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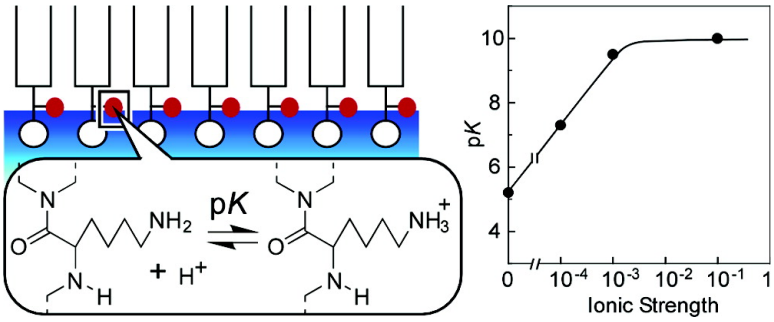

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Tunable p*K* of Amino Acid Residues at the Air−Water Interface Gives an L-zyme (Langmuir Enzyme)

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Abstract: Various amino acid-carrying amphiphiles were synthesized, and the p*K* values of the attached amino acid residues were investigated at the air−water interface and in aqueous vesicles using $\pi-A$ isotherm measurements, ^{1}H NMR titration, and IR spectroscopy in reflection−adsorption mode. The ϵ-amino group of the Lys residue embedded at the air−water interface displays a significant p*K* shift (4 or 5 unit) compared with that observed in bulk water, while the p*K* shift in aqueous vesicles was not prominent (ca. 1 unit). Moreover, p*K* values of the amino acids at the air−water interface can be tuned simply by control of the subphase ionic strength as well as by molecular design of the amphiphiles. A simple equation based on the dominant contribution by the electrostatic energy to the p*K* shift reproduces well the surface pressure difference between protonated and unprotonated species, suggesting a reduction in the apparent dielectric constant at the air−water interface. Hydrolysis of a *p*-nitrophenyl ester derivative was used as a model reaction to demonstrate the use of the Lys-functionalized monolayer. Efficient hydrolysis was observed, even at neutral pH, after tuning of p*K* for the Lys residue in the monolayer, which is a similar case to that occurring in biological catalysis.

Introduction

Highly selective and efficient material conversions conducted by biomolecular catalysts (i.e., enzymes) originate from the sophisticated organization of their amino acid residues. The shape and functional groups of their substrates are recognized in the reaction cavities of enzymes, which have been mimicked by well-structured supermolecules called artificial enzymes.[1] Another surprising characteristic of biomolecular catalysts can be found in the fine-tuning of the physicochemical nature of the amino acid residues. In particular, control of their dissociation behaviors is one of the key factors influencing a powerful catalysis, which seems to be unusual in aqueous media under ambient pH conditions. Investigations using X-ray crystallography, molecular dynamics, and protein engineering have revealed that the p*K* values of the functional groups in enzymes are perturbed by the proximity of neighboring charges and the hydrophobicity of the surrounding environment.[2]

A straightforward approach for p*K* control of the amino acids is by modification of biomolecules using protein engineering methods such as point mutation,[3] which sometimes leads to successful p*K* control of the desired residues. However, prediction of the appropriate modification of the amino acid sequences is not always easy. Rather than modifying the enzymes themselves, supply of an appropriate environment to the target residues by use of a molecular assembly could facilitate p*K*

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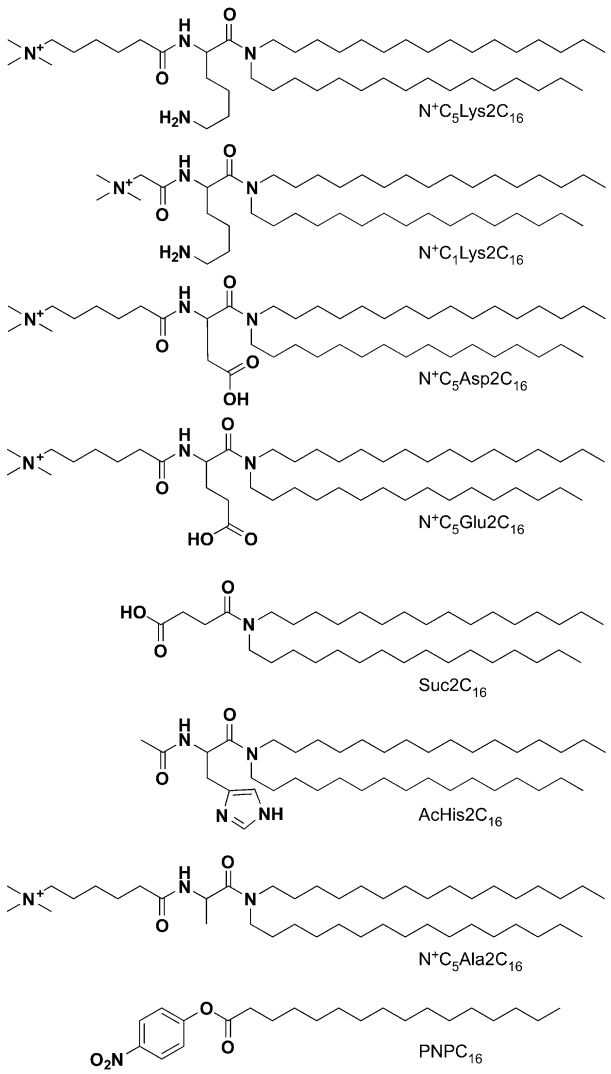
control, because p*K* perturbations have been reported at the surfaces of micelles,[4] lipid bilayer vesicles,[5] self-assembled monolayers,[6] and Langmuir monolayers.[7] However, the p*K* behaviors at different interfaces have thus far been discussed separately, and there has been no systematic interpretation.

One of us reported significant differences in molecular interaction at molecular, microscopic, and macroscopic interfaces, in which the most efficient modification of the molecular interaction was achieved at a macroscopic interface, such as the air−water interface.[8] On the basis of that knowledge, we have recently investigated the p*K* behaviors of amino acid residues (usually the ϵ-amino group in the Lys residue, both at the air−water interface and at a vesicle surface[9]) and have found that the air−water interface provides an appropriate microenvironment for fine-tuning of p*K* and for an enzyme-like reaction. Here, we report three important findings: (i) superior p*K* control at the air−water interface when compared with a vesicle system; (ii) p*K* tuning by 4 or 5 units by control of ionic strength in the subphase and prediction of the interfacial dielectric nature by a simple equation; (iii) demonstration of ester hydrolysis by the p*K*-controlled Lys residue at the air−water interface. These findings should lead to development of a novel concept for artificial enzymes that is proposed to be referred to as the Langmuir-enzyme (L-zyme).

Experimental Section

1. Materials. Structures of the amino acid-carrying amphiphiles and related molecules used in this research are shown in Chart 1. Syntheses of $N^+C_5Lys2C_{16}$,[10] $N^+C_5Asp2C_{16}$,[11] and $N^+C_5Ala2C_{16}$[12] have been described previously. Compounds $N^+C_1Lys2C_{16}$, $N^+C_5Glu2C_{16}$, $Suc2C_{16}$, and $AcHis2C_{16}$ were newly synthesized by coupling of a dialkylamine to the *C*-terminal and a polar headgroup to the *N*-terminal of the corresponding amino acid residue. The detailed descriptions of the synthesis are summarized in Supporting Information. Hexadecanoic acid 4-nitrophenyl ester ($PNPC_{16}$) was purchased from Sigma. Water used for the subphase was distilled using an Autostill WG220 (Yamato) and deionized by a Milli-Q Lab (Millipore). Spectroscopic grade benzene and ethanol (Wako Pure Chem.) were used as the spreading solvents. High-purity sulfuric acid, sodium hydroxide, and sodium sulfate were used as received.

Chart 1. Structures of Amino Acid-Carrying Amphiphiles and Related Compounds



2. π−*A* Isotherm Measurement. π−*A* isotherms were measured using an FSD-300 computer-controlled film balance system (USI System). A solution (benzene/ethanol (70/30 v/v)) of the amphiphile (ca. 1 mM, and ca. 100 μL) was spread on the subphase at 20.0 ± 0.2 °C and compressed at a rate of 0.2 mm s^{-1} (or 20 mm^2 s^{-1} based on area). The subphase pH was adjusted by addition of the minimum amount of aqueous sulfuric acid or sodium hydroxide with fluctuation of subphase pH during a single measurement being less than 0.2 pH units because of the shielded atmosphere. The ionic strength of the subphase was adjusted with sodium sulfate.

3. Evaluation of p*K* Values. Dissociation constants (p*K*) at the air−water interface were evaluated from the pH dependence of the apparent molecular areas. The apparent total molecular area (A_{obsd}) is given, assuming the absence of condensation effects, with the molecular areas A_B and A_{BH} of the unprotonated and protonated species respectively, and the B content of *x*, where B is NH_2, COO^-, or imidazole free base.

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$$A_{obsd} = (1 - x)A_B + xA_{BH} \quad (1)$$

Combining eq 1 with dissociation constant, $K = ([B] + [H^+])/[BH]$ results in eq 2.

$$\log\left[\frac{(A_{BH} - A_{obsd})}{(A_{obsd} - A_B)}\right] = pH - pK \quad (2)$$

The pK value of the $N^+C_5Lys2C_{16}$ monolayer was also evaluated using reflection−absorption infrared (RA-IR) spectra of a single monolayer transferred at 20 mN m^{-1} by the horizontal drawing-up method[13] on a gold-deposited glass slide (prepared with a VPC-260 vapor-deposition apparatus (ULVAC)), using an FT-IR spectrometer Magna 560 (Nicolet) equipped with an MCT detector. The pK value was estimated from the pH-dependence of the intensity of the N−H vibration at 3250 cm^{-1} relative to that of the CH_2 vibration at 2925 cm^{-1}, which is insensitive to pH change.

For pK determination in the vesicular state, $N^+C_5Lys2C_{16}$ was dispersed in D_2O (10 mM) using a vortex mixer for 2 min, followed by sonication (30 W, 3 min). DCl or NaOD was added to the aqueous vesicle, and the solution was equilibrated to a stable pD for 2 h. The addition of 0.4 to the reading from the pH meter gave the corrected pD.[14] ^{1}H NMR spectra were measured using a JEOL JNM-270 spectrometer.

4. Evaluation of $PNPC_{16}$ Hydrolysis. A mixed monolayer of the amino acid-carrying amphiphiles ($N^+C_5Lys2C_{16}$ or $N^+C_5Ala2C_{16}$) and $PNPC_{16}$ (2:1 in molar ratio) was spread and compressed to 20 mN m^{-1}. The hydrolysis of $PNPC_{16}$ was evaluated from the decrease in absorbance at 300 nm ascertained by surface reflection−absorption spectroscopy, because the hydrolyzed product *p*-nitrophenolate group escapes deep into the subphase. The amount of unreacted *p*-nitrophenyl group was quantified on the basis of the calibration curve of $PNPC_{16}$ at 300 nm, and conversion of the $PNPC_{16}$ hydrolysis could be calculated. The surface-reflective UV−vis spectra were measured using a photodiode array-equipped spectrometer (Otsuka Electronics, model MCPD-7000).

Results and Discussion

1. Enhanced pK Shifts at the Air−Water Interface Compared with Vesicular System. π−A isotherms of the amphiphiles at 20 °C on pure water are summarized in Figure 1. All of the isotherms except $Suc2C_{16}$ display an expanded profile. To select an interfacial medium suitable for efficient pK control, we compared pK values both at the air−water interface and at an aqueous vesicle surface. As a model amphiphile, $N^+C_5Lys2C_{16}$ was used, which can form a bilayer vesicle in bulk water or a Langmuir monolayer. First, the dissociation property of the aqueous bilayer vesicle of $N^+C_5Lys2C_{16}$ was monitored by observation of the chemical shift of the ϵ-methylene protons in the ^{1}H NMR spectra (Figure 2A(a)), and its linear plot provides a pK value of 9.4 (Figure 2B(a)). The correlation coefficient close to unity (0.99) in the plot indicates that the bilayer system has a single apparent pK value. Since the pK of the aqueous Lys side chain is 10.5, the pK observed in an aqueous vesicle has a shift of only ca. 1 pK unit.

Next, the pK value of the same lipid composing a Langmuir monolayer was evaluated on the basis of the molecular area change in their π−A isotherms under pH control. The molecular area of the $N^+C_5Lys2C_{16}$ monolayer in an expanded phase in the whole pH region at 20 °C increased as pH decreased because

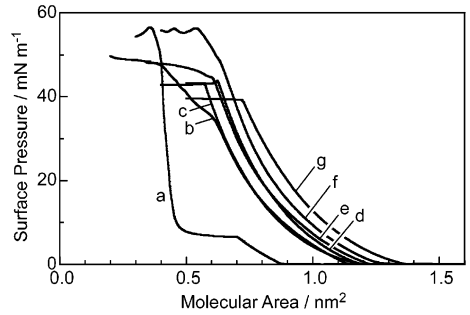

Figure 1. π−A Isotherms on pure water at 20 °C: (a) $Suc2C_{16}$; (b) $AcHis2C_{16}$; (c) $N^+C_5Lys2C_{16}$; (d) $N^+C_5Glu2C_{16}$; (e) $N^+C_5Ala2C_{16}$; (f) $N^+C_5Asp2C_{16}$; (g) $N^+C_1Lys2C_{16}$.

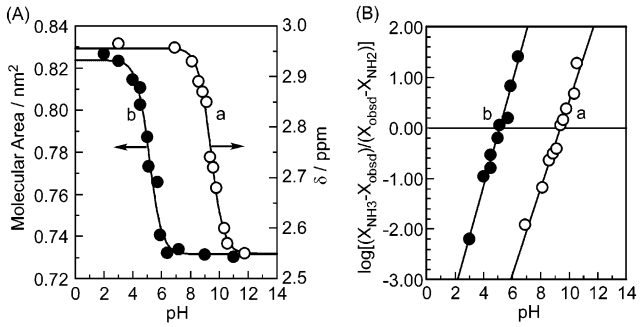

Figure 2. (A) (a) Chemical shifts (δ) of ϵ-methylene protons of $N^+C_5Lys2C_{16}$ in aqueous vesicles at various pH conditions; (b) molecular areas at 20 mN m^{-1} of $N^+C_5Lys2C_{16}$ in monolayer on water at various pH conditions. (B) Linear plots of the data presented in (A): (a) $X = \delta$ for vesicular system; (b) X = molecular area of the monolayer system. In both the plots, X_{obsd}, X_{NH3}, and X_{NH2} represent the corresponding parameters of the observed samples, the value for the protonated species, and the value for the unprotonated species, respectively. The intercepts of the abscissa provide the pK values.

of electrostatic repulsion between protonated amine groups. The pH profile of the molecular areas at 20 mN m^{-1} (Figure 2A(b)) was converted to the linear plot (Figure 2B(b)), resulting in a significantly shifted pK of 5.1. Similar pK values of 5.3 and 5.2 were obtained from the molecular areas at 5 and 35 mN m^{-1}, respectively. Conversely, π−A isotherms of the Ala-functionalized amphiphile, $N^+C_5Ala2C_{16}$, did not show any changes in molecular area in the pH range from 2 to 12.

The monolayer of another Lys-functionalized amphiphile, $N^+C_1Lys2C_{16}$, was also investigated, giving results in pK values of 6.1, 6.1, and 6.0 at 5, 20, and 35 mN m^{-1}, respectively (see Table 1). Similarly, large pK shifts at the air−water interface were confirmed for the two kinds of Lys amphiphiles. More direct evidence for this pK shift was obtained by protonation of the amino group and IR analyses for the monolayers transferred to a gold-coated glass slide. Peaks at around 3250 cm^{-1}, mostly originating from ν(N−H), intensified as pH increased, while the sharp peak for $\nu_{as}(CH_2)$ at 2925 cm^{-1} was unchanged (Figure 3A). IR absorbance intensities at 3250 cm^{-1} relative to those at 2925 cm^{-1} were converted to a linear plot (Figure 3B), giving a pK value of 6.1, which is identical with that determined from the π−A isotherm measurements.

A comparison of pK values in an aqueous vesicle and in a Langmuir monolayer indicates that a remarkable difference exists between these environments. Several researchers have investigated microenvironments at the air−water interface using chromophores to demonstrate a decrease in the dielectric constant.[15] A hydrophobic environment at the water surface

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Table 1. Observed pK Values for Amino Acid and Related Residues at the Air−Water Interface (20 °C)

lipid	functional group	ionic strength	surface pressure (mN m^{-1})	p*K*	lipid	functional group	ionic strength	surface pressure (mN m^{-1})	p*K*
				Monolayer					
$N^+C_5Lys2C_{16}$	NH_2	0	5	5.3	$N^+C_1Lys2C_{16}$	NH_2	0	35	6.0
$N^+C_5Lys2C_{16}$	NH_2	0	20	5.1	$N^+C_1Lys2C_{16}$	NH_2	0.0001	35	7.9
$N^+C_5Lys2C_{16}$	NH_2	0	35	5.2	$N^+C_1Lys2C_{16}$	NH_2	0.01	35	9.6
$N^+C_5Lys2C_{16}$	NH_2	0.0001	35	7.3	$N^+C_5Asp2C_{16}$	COOH	0	5	5.4
$N^+C_5Lys2C_{16}$	NH_2	0.001	35	9.5	$N^+C_5Asp2C_{16}$	COOH	0	20	5.3
$N^+C_5Lys2C_{16}$	NH_2	0.1	5	9.8	$N^+C_5Asp2C_{16}$	COOH	0	35	5.3
$N^+C_5Lys2C_{16}$	NH_2	0.1	20	10.5	$N^+C_5Asp2C_{16}$	COOH	0.1	35	5.8
$N^+C_5Lys2C_{16}$	NH_2	0.1	35	9.8	$N^+C_5Glu2C_{16}$	COOH	0	35	4.6
$N^+C_1Lys2C_{16}$	NH_2	0	5	6.1	$Suc2C_{16}$	COOH	0	5	10.0
$N^+C_1Lys2C_{16}$	NH_2	0	20	6.1	$AcHis2C_{16}$	imidazolyl	0	35	3.6
				Vesicle					
$C^+C_5Lys2C_{16}$	NH_2	0	−	9.4					

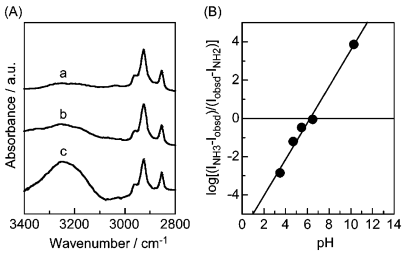

Figure 3. (A) Reflection−absorption infrared (RA-IR) spectra of the $N^+C_5Lys2C_{16}$ monolayer transferred onto a gold-covered glass slide: (a) at pH 4.4; (b) at pH 6.5; (c) at pH 10.3. (B) Linear plots for the p*K* determination using IR intensities (absorbances) at 3250 cm^{-1} relative to those at 2925 cm^{-1}, where I_{obsd}, I_{NH3}, and I_{NH2} represent the corresponding values of the observed films, the value for the protonated species, and the value for the unprotonated species, respectively. The intercept of the abscissa provides the p*K* value.

would suppress protonation of the ϵ-amino group of Lys because of destabilization of charged species in the nonpolar medium through increased electrostatic potentials. On the basis of the nonlinear Poisson−Boltzmann equation Smart and McCammon estimated the p*K* value of a long-chain alkylamine at the air−water interface and demonstrated that a distance of a few angstroms above the water surface results in a shift of several units in the p*K* of the amino group.[16] According to their estimation, the distance between the polar head and the Lys amino group in $N^+C_5Lys2C_{16}$, as estimated by molecular modeling (0.8−1.4 nm), corresponds to a positioning of the Lys amino group appropriate for a significant shift of the p*K*. The importance of interface structure on electrostatic interaction was similarly estimated by Sakurai et al. on the basis of quantum chemical calculations in which the interaction between cationic guanidinium and anionic phosphate could be significantly strengthened by the influence of the hydrophobic lipid phase.[17] If the water−lipid interface is more disordered as expected for aqueous vesicles, the influence of the hydrophobic phase would not be effectively communicated to functional groups embedded at the interface. In fact, the difference in efficiency of molecular interaction between these interfaces was experimentally demonstrated by Onda et al. who reported that the binding constants between guanidinium and phosphate ions at the air−water interface are 10^3-10^4 times larger than those at the surface of an aqueous bilayer.[8] This report also supports an enhanced electrostatic effect at the air−water interface. Interestingly, the difference in guanidinium/phosphate binding constant in Onda's work corresponds roughly to the p*K* difference of the Lys amphiphile between the two interfaces, although the molecular interactions examined are opposing. Some literatures reported differences of hydrogen bonding capability between the air−water interface and the micelle surface, which may be also related with the above-mentioned phenomena.[18] Very recently, we found a significant p*K* difference in phosphoric acid dissociation between the Langmuir monolayer and the aqueous vesicle, where the former interface induces a larger shift than that at the latter one, and their difference is ca. 4 units.[19]

The experimental and theoretical information listed here indicates that a more efficient p*K* shift can be obtained at the air−water interface than in aqueous vesicles. This may be caused by enhanced molecular interactions at the air−water interface where influence of the hydrophobic phase would be effectively communicated to the interfacial molecular species probably because of the smoothness of the interface.

2. Tuning of p*K* Values at the Air−Water Interface. Table 1 summarizes the p*K* values for the Langmuir monolayers investigated. The p*K* values changed significantly, depending on ionic strength of the subphase, while the influence of the surface pressure can be neglected. As plotted in Figure 4, the Lys p*K* values in both the $N^+C_5Lys2C_{16}$ and $N^+C_1Lys2C_{16}$ monolayers increase as ionic strength increases and approach closely the value reported in a bulk aqueous medium. The effect of electric repulsion between the trimethylammonium group and Lys ammonium or between Lys ammonium groups themselves would be shielded by addition of salts. Our most important finding is that the p*K* of the Lys residue can be tuned by ca. 4 or 5 units simply by changing the ionic strength of the aqueous subphase.

The p*K* values of various amino acids can be also tuned by molecular design. The p*K* of the $N^+C_1Lys2C_{16}$ monolayer is lower by ca. 1 unit than that of the $N^+C_5Lys2C_{16}$ monolayer.

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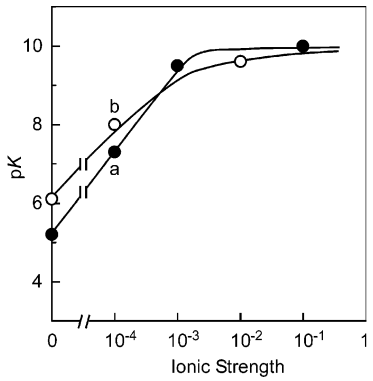

Figure 4. Effect of ionic strength of the aqueous subphase on p*K* values of the lysine residue at 20 °C: (a) $N^+C_5Lys2C_{16}$; (b) $N^+C_1Lys2C_{16}$.

The Lys residue in the $N^+C_1Lys2C_{16}$ monolayer may locate in a more hydrophilic environment than that in the $N^+C_5Lys2C_{16}$ monolayer because of the short spacer between the Lys group and the polar head. The p*K* behaviors of $N^+C_5Asp2C_{16}$ and $N^+C_5Glu2C_{16}$, which contain COOH as a dissociation group, are somewhat different from those observed for the Lys-based amphiphiles. The p*K* values obtained lie within a range from 4.6 to 5.8 and are similar to those of carboxylates in water. In addition, an increase in the ionic strength did not significantly change the p*K* value. In these monolayers, destabilization of the carboxylate group might be compensated by charge attraction from the cationic polar head. In sharp contrast, the monolayer of $Suc2C_{16}$, which contains no cationic headgroup, showed a significant p*K* shift (10.0), where the effect of strong destabilization of the charged species at the air−water interface becomes apparent. Similarly, $AcHis2C_{16}$ does not contain a charged headgroup, and its monolayer displays a large decrease in p*K* of its imidazolyl group compared with the corresponding value observed in bulk water. All the results obtained here suggest the crucial role of electrostatic interactions for p*K* shifts at the air−water interface.

Evaluation of the apparent dielectric constant at the air−water interface provides indispensable information for further tuning of the surface p*K*. We adopted a simplified model for this purpose although many detailed but complicated equations have been thus far proposed.[20] In analogy with the van der Waals equation in two dimensions, the following equation holds between surface pressure π and molecular area A with excluded area (A_o), Boltzmann constant (k), and absolute temperature (T).[21]

$$(\pi - \pi^*)(A - A_o) = k\mathrm{T} \tag{3}$$

The term π^* represents the contribution of molecular interaction to the surface pressure and has dimensions of energy per area. The energy term from molecular interaction may be given generally by a polynomial expression of the averaged molecular distance (r), giving the general expression of π^* as $\sum(a_i/r^i)(1/r^2)$. Therefore, surface pressure π is given by eq 4.

$$\pi = \sum\left(\frac{a_i}{r^i}\right)\left(\frac{1}{r^2}\right) + \frac{kT}{A - A_o} \tag{4}$$

Differences in surface pressure between protonated (π_{BH}) and unprotonated species (π_B) at a given molecular area are given by eq 5.

$$\pi_{BH} - \pi_B = \Delta\left[\sum\left(\frac{a_i}{r^i}\right)\left(\frac{1}{r^2}\right)\right] \tag{5}$$

If the difference in surface pressure between protonated and unprotonated species is dominated by electrostatic interaction contributions, the energy term in $(C/\epsilon) \times (1/r)$ becomes the main attribute in $\sum$ terms, where ϵ is the dielectric constant and C is the constant depending on the two-dimensional geometries of amphiphiles. This assumption simplifies the eq 5 with constant term C' as follows.

$$\pi_{BH} - \pi_B = \left(\frac{C}{\epsilon}\right)\left(\frac{1}{r^3}\right) \tag{6}$$

$$(\pi_{BH} - \pi_B)A^{3/2} = \frac{C'}{\epsilon} \tag{7}$$

This consideration was applied to the actual $\pi-A$ isotherms of $N^+C_1Lys2C_{16}$ in the protonated (Figure 5A(a)) and unprotonated states (Figure 5A(b)). A plot of $(\pi_{NH3} - \pi_{NH2})A^{3/2}$ as a function of molecular area (Figure 5B) becomes constant between the lift-off pressure and the collapse pressure. Influence of a double-layer potential on surface acidic environment has been discussed on the basis of Gouy−Chapmann theory.[22] Consideration of the surface pressure difference based on the Gouy−Chapmann theory gives eq 8, which contains the constants (D and D') and bulk concentration of ions (c).[21]

$$\pi_{BH} - \pi_B = Dc^{1/2}\left[\cosh\left\{\sinh^{-1}\left(\frac{D'}{Ac^{1/2}}\right)\right\} - 1\right] \tag{8}$$

Davies proposed a simplification of this term to $2kT/A$.[19a] However, a plot of $(\pi_{NH3} - \pi_{NH2})A$ does not show the constant behavior as effectively as a $(\pi_{NH3} - \pi_{NH2})A^2$ plot (Figure 5C). In addition, plotting of logarithms of $\pi_{NH3} - \pi_{NH2}$ against A shows a slope of 1.49 with a correlation coefficient of 0.995 (Figure 5D). These results prove the viability of eq 7.

Plots based on eq 7 provide relative values of the apparent dielectric constant (ϵ). Figure 6 shows values calculated for the $N^+C_5Lys2C_{16}$ and $N^+C_1Lys2C_{16}$ monolayers at various ionic strengths. These dielectric constants are values relative to those observed for the $N^+C_5Lys2C_{16}$ monolayer with ionic strength of 0.1 where the observed p*K* value is close to that of amino groups in bulk water. If we assume that dielectric constant at high ionic strength equals that of bulk water (80 at 20 °C), dielectric constants at the surfaces of $N^+C_5Lys2C_{16}$ and $N^+C_1Lys2C_{16}$ monolayers on pure water are estimated to be 52 and 66, respectively. These values are in good agreement with those estimated experimentally by Grieser et al.[15a] and Petrov and Möbius.[15c] However, determination of absolute

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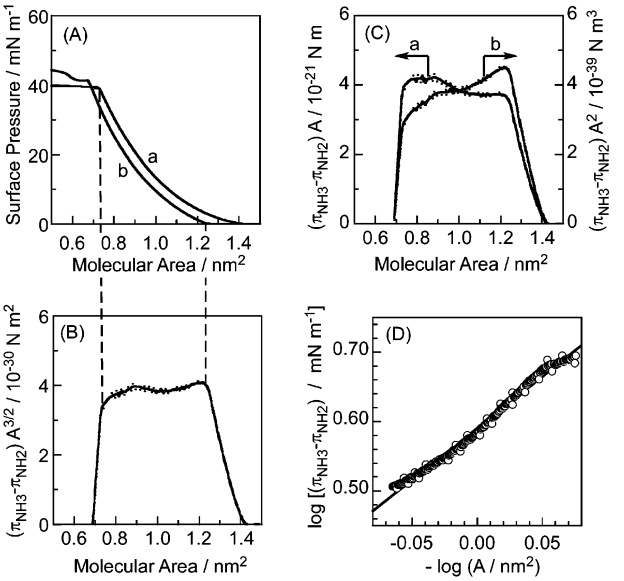

Figure 5. (A) $\pi-A$ Isotherms of $N^+C_1Lys2C_{16}$ (20 °C): (a) at pH 3; (b) at pH 11.0. (B) Plot of the parameter in eq 7 $[(\pi_{NH3} - \pi_{NH2}) \times A^{3/2}]$ as a function of the molecular area using the data presented in (A). (C) Plots of the parameter in (a) $[(\pi_{NH3} - \pi_{NH2}) \times A]$ and (b) $[(\pi_{NH3} - \pi_{NH2}) \times A^2]$ as a function of the molecular area using the data presented in (A). (D) Linear relation between $\log(\pi_{NH3} - \pi_{NH2})$ and $-\log A$ where the slope and intercept are 1.49 and 0.59, respectively, with the correlation coefficient of 0.995.

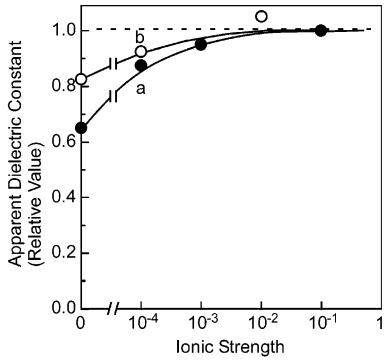

Figure 6. Effect of ionic strength on apparent dielectric constants at the monolayer surface: (a) $N^+C_5Lys2C_{16}$; (b) $N^+C_1Lys2C_{16}$. These dielectric constants are values relative to that observed for the $N^+C_5Lys2C_{16}$ monolayer at an ionic strength of 0.1.

values of interfacial dielectric constants still needs careful consideration as seen in discussions in some pioneering reports.[23]

3. Ester Hydrolysis Catalyzed by p*K*-Tuned Amino Groups. Basic amino groups are able to catalyze hydrolysis of esters, but their protonated forms are not. Therefore, p*K* shifts of the amino groups to the neutral pH region are indispensable for ester hydrolyses under biological conditions and are commonly accomplished in enzyme systems. Tuning of the p*K* of Lys residues at the air−water interface could mimic this biological activity.

A model system contains hexadecanoic acid 4-nitrophenyl ester ($PNPC_{16}$) as hydrolysis substrate in the monolayer of $N^+C_5Lys2C_{16}$ as the catalyst. Changes of surface-absorption spectra, upon dissolution of *p*-nitrophenyl residue, were monitored for evaluation of the hydrolysis of $PNPC_{16}$ (see typical example in Figure 7A). Conversion of the hydrolysis was plotted

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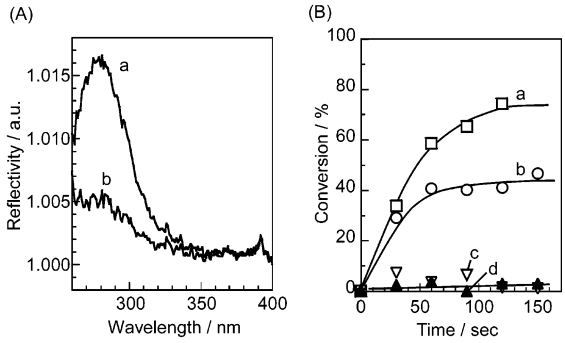

Figure 7. (A) Surface absorption spectra of the mixed monolayer of N^+C_5-$Lys2C_{16}$ and $PNPC_{16}$ (2:1 in molar ratio) at pH 5.1 and at ionic strength of 0 (20 °C, 20 mN m^{-1}): (a) 0 s; (b) after 120 s. (B) Time-course of conversion of the $PNPC_{16}$ hydrolysis within the mixed monolayer [lipid X:$PNPC_{16}$ with 2:1 in molar ratio] (20 °C, 20 mN m^{-1}): (a) lipid X = $N^+C_5Lys2C_{16}$ at pH 5.1 and at ionic strength of 0; (b) lipid X = $N^+C_5Lys2C_{16}$ at pH 8.0 with ionic strength of 0; (c) lipid X = $N^+C_5Lys2C_{16}$ at pH 8.0 and at ionic strength of 0.1; (d) lipid X = $N^+C_5Ala2C_{16}$ at pH 8.0 and at ionic strength of 0.

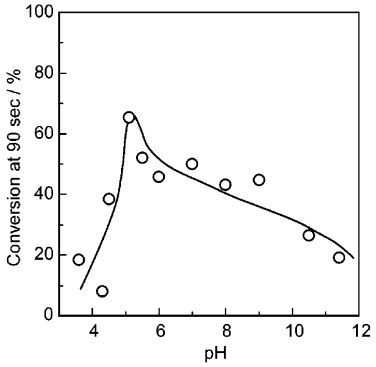

Figure 8. Conversion of the $PNPC_{16}$ hydrolysis within the mixed monolayer [$N^+C_5Lys2C_{16}$: $PNPC_{16}$ with 2:1 in molar ratio] after a reaction time of 90 s under various pH conditions (20 °C, 20 mN m^{-1}).

as a function of reaction time (Figure 7B). The $N^+C_5Lys2C_{16}$ monolayer promoted the $PNPC_{16}$ hydrolysis at pH 5.1 (a) and pH 8.0 (b), while the $N^+C_5Ala2C_{16}$ monolayer did not show any hydrolytic activity (d). The latter fact suggests that hydroxyl anions in the vicinity of the quaternary ammonium headgroup, if any, do not contribute to the hydrolysis, and the lysine amino groups play an indispensable role in the hydrolysis. Interestingly, increase of ionic strength (0.1) drastically suppressed the $PNPC_{16}$ hydrolysis (c). Lower population of free amine under this condition leads to inactivation of the $N^+C_5Lys2C_{16}$ catalyst, because the p*K* value of the lysine residues at the interface are shifted to 10.5 at the ionic strength of 0.1 (see Table 1).

The $PNPC_{16}$ hydrolysis catalyzed by $N^+C_5Lys2C_{16}$ was investigated under various pH conditions, and the conversion at 90 s was plotted as a function of subphase pH (Figure 8). The maximum conversion was observed at around pH 5 although the data were scattered. Because the p*K* value of the Lys residue at the corresponding condition is 5.1, coexistence of free amine and protonated ammonium seems to more effectively promote the reaction. Cooperative action of NH_2 and NH_3^+ groups, where electrostatic interaction between ester carbonyl oxygen and NH_3^+ group assists nucleophilic attack by the NH_2 group at the ester carbonyl carbon (see Figure 9), may play an important role in the $PNPC_{16}$ hydrolysis in the present case.

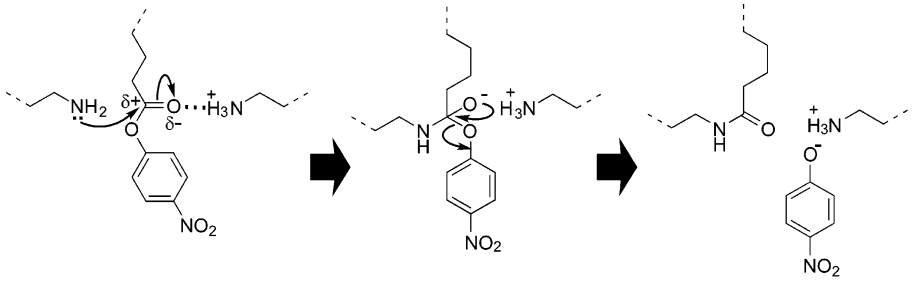

Figure 9. Plausible mechanism of the $PNPC_{16}$ hydrolysis by lysine residues at the air−water interface.

Conclusion

The results obtained impressively illustrate that the air−water interface is an appropriate medium for control of p*K* of amino acid residues, where the p*K* values can be tuned within the range of 4 or 5 units simply by adjusting the ionic strength in the subphase. The origin of the effective p*K* shift at the air−water interface when compared with the aqueous vesicle medium is still unclear, but the smooth and undisturbed nature of the air−water interface may enhance the influence of the low dielectric phase on functional groups embedded at the air−water interface. The hydrolysis by amino groups over the neutral pH region, which is commonly seen in biological catalysts, was demonstrated through p*K* control at the air−water interface. Rich possibilities in design of amino acid-carrying amphiphiles and the free mixing nature of Langmuir monolayers should lead to development of a more sophisticated catalysis as seen in enzyme reaction pockets. Elaborate functions, that can be referred to as a "Langmuir enzyme (L-zyme)" will be realized as the end result of the concept proposed in this research,[24] and demonstration of other reactions is now under investigation for generalization of this concept.

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Supporting Information Available: Syntheses of $N^+C_1Lys2C_{16}$, $N^+C_5Glu2C_{16}$, $Suc2C_{16}$, and $AcHis2C_{16}$. This material is available free of charge via the Internet at http://pubs.acs.org.

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