Remarkable Microenvironmental Difference between Monolayer and Bilayer Membrane Interfaces. Dissociation Behavior of a Lysine Residue Placed on the Membrane Surface

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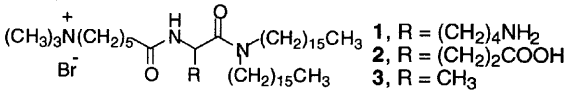
The dissociation constant (p*K*) of an ε-amino group of the L-lysine residue in a cationic peptide lipid was evaluated for both the bilayer vesicle formed in aqueous media and monolayer membrane assembled at the air-water interface. The p*K* value of 9.4 in the aqueous vesicle evaluated using ^{1}H-NMR spectroscopy was relatively close to that observed for the ε-amino group of lysine in water (10.5). In contrast, π-A isotherm measurements for the monolayer membrane on pure water revealed a remarkably shifted p*K* (5.1).

The excellent catalytic abilities of enzymes are due to the sophisticated design of their reaction sites. Control of their dissociation behaviors is one of the key factors to exhibit a powerful catalysis which apparently seems to be unusual in aqueous media under ambient pH conditions. Researches with X-ray crystallography, molecular dynamics, and point mutation have revealed that p*K* values of the functional groups in enzymes can be perturbed by the proximity of neighboring charges[1] and hydrophobicity of the surrounding environments.[2] Changing the surrounding environments upon modification of the amino acid sequences by point mutation is currently one of the successful approaches to control the p*K* value of a desirable group. However, it is not always easy to predict a suitable mutation. An easier methodology to place functional groups in desirable microenvironments must be established to develop enzyme mimetic catalysts. Recent researches on molecular recognition at interfaces revealed that the interfaces provide an environment quite different from bulk water.[3] The accumulated results on the interfacial phenomenon encourage us to study the modification of the p*K* of amino acids at the artificial interfaces. In this article, we will demonstrate that the p*K* value of an amino acid residue can be significantly controlled by selecting interfaces.



The molecular assembly of amphiphiles provides hydrophobic environments. We have been investigating the catalytic property of the bilayers of peptide lipids which have amino acid residues between the polar head and aliphatic chains. A hydrophobic microenvironment and side-by-side arrangement of the functional groups result in enzyme-like specific catalyses.[4] A recent report showed that catalytic properties were observed for the monolayer and LB film[5] although they had been believed to have too small a specific area to obtain a detectable amount of product. Therefore, we now have two molecular assembly candidates to construct the membrane-type enzyme mimic. Properties of the side chains of amino acids must be systematically compared both in the bilayer and monolayer membranes.

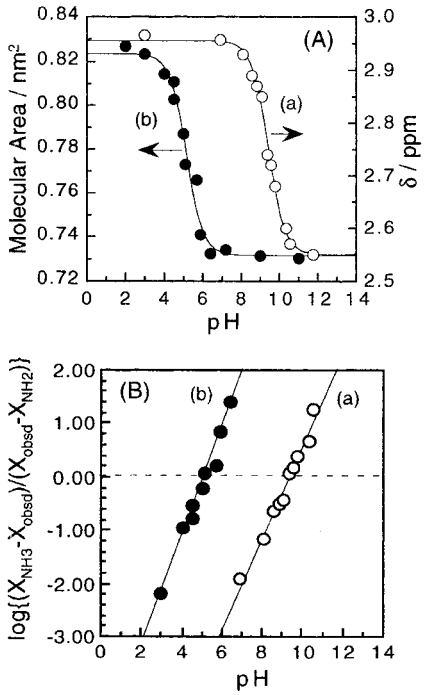


Figure 1. (A) a, Chemical shifts (δ) of ε-methylene protons of **1** in aqueous vesicle at various pH conditions; b, Molecular areas at 20 mN m^{-1} of **1** in monolayer on water at various pH conditions. (B) Linear plots of the data represented in (A): a, X = δ for the bilayer system; b, X = Molecular area for the monolayer system. In both plots, X_{obsd}, X_{NH3}, and X_{NH2} represent the corresponding parameters of the observed value, the value for the protonated species, and the value for the unprotonated species, respectively. The intercept of the abscissa gives the p*K* value.

For such a purpose, we selected two kinds of peptide lipids as model compounds. They have Lys (**1**)[6] and Asp (**2**)[7] residues between a cationic head and a double-alkyl chain. First, the dissociation property in the aqueous bilayer of **1** was monitored from the chemical shift of the ε-methylene protons in the ^{1}H-NMR spectra.[8] Figure 1(A) shows the pH dependence of the chemical shift and linear plot of these data gives a p*K* value of 9.4 (Figure 1(B)). The correlation coefficient close to unity (0.99) in the plot indicates that the system has a single apparent p*K* value. Since the p*K* of the aqueous Lys side chain is 10.5,[2a] the p*K* observed in an aqueous vesicle has a shift of only ca. 1 p*K* unit. Next, p*K* of the same lipid in a monolayer membrane was evaluated based on the molecular area change in π-A isotherms under pH control with a minimum amount of NaOH and H_2SO_4.[9] The lipid **1** showed an expanded monolayer in the whole pH region tested at 20 °C (see molecular areas in Table 1). The molecular areas at 20 mN m^{-1} are plotted versus pH in Figure 1(A) which shows that protonation of the side chain expand the monolayer. The

Table 1. p*K* Values of **1** and **2** in bilayer and monolayer

Lipid	State	Ionic Strength	p*K*	A_1[b]	A_2[b]
1	Bilayer[a]	≅ 0	9.4	—	—
1	Monolayer[c]	≅ 0	5.1	0.824	0.729
1	Monolayer[c]	0.1[d]	10.4	0.810	0.750
2	Monolayer[c]	≅ 0	5.3	0.794	0.892
2	Monolayer[c]	0.1[d]	5.7	0.760	0.850

[a] The p*K* values are determined from chemical shift of CH_2NH_2 in ^{1}H-NMR spectra. [b] A_1 and A_2 represent molecular areas at low and high pH values, respectively. [c] The p*K* values are determined from molecular areas at 20 mN m^{-1}. [d] Ionic strength of subphase was adjusted with Na_2SO_4.

p*K* of the monolayer was determined as 5.1 from the linear plot (Figure 1(B)). Similarly, p*K* values of 5.3 and 5.2 were obtained from the molecular areas at 5 and 35 mN m^{-1}, respectively. These results indicate that a remarkable difference exists between the monolayer and bilayer microenvironments.

In order to evaluate the contribution of the electrostatic factor to this remarkable p*K* shift, the same measurements were carried out under an ionic strength of 0.1. The ionic strength was adjusted by the addition of Na_2SO_4 to the subphase. The increase in the ionic strength of the subphase shifted the p*K* value to 10.4 which is close to that in water (Table 1). This result indicates that electrostatic interaction is crucial in the p*K* shift observed in the monolayer. We also investigated the dissociation behavior of **2** in the monolayer. The obtained p*K* was 5.3, relatively similar to those of carboxylates in water (Table 1). An increase in the ionic strength did not significantly change the p*K* value. π-A Isotherms of the Ala-functionalized lipid (**3**)[10] was also investigated as a control experiment, but a significant change in molecular area was hardly observed in the pH range from 2 to 12.

Decreased proton concentration at the positively charged interface might be part of the reason of the shifted p*K*. However, the difference in p*K* between the two interfaces cannot be simply explained by this effect. The p*K* difference would be originated in microenvironmental difference of the interfaces. The perturbed p*K* values of functional groups on molecular assemblies have been similarly reported for micelles,[11] bilayers,[12] Langmuir monolayers,[13] and self-assembled monolayers.[14] Grieser *et al.*[15] and Petrov and Möbius[16] investigated the microenvironments at the air-water interface using a fluorescent probe, and estimated the lowering of the dielectric constant at monolayer-water boundary. A hydrophobic environment at the water surface would suppress protonation to the ε-amino group of Lys. Quantum chemical calculations by Sakurai *et al.* indicated that molecular interactions at the lipid-water interface are strengthened by influence from the hydrophobic lipid phase.[17] Strengthened electrostatic repulsion near the hydrophobic phase unstabilized cationic species and lowered significantly p*K* of **1**. Increase of ionic strength probably removes this effect. The absence of p*K* shifts in the monolayer of **2** might come from the lack of charge repulsion from polar head. Effects of the position of the amino group on the p*K* perturbation at the air-water interface were estimated by Smart and McCammon.[18] They calculated the p*K* value of a long-alkyl amine at the air-water interface based on the nonlinear Poisson-Boltzmann equation. Their calculation indicated that a distance of a few angstroms above the water surface results in a several-unit shift in the p*K* of the amino group. In our case, a simple molecular model of lipid **1** revealed that the distance between the polar head and Lys amino group is 0.8-1.4 nm. Although conformational ambiguity remains, the side chain of **1** is probably placed apart from the water phase. This positioning of the Lys amino group would induce a significant negative shift of the p*K* through unstabilization of the charged group in the dehydrated medium. These effects would be more emphasized at clear air-water interface than bilayer surface.

What we have noticed in this study is the remarkable difference in the p*K* values observed between the monolayer and bilayer membranes. Adjustment of the ionic strength in subphase is an efficient factor for the p*K* shift. Systematic researches based on appropriate molecular design will generalize these findings, leading to desirable control of dissociation of amino acids.

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