanger columns, an activated charcoal column and an ultrafilter in a Milli-Q water-purifying system (Bedford, MA). The specific resistance of the obtained water was maintained above 16 Mohm · cm, and the absence of surface active impurities was checked by the dynamic surface tension measurement as previously reported [9].

Surface pressures were measured by the Wilhelmy method with a glass plate and a high sensitivity force transducer (Shinko Co., Kanagawa, Japan) as previously described [9]. The glass plate was cleaned in dichromate-sulfuric acid solution.

Adsorption of 1-alkanols on an air/water interface was measured in the presence and absence of polypeptides. The concentrations of 1-alkanols were estimated from the fluid density using an Anton-Paar oscillation densimeter DMA60/601HT (Mettler, Hightstown, NJ), which was calibrated by water, heptane and air at 20.00°C. The temperature of the densimeter cell was maintained by circulating water from a Hart 5001 constant-temperature water bath (Provo, UT) with ±0.005°C stability, and monitored by a microprocessor-controlled thermistor thermometer (Micro-Therm 1006, Hart, Provo, UT) with 0.0001°C resolution.

For measurement of the equilibrium adsorption of 1-alkanols on the interface, a trough measuring 90 mm in diameter and 15 mm in depth was milled from a Teflon block. The temperature was controlled by circulating water from a constant-temperature water-bath through a glass tube immersed in the trough, and was measured by a thermistor thermometer (United Systems, Dayton, OH) with 0.01°C resolution. The water surface was cleaned by sweeping with a Teflon barrier, followed by suction.

In the absence of the polymer monolayer, 1-alkanols of varying concentrations were added to the trough in an aliquot of 1.0 ml by a volumetric

pipet. An equal amount of water was removed from the trough prior to the addition of the 1-alkanol solution in order to avoid a change in the water level, which would change the buoyancy of the glass plate.

The polypeptides were dissolved in a 95 : 5 (v/v) mixture of dichloromethane and *N*,*N*-dimethylformamide and were applied to the water surface by a microsyringe. After 10 min, the 1-alkanol solutions were added to the subphase. The subphase was mixed by a magnetic stirrer for about 1 min to insure complete mixing. The equilibrium criterion for mixing and penetration into the polypeptide membrane was constancy of the surface tension over 30 min. The initial surface pressure of the polypeptide monolayer was 5 ± 0.1 dyn $\cdot$ cm^{-1}.

Dynamic surface tension was studied by an Acoma surface tensiometer (Tokyo, Japan) using a Teflon trough ($250 \times 50 \times 10$ mm) and a Teflon compression bar. The monolayer was spread on one side of the compression bar with the initial area being 45 Å^2 per one residue of the polypeptide. The 1-alkanol solution was added to the subphase from the other side of the barrier and the subphase was mixed with a magnetic stirrer for 5 min. After standing for 30 min, the monolayer was compressed at a rate of 0.5 Å^2 $\cdot$ (residue)$^{-1}$ $\cdot$ min^{-1} by a servomotor. The position of the barrier was detected by a followup linear potentiometer, and an *X*-*Y* recorder recorded the output as well as the output of the carrier amplifier of the force transducer for the surface tension. All measurements were performed at 25.0 ± 0.2°C.

Results

1-Alkanol adsorption on air/water interface

Surface pressure, π, is defined as the difference between the surface tension of water in the absence, γ_0, and presence, γ, of solute molecules.

$$\pi = \gamma_0 - \gamma \qquad (1)$$

The surface pressures of the solutions of 1-alkanols are shown in Fig. 1 as a function of the logarithm of their subphase bulk concentrations. Adsorption of the 1-alkanols on the air/water interface is promoted by the increase in the length of the alkyl chain.

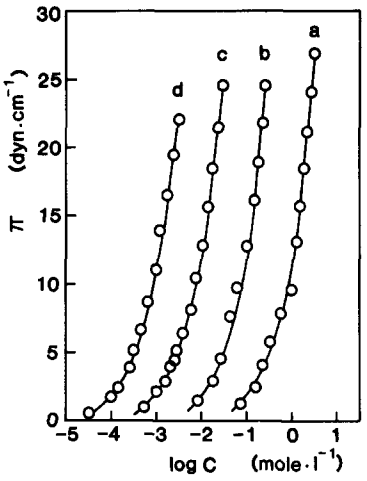

Fig. 1. The surface pressure as a function of the logarithm of 1-alkanol concentration at 25°C. a, Ethanol; b, 1-butanol; c, 1-hexanol, and d, 1-octanol.

The change in the free energy, ΔG, of the adsorbed molecules by transfer from the bulk solution in infinite dilution to the interface in two-dimensional infinite dilution is often written [10,11] as

$$\Delta G = RT \ln(\pi / x_2) \qquad (2)$$

where R is the gas constant, T is the absolute temperature and x_2 is the mole fraction of the

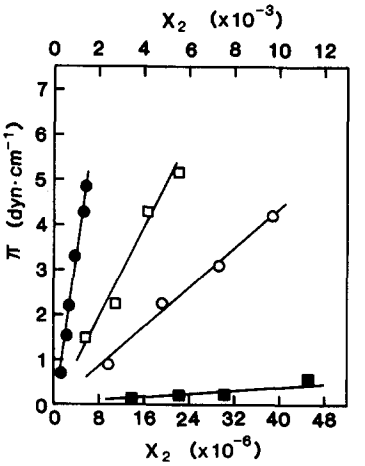

Fig. 2. The surface pressure as a function of 1-alkanol mole fraction at 25°C. The upper scale is for ethanol (□). The lower scale is for 1-butanol (■), 1-hexanol (○), and 1-octanol (●).

additive at low concentrations. This equation, however, does not consider the change in the state of ligand molecules when transferred from bulk solution to the interface. Hence, we express the free energy change by the following equation.

$$\Delta G = -RT\ln(\pi/x_2) + RT\ln(kT\Delta l/\Delta v) \quad (3)$$

The meaning of the second term on the right hand side will be discussed in the Theory section.

The value of ΔG can be obtained from the slope of a plot between the surface pressure and the mole fraction of the 1-alkanol in the bulk solution.

Fig. 2 shows the relationship between the surface pressure and the mole fraction of the 1-alkanols in the low concentration range. The surface pressure increased linearly with the increase in the subphase 1-alkanol mole fraction, to the limit of dilute 1-alkanol concentrations where $\pi \leq 5$ dyn · cm^{-1}.

Fig. 3 shows the relationship between the free energies of adsorption and the number of carbon atoms in the alkyl chain. The free energy of adsorption from solution to the air/water interface obeyed Traube's rule. The increment per methylene group was -0.68 kcal · mol^{-1} at 25°C, which agrees with the literature values [11–14]. The free energy of adsorption of the hydroxyl group of the 1-alkanol molecule can be estimated from the intercept in this figure, and was 0.15 kcal · $mole^{-1}$.

Dynamic surface tension of polylysine monolayer

Fig. 4 shows the π-a curves for PLL(Z) and PDLL(Z). The π-a curves were reproducible to

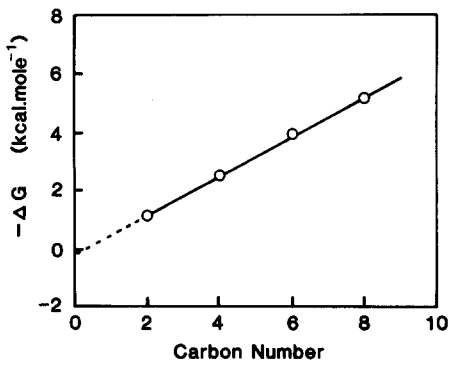

Fig. 3. The adsorption free energies as a function of the number of carbon atoms of the 1-alkanols at 25°C.

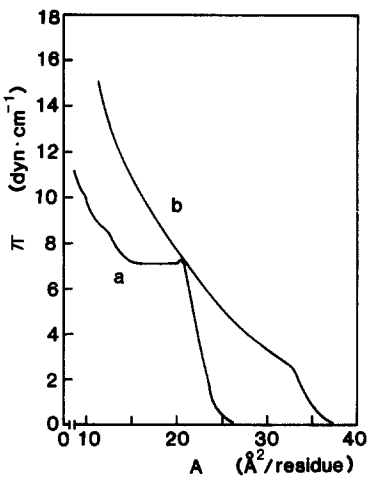

Fig. 4. The surface pressure-area curves of PLL(Z) (a) and PDLL(Z) (b) monolayers at 25°C.

± 0.2 Å^2 · $(\text{residue})^{-1}$. The slope of the π-a curve for PLL(Z) indicates that the monolayer is in the condensed state. The curve contains a plateau portion, which is characteristic of a number of synthetic polypeptide monolayers. The plateau is generally considered to be formed by collapse of the membrane or transition of the monolayer from a two-dimensional oriented state to a three-dimensional disoriented state [15]. At the transition point, PLL(Z) monolayers are believed to be compressed into the disoriented state from the orderly array of the helical polypeptide lying flat on the interface. The limiting area was obtained by extrapolating the slope of the steep region to zero surface pressure. The value was 23.9 Å^2 · $(\text{residue})^{-1}$. The surface pressure at this point was about 7 dyn · cm^{-1}. These values are in good agreement with those reported by others [16,17].

The behavior of PDLL(Z) in the monolayer state was significantly different from that of PLL(Z). Under identical conditions, the PDLL(Z) monolayer is more expanded than the PLL(Z) monolayer over the entire range of the present experiment. PDLL(Z) showed a transition at a lower pressure (about 3.5 dyn · cm^{-1}) than PLL(Z) and the surface area at the transition was about 33 Å^2 · $(\text{residue})^{-1}$.

The compressibility, δ, of an insoluble monolayer is expressed as

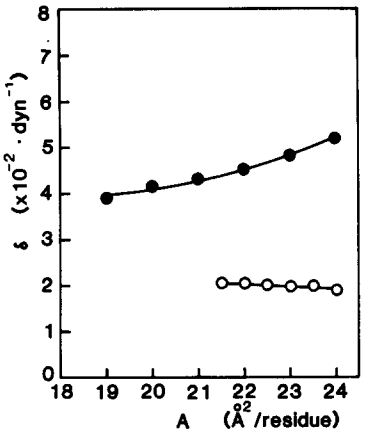

Fig. 5. The compressibilities of PLL(Z) (○) and PDLL(Z) (●) as a function of the molecular area at 25°C.

$$\delta = (1/a)(\partial a/\partial \pi)_T \tag{4}$$

where a is the area per each polypeptide molecule at the surface and π is the surface pressure. The compressibility is derived from the slope of the π-a curve. Fig. 5 shows the compressibilities of PLL(Z) and PDLL(Z) at varying surface areas.

1-Alkanol penetration into the polylysine monolayer

The mode of adsorption of 1-alkanol on the monolayers of PLL(Z) and PDLL(Z) is different from that on the vacant air/water interface. The observed π of 1-alkanols in the presence of the polypeptide monolayers can be fitted by the equation

$$1/\pi = (1/\pi_m) + (B/x_2) \tag{5}$$

where π_m and B are the constants described in the next section. This plot ($1/\pi$ vs. $1/x_2$) produced straight lines but the Gibbs plot (π vs. x_2) did not. The experimental data are shown in Figs. 6–9 for ethanol to 1-octanol.

Figs. 10 and 11 show the π-a curves for PLL(Z) and PDLL(Z) with varying bulk concentrations of 1-octanol. The transition pressures of both PLL(Z)

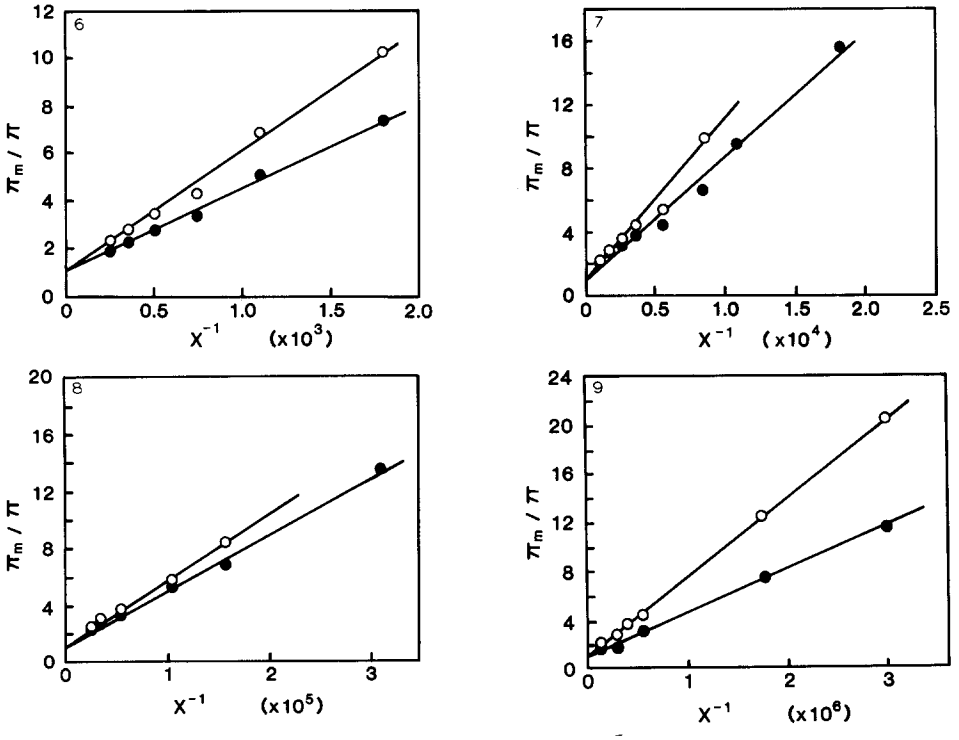

Fig. 6–9. The reciprocal of surface pressure increment of PLL(Z) (○) and PDLL(Z) (●) monolayers as a function of the reciprocal of the concentration of 1-alkanol at 25°C. The ordinate is normalized to dimensionless values by expressing π_m/π. Fig. 6. 1-Ethanol. Fig. 7. 1-Butanol. Fig. 8. 1-Hexanol. Fig. 9. 1-Octanol.

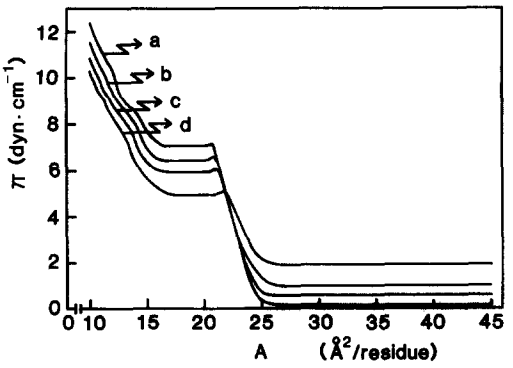

Fig. 10. The surface pressure-area curves of PLL(Z) monolayers after penetration by 1-octanol at 25°C. a, Pure water; b, $3.52 \cdot 10^{-5}$ M; c, $7.05 \cdot 10^{-5}$ M, and d, $1.41 \cdot 10^{-4}$ M.

and PDLL(Z) monolayers decreased with the increasing concentrations of 1-octanol.

Theory

The mode of adsorption of 1-alkanols on the polypeptide monolayer was quite different from that on the vacant water surface. The water surface adsorption is characterized by a linear relationship between the surface pressure and the mole fraction of 1-alkanols (Fig. 2) in the low concentration limit, indicating the Gibbs surface excess which assumes no binding site. In the presence of the polypeptide monolayer, the plot between inverse surface pressure versus inverse mole fraction of 1-alkanols was linear, as shown in Figs. 6 to 9.

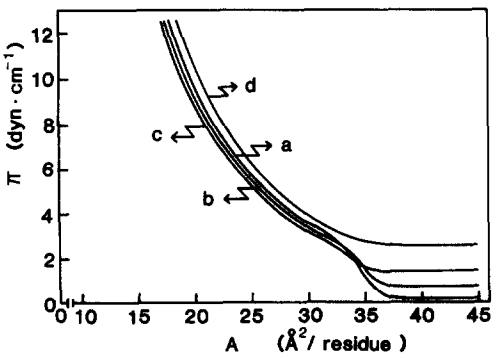

Fig. 11. The surface pressure-area curves of PDLL(Z) monolayers after penetration by 1-octanol at 25°C. a, Pure water; b, $3.52 \cdot 10^{-5}$ M; c, $7.05 \cdot 10^{-5}$ M, and d, $1.41 \cdot 10^{-4}$ M.

This result implies that the adsorption on the protein monolayer follows the Langmuir adsorption isotherm; a finite number of saturable binding sites exists on the polypeptide monolayer for the adsorption of 1-alkanols.

First, we formulate the adsorption of 1-alkanols (or any additives) on the water surface by simple statistical mechanics. Let X and N be the total number and the adsorbed number of 1-alkanols (or any additives), respectively. The partition function Z of the total system is written as

$$Z = \frac{1}{N!}(\mathrm{pf})_{\mathrm{A}}^{N} \cdot \frac{1}{(X-N)!}(\mathrm{pf})_{\mathrm{B}}^{X-N} e^{-\gamma_0 A/kT} \tag{6}$$

where γ_0, A, k, and T are surface tension of water, total surface area, the Boltzmann constant, and absolute temperature, respectively. Rigorous expression of the surface area A should be $A - Na_0$ instead, where a_0 is the co-area of 1-alkanol. However, numerical computations showed that the effect of co-area exclusion was negligible in the present analysis for estimation of the adsorption free energy at the limit of the low concentration range. The factors $(\mathrm{pf})_{\mathrm{A}}$ and $(\mathrm{pf})_{\mathrm{B}}$ are the molecular partition functions of 1-alkanols adsorbed on the surface and dispersed in the bulk aqueous solution, respectively, and

$$(\mathrm{pf})_{\mathrm{A}} = \lambda_{\mathrm{A}}^{-3} \Delta l A e^{-\Delta g_0/kT} \tag{7}$$

$$(\mathrm{pf})_{\mathrm{B}} = \lambda_{\mathrm{A}}^{-3} \Delta v N_{\mathrm{w}} \tag{8}$$

$$\lambda_{\mathrm{A}}^{-3} = (2\pi mkT)^{3/2}/h^3 \tag{9}$$

where, h, Δl, Δv, and N_{w}, respectively, are the Planck constant, the translational length of a 1-alkanol molecule in the perpendicular direction on the surface of the aqueous solution, the free volume of a 1-alkanol molecule in water, and the number of total water molecules. The factor Δg_0 in Eqn. 7 is the free energy change of a 1-alkanol molecule associated with the transfer from the aqueous solution to the air/water interface.

The adsorbed number, N, is determined so as to minimize the free energy, F, defined as

$$F = -kT \ln Z \tag{10}$$

By the use of Eqns. 6–10, N is determined so as to minimize F.

$$\frac{\partial F}{\partial N} = \Delta g_0 - kT \ln\left(\frac{\Delta l}{N}\frac{A}{\Delta v}\cdot\frac{X-N}{N_w+X}\right) = 0 \tag{11}$$

The surface tension, γ, of the adsorbed interface is derived from F according to the definition

$$\gamma = \frac{\partial F}{\partial A} = \gamma_0 - \frac{NkT}{A} \tag{12}$$

Because the very high surface excess region is not considered at present, we neglected the effect of the surface area excluded by the presence of 1-alkanol molecules, as previously discussed in the explanation of Eqn. 6. Preliminary application of the above formalism has been reported [18,19] in analyzing the stability of the lipid bilayer membranes.

According to the definition of surface pressure (Eqn. 1), and from Eqn. 12,

$$\pi = NkT/A \tag{13}$$

Because $X \gg N$, Eqn. 11 can be rewritten by the use of Eqn. 13

$$\Delta g_0 = -kT \ln(\pi/x_2) + kT \ln(\Delta l kT/\Delta v) \tag{14}$$

where $x_2 = X/(N_w + X)$ is the mole fraction of 1-alkanols in the solution.

From the data in Fig. 2, one can estimate the molar free energy change, $\Delta G_{0(m)}$ in the absence of monolayers by

$$\Delta G_0 = -RT \ln(\pi/x_2) + RT \ln(\Delta l kT/\Delta v) \tag{15}$$

for respective 1-alkanols. The obtained results are listed in Table I where we used the following parameters: $\Delta l = 1$ Å [18] and $\Delta v = 3 \cdot 10^{-24}$ cm^3.

Next, the adsorption of 1-alkanols from the aqueous solution to the polypeptide monolayer is analyzed. We postulate that there are finite binding sites on the monolayer for 1-alkanols and designate N_s for the number. In this case, the partition function Z_p of the system is written,

$$Z_p = \binom{N_s}{N}(\mathrm{pf})_{A_p}^{N}\cdot\frac{1}{(X-N)!}(\mathrm{pf})_B^{X-N}e^{-\gamma_p A/kT} \tag{16}$$

$$(\mathrm{pf})_{A_p} = \lambda_A^{-3}\Delta l\frac{A}{N_s}\cdot e^{-\Delta g/kT} \tag{17}$$

where γ_p is the surface tension of the spread monolayer of polypeptides. Other notations are identical with those in the previous analysis. The first factor in the right-hand side of Eqn. 16 is the number of combinations of N-adsorbed 1-alkanols to N_s sites.

$$\binom{N_s}{N} = \frac{N_s!}{N!(N_s-N)!} \tag{18}$$

In Eqn. 17, Δg is the free energy change of a 1-alkanol molecule when transferred from the aqueous solution to the polypeptide monolayer.

Similar to the previous analysis, we define the free energy F_p, of the system as

$$F_p = -kT \ln Z_p \tag{19}$$

The adsorbed number, N, and the surface tension, γ, are determined by the following equations.

$$\frac{\partial F}{\partial N} = \Delta g + kT \ln\left(\frac{N_s}{N_s-N}\right) - kT \ln\left(\frac{\Delta l}{\Delta v}\frac{A}{N}\cdot\frac{X-N}{N_w+X}\right) = 0 \tag{20}$$

$$\lambda = \frac{\partial F}{\partial A} = \gamma_p - \frac{NkT}{A} \tag{21}$$

$$\pi = NkT/A \tag{22}$$

By rearranging Eqn. 20 by the use of Eqn. 22, one obtains the relationship among Δg, N, π in the following form;

$$\frac{N_s-N}{N_s}\cdot\frac{x_2 kT\Delta l}{\pi\Delta v} = e^{\Delta g/kT} \tag{23}$$

TABLE I

ADSORPTION FREE ENERGIES OF 1-ALKANOLS ONTO THE AIR/WATER INTERFACE, $-\Delta G_0$, AND ONTO PLL(Z) AND PDLL(Z) MONOLAYERS, $-\Delta G$

Values are expressed in kcal·mol^{-1}.

	$-\Delta G_0$	$-\Delta G$	
		PLL(Z)	PDLL(Z)
Ethanol	1.18	1.70	1.85
1-Butanol	2.55	3.02	3.19
1-Hexanol	3.99	4.60	4.74
1-Octanol	5.21	5.78	5.99

We define the maximum value of π_m as

$$\pi_m = N_s kT/A \tag{24}$$

Then, Eqn. 23 is rewritten as

$$\frac{1}{\pi} = \frac{1}{\pi_m} + \left(\frac{\Delta v}{kT\Delta l} \cdot e^{\Delta g/kT}\right)\frac{1}{x_2} \tag{25}$$

Therefore, when $1/\pi$ is plotted against $1/x_2$, a linear line is obtained and the numerical values of π_m and Δg are estimated from the intercept and the gradient. The obtained values of ΔG ($= N_0 \Delta g$, N_0 being Avogadro's number) are listed in Table I. The plots shown in Figs. 6–9 are normalized to dimensionless values by expressing the ordinate with π_m/π. The values of the free energy change for the adsorption of 1-alkanols on the PLL(Z) monolayer were more negative than those on the vacant air/water interface by 0.54 kcal · mol^{-1} in average. The values were further negative with PDLL(Z) than with PLL(Z), the difference being 0.17 kcal · mol^{-1} in average.

Discussion

1-Alkanol adsorption onto air/water interface

As shown in Fig. 1, the surface pressure increases steeply with increasing bulk concentrations of 1-alkanols. The slopes in the higher concentrations are linear and parallel to each other. This indicates that the same number of 1-alkanol molecules is adsorbed per unit area at the interface, independent of the length of the alkyl chain of the 1-alkanol molecules. The lines in this area are relatively linear, indicating that the surface concentration of these 1-alkanols is not significantly increased by the increase of their bulk concentrations according to the Gibbs surface excess. The adsorbed 1-alkanol molecules are closely packed in this region, orienting themselves perpendicular to the interface.

The area occupied by each molecule on the interface is obtained from the surface excess, Γ, which can be estimated according to the Gibbs equation as follows.

$$\Gamma = (1/RT) \cdot (d\pi/d \ln c) \tag{26}$$

where π is the surface pressure and c is the bulk concentration of the 1-alkanols. The occupied area per molecule for ethanol, 1-butanol, 1-hexanol and 1-octanol were 28.0 ± 1.4, 27.2 ± 1.3, 27.5 ± 1.4, 26.0 ± 1.4 Å^2, respectively. It is clear that the occupied area is independent of the chain-length of the 1-alkanol molecules.

By the use of the spread monolayers of the water-insoluble long-chain 1-alkanols, the cross sectional area of a hydrocarbon chain has been estimated to be 20.5 Å^2/molecule (see, for instance, Adamson [20]). The comparison of this value with those obtained in the present study supports the view that the 1-alkanol molecules are oriented perpendicular to the interface when the bulk concentration of the 1-alkanol is high.

Kipling [21] reported that even at concentrations close to the solubility limit, the surface area of the adsorbed 1-butanol molecule does not approach that of the close-packed insoluble monolayer. The surface area consisted of approximately a 70 : 30 proportion of 1-butanol and water. From the value of the cross-sectional area estimated from the insoluble 1-alkanols, the present results indicate that the 1-butanol molecule occupies about 75% of the surface, which is in reasonable agreement with the above value [21].

The free energy of adsorption of the 1-alkanols (in the region where $\pi \leq 5$ dyn/cm) was linearly related to the number of carbon atoms in the aliphatic chain (Fig. 3). The constancy of the value of ΔG_0 per methylene residue indicates that the forces of interaction of each carbon atom with the surface are similar. Therefore, 1-alkanols are adsorbed to the surface with their main axis of the hydrocarbon chain parallel to the surface at low concentrations. If not parallel, the distance between each carbon atom and the surface varies, and ΔG_0 cannot assume a constant value.

Polylysine monolayers

The behavior of PDLL(Z) in the monolayer state differs significantly from that of PLL(Z) (Fig. 4). The conformations of DL-copolymers are closely related to the way in which the D- and L-residues are arranged along the peptide chain. If the D- and L-residues are distributed in groups, the properties of the PDLL(Z) monolayer would be expected to show some similarity with that of racemic poly(ε-benzyloxycarbonyllysine).

For the polypeptides with short side chains, a change in the helix sense would alter the orientation of the side chain groups with respect to the backbone. This in turn would modify the interaction between the side chain and water, and the resulting properties would be different between the enantiomorphs.

However, Malcolm [22] reported that the area per residue and the height and shape of the plateau in the π-a curve for the racemic poly(ε-benzyloxycarbonyllysine) is not significantly different from those of the enantiomorphs. In other words, the monolayer consisting of the mixture of the two enantiomorphs behaved as if it consisted of a single species. The interaction energies between the molecules of each species were identical. This indicates that the four methylene groups close to the backbone provide sufficient flexibility to the side chains, and the conformation of the backbone is little affected.

On the other hand, if the D- and L-residues are randomly distributed in the PDLL(Z) molecule, the helix structure would be destabilized. As shown in Fig. 5, the compressibility of PDLL(Z) is larger than that of PLL(Z). The difference in the compressibility between the two should arise from the difference in the flexibility of the backbone rather than that of the side chains. The backbone of the DL-copolymer presumably consists of random-coil portions and α-helix portions. The random-coil portions of PDLL(Z) are apparently responsible for the increased flexibility of this polymer. Also, this part may be a reasonable model for the irregular portion of a protein, even though these are not truly random as the term implies. The term random indicates a non-regular and non-repeating structure in a polypeptide molecule.

1-Alkanol interactions with polylysine monolayers

In the dynamic surface tension studies, the transition pressures for both PLL(Z) and PDLL(Z) monolayers decreased with the increasing concentration of 1-octanol. This effect is also observed with other 1-alkanols. From the surface phase rule [23], this phenomenon indicates that the interaction occurs between the polypeptide monolayer and 1-alkanol molecules. In Table I, the free energy changes in the adsorption of 1-alkanols onto the interface are compared. The values were in the order of air/water interface > PLL(Z) monolayer > PDLL(Z) monolayer. The polypeptide monolayers promote adsorption of 1-alkanol molecules from the aqueous phase to the interface. The affinity of 1-alkanols to PDLL(Z) monolayer is stronger than that of the PLL(Z) monolayer.

It appears that the affinity of 1-alkanols to polypeptide monolayers depends on the following factors: (1) The conformation of the polypeptide at the interface and the stereochemical configuration of 1-alkanol in the subphase. (2) The van der Waals force between the nonpolar moieties of the polypeptide and the 1-alkanol. (3) The number and chemical composition of polar groups in the two species. (4) The surface pressure of the monolayer. (5) The bulk concentration of the 1-alkanol.

We consider that the differences in the interaction energies between PLL(Z)-alkanol and PDLL(Z)-alkanol systems arise only from the difference in the conformation of polypeptide molecules at the interface because PLL(Z) and PDLL(Z) have the same interaction factors (2) to (5) in the above arguments.

The radial distribution of the side-chains in the α-helical PLL(Z) molecule means that the helical peptide is essentially covered by the layer of the side-chains and orientation of the peptide is governed by the hydrophobicity of the side-chains. Therefore, the polar moieties of the peptide backbone of the PLL(Z) molecule may not interact with the dipole of the 1-alkanols. The random-coil portion of PDLL(Z) is expected to expose the polar moieties of the backbone to the aqueous phase. Adsorption of the 1-alkanols to the PDLL(Z) monolayer may include dipole interaction in addition to hydrophobic interaction and this may account for the increased adsorption of the 1-alkanols to the random-coiled peptide. The interaction of 1-alkanols with macromolecules is not a simple function of the so-called hydrophobic effect, and dipole interactions may be a significant factor.

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