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Interaction of indole derivatives and tryptophan peptides with interfaces of sodium dodecyl sulfate micelles

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Abstract: The free energies of transfer for indole and tryptophan derivatives and pentapeptides having single tryptophan residues from aqueous to sodium dodecyl sulfate (SDS) micellar phases have been systematically studied using the conventional method of ultraviolet absorption spectrophotometry. The free energies for the position isomers of methyl indoles varied depending on the substitution positions. Thus, the contribution of the methyl group to the binding affinity of the 4-methyl indole to the micelle was about twice that of the 2- and 7-methyl indoles. The free energy changes with the introduction of halogen groups to the indole rings were correlated to the nonpolar water-accessible surface area (ΔA_{np}) of the halogen moieties, which were regarded as hydrophobic. The relationships followed straight lines passing through the origins. Position dependence having tendencies similar to the methyl indoles was observed among the magnitudes of the slopes of the straight lines. These results strongly suggest that the indole rings of the derivatives residing in the micellar interface regions direct their imino moieties –NH– toward the micellar surfaces. Experiments using model tryptophan pentapeptides showed that the magnitude of free energy change per methylene unit of an alkyl amino acid residue in the pentapeptide increased with elongation of the alkyl moiety and was not a constant value as reported for various alkyl compounds. When the peptides distribute to the SDS micelles, the peptide backbones are anchored in aqueous phases and the amino acid side chains in the interfaces extend their alkyl groups toward the micellar centers. Thus, the free energy changes can be connected to the positions of the alkyl groups of the amino acid residues in the micelles.

Keywords: methyl indoles; tryptophan peptides; indole halides; UV absorption; free energy of transfer; SDS micelle; micellar interface; nonpolar water-accessible surface area

INTRODUCTION

Membrane–peptide interaction has been studied from a variety of perspectives owing to its biological significance. It is generally accepted that hydrophobic interaction is responsible for the distribution of the peptide from the aqueous phase to the membrane [1]. Nevertheless, many hydrophobic amino acid residues in the peptides, especially aromatic residues such as tryptophan and phenylalanine, seem to distribute favorably to the interface region of the membrane [1–4]. It is important to describe in detail how the peptide interacts with the membrane interface and how the hydrophobic and other interactions contribute to interface distribution. The membrane interface is a complicated system in which many species of functional groups of membrane constituents coexist in a narrow region of about 1.5 nm in thermal thickness [1]. In addition, the peptide is a complex cluster covalently bonded by amphiphilic groups. In order to elucidate the mode of the interaction in such a complicated system, it is necessary to collect fundamental data, using the detergent micelles such as sodium dodecyl sulfate (SDS) that can be regarded as a membrane-mimetic media and the small molecules constituting the peptide.

The free energy of transfer for a compound is a measure of the difference in its affinity to the membrane or the detergent micelle. The value can be taken as the total contribution of all functional groups of the compound and the value reflects the result of accommodation among the groups. An alkyl group is a typical hydrophobic constituent. From experiments of the solubilization or binding of alkyl compounds to SDS micelles, it has been observed that the free energies are proportional to the number of methylene units [5–10]. The slope of the relationships representing the contribution per methylene unit lies over a relatively wide range. However, the magnitudes of these reported values are low compared to the free energies of transfer of the methylene units from water to organic solvents [6,10]. It is assumed that the contribution of a functional group varies depending on its position in the micelles. In order to evaluate the contribution of the group to the free energy, therefore, systematic investigation is necessary using a compound that has many types of derivatives.

Indole derivatives were used in this study. Distinct from the benzene ring, the indole ring, which is a major side chain of the tryptophan residue, is structurally and electrostatically anisotropic because of its imino group moiety. On the basis of a simple pseudophase equilibrium theory, the free energies of transfer of the indole derivatives to the SDS micelles were obtained

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from changes in the ultraviolet (UV) absorption spectra. Contributions of methyl (methylene) groups of the alkyl indoles to the free energies of transfer were systematically investigated and discussed relative to their dissolving sites and to the orientation of the indole rings in the SDS micelles. A tryptophan residue with the indole ring can also play a role as a probe for measuring UV absorption. Model pentapeptides having single tryptophan residues were synthesized in order to investigate the free energy change accompanied by elongation of the methylene group on an amino acid residue of the peptide. We attempted to visualize the mode of interaction between the peptides and the SDS micelles.

MATERIALS AND METHODS

Materials

The following indole derivatives were used: indole, 3-methyl indole, and 5-methoxy indole from Nacalai Tesque; 2-ethyl, 5-ethyl, 6-hydroxy, 4-fluoro, 4-bromo, 7-fluoro, and 7-bromo indoles, 5-methyl indolecarboxylate, 7-methyl indole carboxylate, 6-indolol and 2-methyl-5-indolol from Wako Chemicals; 7-ethyl and 3-acetyl indoles from Acros Organics; 4-methoxy indole from Tokyo Kasei Kogyo; 3-acetoxy indole and tryptophan alkyl esters from Sigma; and 2-acetyl indole from TRC Inc. All the other indole derivatives were from Aldrich. Sodium dodecyl sulfate (99% pure) was obtained from Nacalai Tesque. The model pentapeptides were synthesized by the standard Fmoc method [11] and purified using an HPLC-ODS column (Tosoh) with a linear gradient from 15 to 50% acetonitrile containing 0.1% trifluoroacetic acid. The resulting peptides appeared as a single peak (above 95%) on the HPLC column and showed the expected mass by mass spectrometry.

Measurements of UV Absorption

Samples of the indole derivatives and tryptophan peptides were dissolved in 50 mM phosphate buffer, pH 6.0. The sample solutions containing various concentrations of SDS for the UV absorption measurements were prepared by adding a concentrated stock solution of SDS. The absorption spectra were measured with a Hitachi U-3310 spectrophotometer using a 1-cm cell in a temperature-controlled holder at 25 °C. The samples were prepared at a concentration that kept absorbance for the major peak around 280 nm at 0.854 in the buffer without SDS, corresponding to a concentration of the tryptophan derivatives at 0.150 mM [12]. Figure 1 shows the UV absorption spectra (A) for 3-methyl indole in the phosphate buffer containing different concentrations (0–0.14 M) of SDS and the differential spectra (B) between those for 0 mM and for the other concentrations of SDS. A fixed wavelength (292 nm for 3-methyl indole) at which the differential spectra gave a major peak was used for the analysis. The absorbance at this wavelength is linearly related to the amount of the compound distributed to the SDS micelle [13].

Data Treatments

The equilibrium distribution of the indole derivatives and tryptophan peptides from aqueous to SDS micellar phases is given by the following distribution coefficient:

$$K_d = C_M/C_A \quad (1)$$

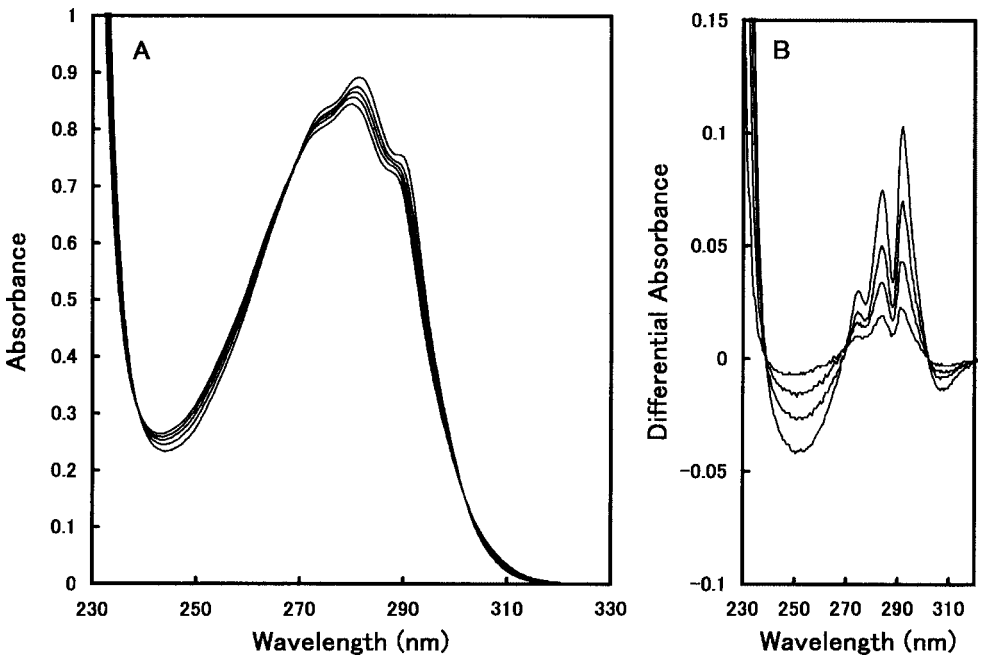

Figure 1 Ultraviolet absorption spectra (A) of 3-methylindole in various concentrations of SDS and their differential spectra (B). (A) SDS concentrations in 50 mM sodium phosphate (pH 6.0) buffer were 0, 3.47, 7.11, 13.9, and 76.3 mM, respectively, from lower to upper at around 280 nm. (B) Difference between spectra for the SDS concentration and those for absence of SDS. Sodium dodecyl sulfate concentrations were 3.47, 7.11, 13.9, and 76.3 mM from lower to upper, respectively.

where C_A and C_M are the local molar concentrations of the compounds in both phases, respectively. Measurements of the changes in the absorbance at a fixed wavelength depending on the micellar concentrations of SDS gave the values of K_d. The values of the changes in the absorbance can be treated using the following equation [5]:

$$A_0 - A = (A_0 - A_m)\upsilon C_m/(\upsilon C_m + 1/K_d) \quad (2)$$

where A, and A_0 are the absorbances measured in a micellar concentration C_m (gl^{-1}), and in a buffer without micelles, A_m is the absorbance for the sample dissolved in the micelles, and υ is the partial specific volume of a SDS micelle, respectively. The dependence of the SDS concentration on absorbance and the double reciprocal plots between $A_0 - A$ and C_m are shown in Figure 2. A steep increase occurred above the cmc of the SDS concentration (Figure 2(A)). The value of cmc determined with stalagmometry as 1.72 mM (0.496 gl^{-1}) in the buffer was used. The double reciprocal plot gave a straight line (Figure 2(B)); distribution coefficient K_d was obtained from the slopes and intercepts of the plots. The free energies of transfer (ΔG) for the derivatives from the aqueous to micellar phases were obtained from the K_d values using the following equation [5]:

$$\Delta G = -RT \ln(n_w \times K_d \times \upsilon_m) \quad (3)$$

where υ_m and n_w were the partial molar volume of SDS (0.249 mol l^{-1}) and the amount of water per the volume of the aqueous phase (55.5 mol l^{-1}), respectively. The obtained transfer of free energies correspond to those treated by the mole-fraction partition coefficients.

Nonpolar Water-accessible Surface Area

In essence, the nonpolar water-accessible surface area (A_{np}) was estimated according to the method of Lee and Richards [15]. The values were calculated by the equation $A_{np} = \Sigma(R/(R^2 - Z_i^2)^{-2})DL_i$, where R is the sum of the van der Waals radius of an atom and the radius of water molecule (0.14 nm), L_i is the length of the arc drawn on a given section i, Z_i is the perpendicular distance from the center of the atom to the section i, and D is the distance (0.001 nm) between the sections [15]. As the imino group of the indole ring was regarded as polar, its surface area was not added to A_{np}. Values of the van der Waals radii for halogen atoms provided by Bondi [16] were employed. The methyl and methylene groups were approximated as a sphere that has a radius of 0.20 nm [16]. The bond distances and angles were cited from Spartan '04 (Wave function, Inc.). By replacing the hydrogen atom (van der Waals radius of 0.12 nm) of the indole ring with a functional group, the increment in the nonpolar water-accessible surface area (ΔA_{np}) is the difference between A_{np} for the group and the loss of A_{np} on the indole ring, caused by the replacement.

RESULTS

Free Energies of Transfer of Alkyl Indoles and Indole Halides

It has been reported that alkyl and halogen groups of a variety of organic compounds facilitate the distribution of compounds from the aqueous to detergent micellar phases [5,9,17]. Table 1 lists the values of the free energies of transfer for the investigated alkyl indoles and indole monohalides. The introduction of a methyl or halogen group to the indole ring stimulated the affinity to the micelle. The values apparently depended on the positions of the groups on the indole rings. For

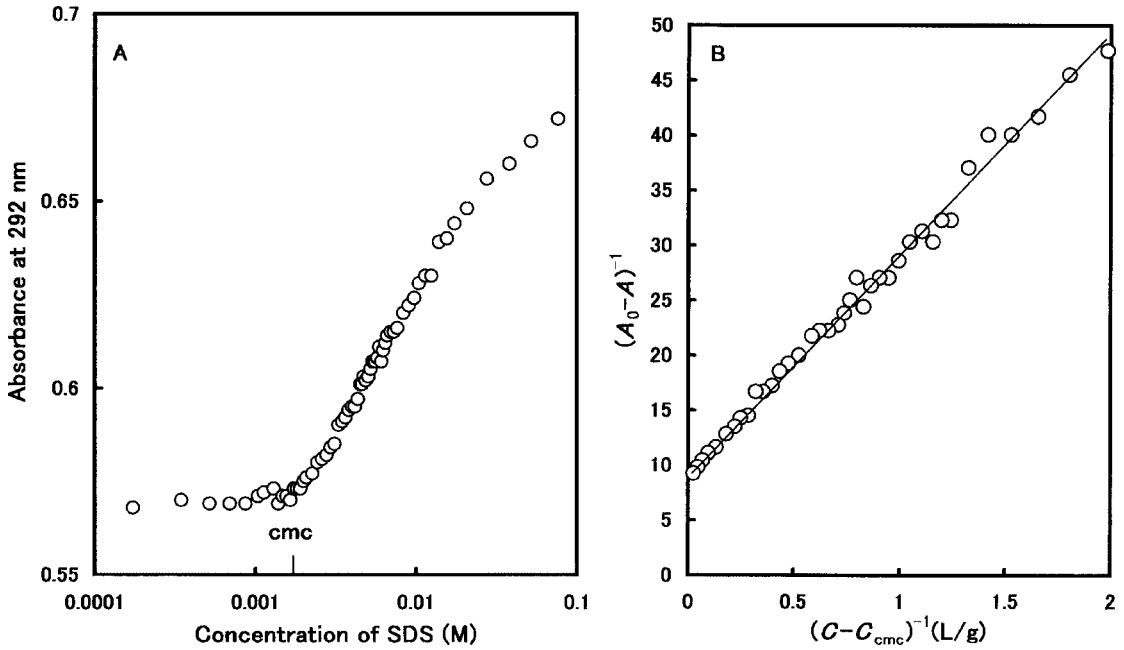

Figure 2 SDS concentration dependence of absorbance at 292 nm for 3-methylindole (A) and their double reciprocal plot between A_0–A and micellar concentrations (B). Distribution coefficient K_d was obtained from the slope and intercept of the double reciprocal plot using υ of 0.863 cm^3/g [14].

Table 1 Free energies of transfer for alkyl indoles and indole halides

Position	Functional group	$-\Delta G$ (kJ mol^{-1})[a]	$-\Delta\Delta G$ (kJ mol^{-1})[b]	ΔA_{np}(nm^2)[c]
	Indole	19.42 ± 0.04	0	0
1	CH_3–	21.49 ± 0.07	2.07	0.585
2	CH_3–	21.16 ± 0.07	1.74	0.376
2	CH_3CH_2–	23.41 ± 0.02	3.99	0.670
3	CH_3–	21.90 ± 0.07	2.48	0.270
4	CH_3–	21.92 ± 0.11	2.49	0.245
5	CH_3–	21.73 ± 0.08	2.31	0.282
5	CH_3CH_2–	23.71 ± 0.16	4.29	0.559
6	CH_3–	21.56 ± 0.06	2.14	0.286
7	CH_3–	21.25 ± 0.09	1.82	0.331
7	CH_3CH_2–	22.49 ± 0.09	3.06	0.636
4	F–	21.03 ± 0.07	1.62	0.079
	Cl–	23.55 ± 0.04	4.12	0.211
	Br–	24.38 ± 0.08	4.96	0.272
5	F–	20.70 ± 0.07	1.27	0.094
	Cl–	23.51 ± 0.07	4.08	0.249
	Br–	24.45 ± 0.09	5.02	0.313
	I–	25.89 ± 0.06	6.47	0.407
6	F–	20.77 ± 0.05	1.35	0.094
	Cl–	23.15 ± 0.03	3.73	0.247
	Br–	24.09 ± 0.06	4.67	0.313
7	F–	20.64 ± 0.08	1.22	0.106
	Cl–	22.93 ± 0.07	3.51	0.273
	Br–	23.65 ± 0.07	4.23	0.346

[a] Values are means ± SD of measurements for three to five different preparations.
[b] Difference between ΔG of indole derivatives and that of indole.
[c] Difference between A_{np} of indole derivatives and that of indole.

example, the magnitude of the free energy change for the transfer per methyl group for the 3- (2.5 kJ mol^{-1}) or 4-methyl (2.5 kJ mol^{-1}) indole was significantly greater than that for the 2- (1.7 kJ mol^{-1}) or 7-methyl (1.8 kJ mol^{-1}) indole. It was observed for the halogen monosubstituents that the affinity increased with an increase in the period of the halogen group. This tendency is consistent with that reported for the benzene halides [9].

Relationships Between Nonpolar Water-accessible Surface Area of Hydrophobic Groups and Their Contributions to Free Energies

The hydrophobic interaction between the groups and hydrocarbon tails of the detergents forming the micelles is a significant factor in the stabilization of compound–SDS micelle complexes. It is accepted that the hydrophobic contributions (ΔG_{np}) for the free energies of transfer of the compounds is proportional to the nonpolar water-accessible surface area (A_{np}) [1].

$$\Delta G_{np} = -\sigma A_{np} \quad (4)$$

where σ is the so-called *solvation parameter*. Because the indole ring is structurally anisotropic and distinct from the benzene ring, it is necessary to compare the free energies per nonpolar water-accessible surface area of the methyl group depending on the substitution positions for the indole rings. If the contributions of the methyl groups for the free energies ($\Delta\Delta G$) are exclusively hydrophobic, the values of $-\Delta\Delta G/\Delta A_{np}$ will be equal to the solvation parameter σ, where ΔA_{np} is the increment in A_{np} by replacement of the hydrogen atom with the methyl group. Figure 3 shows the relationships between $-\Delta\Delta G/\Delta A_{np}$ and the positions of the methyl groups. The magnitude of $-\Delta\Delta G/\Delta A_{np}$ varied remarkably depending on the positions of the methyl groups. The values calculated using ΔG for the alkyl benzenes reported earlier for the SDS micelle systems are 7–9 kJ mol^{-1} nm^{-2} [5,6,8–10]. These values are smaller than that for 4-methyl indole (10 kJ mol^{-1} nm^{-2}) but larger than those for the 2-methyl (4.6 kJ mol^{-1} nm^{-2}) and 7-methyl indoles (5.5 kJ mol^{-1} nm^{-2}). The value for the 5-methyl indole (8.2 kJ mol^{-1} nm^{-2}) was close to those for the alkyl benzenes. These results can be explained as follows. A hydrophilic imino moiety of the indole ring exists on the interface area close to the micellar surface, whereas the 4-position is directed toward the micelle

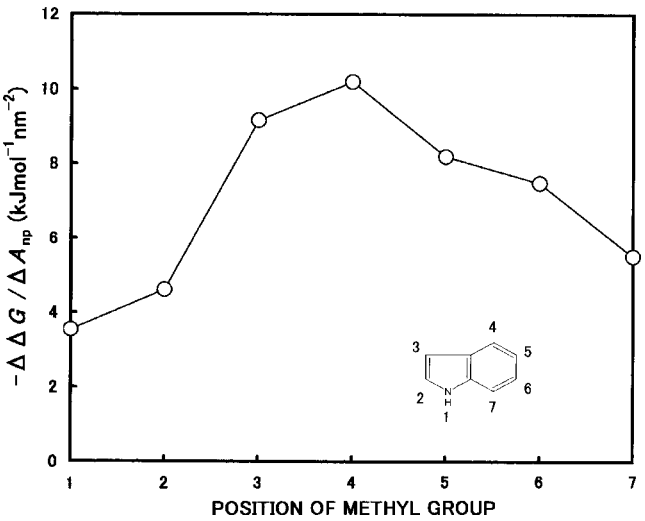

Figure 3 Dependence of $-\Delta\Delta G/\Delta A_{np}$ of methyl groups on substituent position of indole ring for methyl indoles. The differences (ΔA_{np}) between the water-accessible surface area of each methyl indole and that of the indole were calculated as the difference between the surface area of the methyl group of which the van der Waals radius was taken as 0.20 nm and those lost by methyl substitution. The imino group of the indole ring was regarded as polar and its surface area was not added to A_{np}.

center. Under such environmental conditions, the 4-methyl group on the indole ring can contribute more effectively to the stabilization of the compound–SDS micelle complexes than can the methyl group on the benzene ring, which is structurally homotropic. On the basis of the measurement of the change of the heat capacity by the partition of the 3-methyl indole to a model biomembrane, Wimley and White suggested that the –NH– moiety of the compound maintains access to the aqueous phase [2]. Their conclusion is fundamentally consistent with our results, even if there is a certain difference in the structural complexity between the biomembrane and SDS micelle.

Halogen groups can be regarded as hydrophobic [9,17]. Linear correlations were obtained between the $\Delta\Delta G$ values and the ΔA_{np} of the halogen groups, as shown in Figure 4(A). It was shown that the contributions of the halogen groups for the affinities obeyed Eqn (4), i.e. the relationships gave straight lines and coincided with the origins. This means that the contribution in each substitution position depends only on the water-accessible surface area of the halogen groups and indicates that the microenvironment on the substitution position is independent of the kinds of the substituted halogen groups. The slope of the relationship ($-\Delta\Delta G/\Delta A_{np}$) depended on the position of the groups, which showed tendencies similar to those for the methyl groups; however, the magnitudes were fairly large. The contributions of the halogen groups positioned near the imino group of the indole ring were not as large as were those of the halogen groups positioned away from the imino group. If the values of $-\Delta\Delta G/\Delta A_{np}$ could be considered to reflect the positions of the hydrophobic halogen groups in the micelle, then these results for the indole halides also support the explanation that the imino nitrogen moiety of methyl indole distributing to the micellar interface faces in the direction of the aqueous surface.

Contribution of Methyl Groups to the Free Energies of Transfer for Ethyl Indoles

Figure 4(B) shows the dependence of $-\Delta\Delta G$ on ΔA_{np} for the 2-, 5-, and 7-alkyl (methyl and ethyl) indoles. Deviations from the linear relationship were observed for the 2- and 7-alkyl indoles. The deviation by the 5-alkyl indole was small and the slope ($-\Delta\Delta G/\Delta A_{np}$) of the relationship was close to that obtained from the ΔG value reported for the alkylbenzenes, for which the relationship between $-\Delta\Delta G$ and ΔA_{np} was linear. The bends toward the opposite directions of the relationships between $-\Delta\Delta G$ and ΔA_{np} for the 2- and 7- alkyl indoles in Figure 4(B) are also explained by the upper view of the orientation of the indole ring in the SDS micelle. That is, the 2-ethyl indole can take a conformation such that its terminal methyl moiety contributes more favorably to the micelle distribution than that of the 7-ethyl indole.

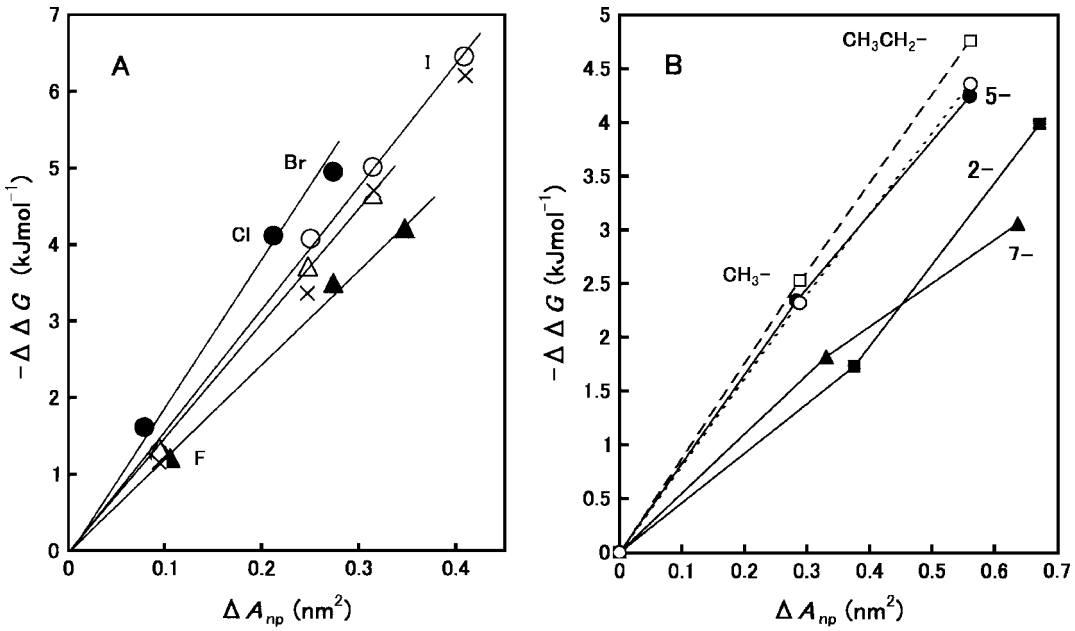

Figure 4 Relationship between the differences in the free energies of transfer($\Delta\Delta G$) and nonpolar water-accessible surface area (ΔA_{np}) for the indole halides (A) and for the alkyl indoles (B). (A): The differences, $\Delta\Delta G$, are ΔG (indole halide) $-\Delta G$ (indole). The nonpolar water-accessible surface area was calculated using the van der Waals radii of F = 0.147 nm, Cl = 0.175 nm, Br = 0.185 nm, and I = 0.198 nm [16]. Data plotted were 4-(closed circles), 5-(open circles), 6-(open triangles), and 7-substituents (closed triangles). The values of $\Delta\Delta G$ for benzene halides (×) were cited from Ref. 9. (B): The values were for 2-(closed squares), 5-(closed circles), and 7-(closed triangles) alkyl (methyl and ethyl) indoles. Open circles and squares are $\Delta\Delta G$ values for alkyl benzenes cited from Refs 9 (open squares) and 10 (open circles), respectively.

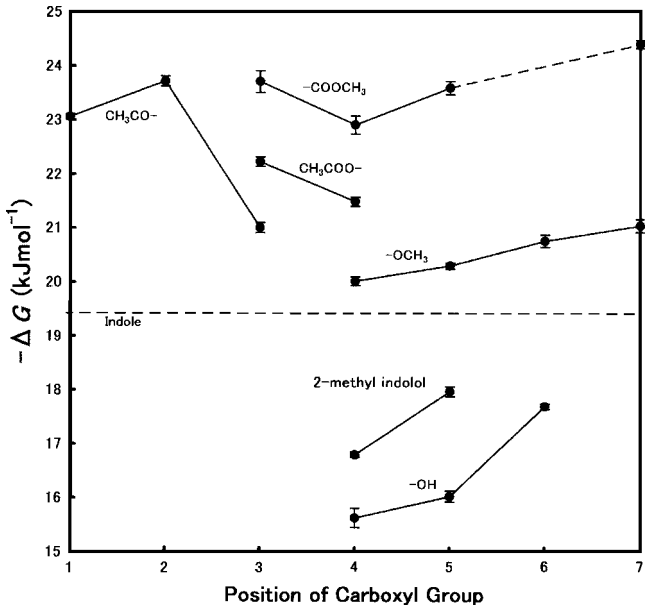

Figure 5 Dependence of ΔG of various indole derivatives on substitution positions of polar functional groups. The dashed line represents the ΔG value of the indole. Data plotted were mean values having standard deviations of measurements for three to five different preparations.

Contribution of Polar Groups to the Free Energies of Transfer of Indole Derivatives

The free energies of transfer of the position isomers of the hydrophilic groups for the indole derivatives were investigated, as shown in Figure 5. The introduction of the hydroxyl groups resulted in decreases in the affinities to the micelle. This phenomenon can be predicted by considering that attractive interactions of the hydroxyl groups of the compounds with water molecules will be lost by distribution to the micellar phase. This action is also presumed by comparison with the methoxy substituents; that is, replacement of –H of the hydroxyl group by $-CH_3$ increases the affinities well beyond the expected contribution of the methyl groups themselves. Similar decreases in the affinities by the hydroxyl groups have also been observed between benzene and phenol [18]. For methoxy indoles, accommodation of the positive contribution of the methyl group and the negative contribution of the polar -*O*- for the affinities to the micelle would result in apparently small changes of ΔG values. Therefore, dependence of ΔG on the substitution positions of the indole rings was also small. Substitution by the acetyl, acetoxy, and methoxycarbonyl groups increased the affinities. Since the methyl moieties of these groups contribute more or less positively to the affinities, the carbonyl moieties seem to inhibit the distribution of the compounds to the micelles only slightly. The $-\Delta G$ values of all the 4-substituents investigated in this experiment could be expected to be at a minimum among their homologs and those for the 2- and 7-substituents to be relatively large. These tendencies are contrary to those for the substituents by methyl and halogen groups. Sepulveda and his coworkers suggested that the factor that tends to favor the transfer of a solute from the aqueous phase to the micelle is the change in the relative positions of the hydrophilic substituents from a diametrical orientation to an adjacent position [5]. Because the 4-position is located on the opposite side of the imino group on the indole ring, a compromise of both hydrophilic groups may result in relative decreases in the values. In contrast, when multiple hydrophilic groups are adjacent to each other, the groups may contribute effectively to the distribution. Consideration for the contributions of the multiple polar groups, which will inherently favor interface or aqueous regions, might be difficult because of an assortment of problems containing interactions among multiple local dipoles.

Interaction of Oligopeptides with SDS Micelles

For the contribution of alkyl groups, the change in the free energies of transfer per methylene unit, alternative to the solvation parameter, which is the slope of the linear relationship, has been often employed in the discussion of the relationship with the manner of distribution of the compound on the micelle [6–10]. Figure 6 shows the relationship between the free energies and the carbon number of the alkyl chain for tryptophan alkyl esters at pH 10.7, at which their amino groups will be deprotonated. The relationship is approximately linear and the change in free energy per methylene unit is 2.0 kJ mol^{-1}. This value is somewhat smaller than that for the alkyl benzene or 4-methyl indole and showed good agreement with the value for the alkylphenone or benzoic acid alkyl ester [9].

Table 2 lists the free energies of transfer of the model pentapeptides. The pentapeptides, acetyl GlyXGlyGlyTrp-NH_2, were designed so that both termini

Table 2 Free energies of transfer for model pentapeptides

Pentapeptide	$-\Delta G$ (kJ mol^{-1})[a]
AcGlyGlyGlyGlyTrp-NH_2	19.99 ± 0.04
AcGlyAlaGlyGlyTrp-NH_2	20.06 ± 0.11
AcGlyAbuGlyGlyTrp-NH_2	20.89 ± 0.18
AcGlyNvaGlyGlyTrp-NH_2	22.71 ± 0.07
AcGlyNleGlyGlyTrp-NH_2	24.86 ± 0.03
AcGlyGlyLeuGlyTrp-NH_2	23.60 ± 0.01
AcGlyAlaLeuGlyTrp-NH_2	23.97 ± 0.10
AcGlyAbuLeuGlyTrp-NH_2	24.91 ± 0.05
AcGlyNvaLeuGlyTrp-NH_2	26.80 ± 0.05

[a] Values are means ± SD of measurements for three to five different preparations.

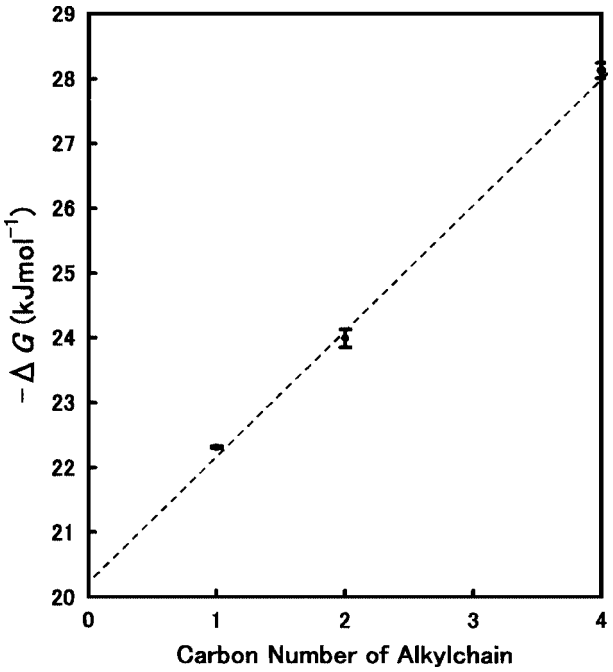

Figure 6 Relationship between free energies of transfer for tryptophan alkyl ester and the number of methylene units. Samples dissolved in 20 mM glycine buffer containing NaCl (total molar concentration of cation was 0.080 M) at pH 10.7 were used in these experiments. Data plotted were mean values having standard deviations of measurements for three different preparations.

were acetylated and amidated in order to eliminate the effect on the electric charges so that the Gly residues having no side chains were arranged before and behind the target residue -X-. To avoid the influence of the tryptophan residue on X, two glycine residues were put between both residues. The contribution to ΔG of the methylene groups in the X residue in the pentapeptides was estimated as is shown in Figure 7(A). The values of $-\Delta\Delta G$ increased concavely, not linearly, with the increase in the number of methylene groups covalently bonded to the peptide backbone. Therefore, it appears that these alkyl groups cannot fully contribute to the affinities. When the peptides distribute to the micelle, their backbones (Gly residues), different from the methyl groups, are preferentially located in the aqueous phase. Because the ΔG of the peptide bond partitioning between water and octanol is estimated at about 8 kJ mol^{-1} [1], the contribution of one or two methyl group(s) is too small to compensate for the cost of the distribution of the peptide backbone. Consequently, it is thought that the peptide bonds of X residues exist in the aqueous phase and that their alkyl moieties in the interface area extend toward the micelle center. AcetylLeuXGlyGlyTrp-NH_2 (closed circles) was designed for investigating the contribution of the hydrophobic residue (Leu) adjacent to the X residue. The introduction of Leu instead of Gly to the position adjacent to the X residue resulted in a slight increase of $-\Delta\Delta G$.

DISCUSSION

On the basis of NMR measurements [3,4] and other physico–chemical data [2], it has been reported that indole and methyl indole molecules preferentially position on the membrane interface. These compounds as well as phenyl compounds also seem to distribute to the SDS micelle interface [10], although the interface region may be simple compared with the membrane interface. When the molecules of indole derivative distribute to the interface region of the SDS micelle, it must be favorable to keep its planar surface perpendicular to the micelle surface. Our systematic investigation for the derivative strongly suggests that the indole ring in the micelle faces its imino moiety to the aqueous phase. On the basis of a molecular dynamic simulation analysis, it was reported that the interface length of the SDS micelle could be calculated to be about 0.45 nm by the definition of the region where the hydrocarbon and water densities pass through 10% of their bulk densities [19]. The thickness is comparable to the size of the indole ring, being about 0.5 nm $\times$ 0.7 nm. However, as the positions of indole rings will be perpendicular to the micelle surface, the microenvironments around its substituent groups will vary significantly with the positions. For example, dielectric constants in the interface region drastically decrease from the micelle surface toward the micelle core. Thus, it can be explained that the changes in the free energies depend significantly on the substitution positions, as shown in Figures 3 and 4.

Contribution of methylene units to partition from the aqueous to the SDS micellar phases was investigated using a model pentapeptide, AcGlyXGlyGlyTrp-NH_2 (X = Gly to Nle) (Figure 7(A)). The values of $-\Delta G$ increased concavely, not linearly, with the increase in the methylene units. When these peptides distribute to the SDS micelle, their backbones are presumably squeezed out to the aqueous phase owing to the high cost of transferring to the interface region, that is, the pentapeptide backbones are strongly anchored to the micellar surface. In fact, the difference between the ΔG values of X = Gly and X = Ala was extremely small, as listed in Table 2, implying that the methyl group adjacent to the peptide backbone (Ala residue) contributed only slightly to the affinity to the micelle and that the group exists in a similar microenvironment, probably, the aqueous phase as the peptide backbones. In contrast, the contribution of the terminal methyl group of the Nleu residue was about 2.2 kJ mol^{-1}, which was the difference between the ΔG value for X = Nva and that for X = Nle. As a result, the values of $-\Delta\Delta G$ per methylene unit increase with elongation of the alkyl chains, which are pulled toward the micelle core.

Figure 7(B) shows the relationship between the contributions of the terminal methyl groups of the

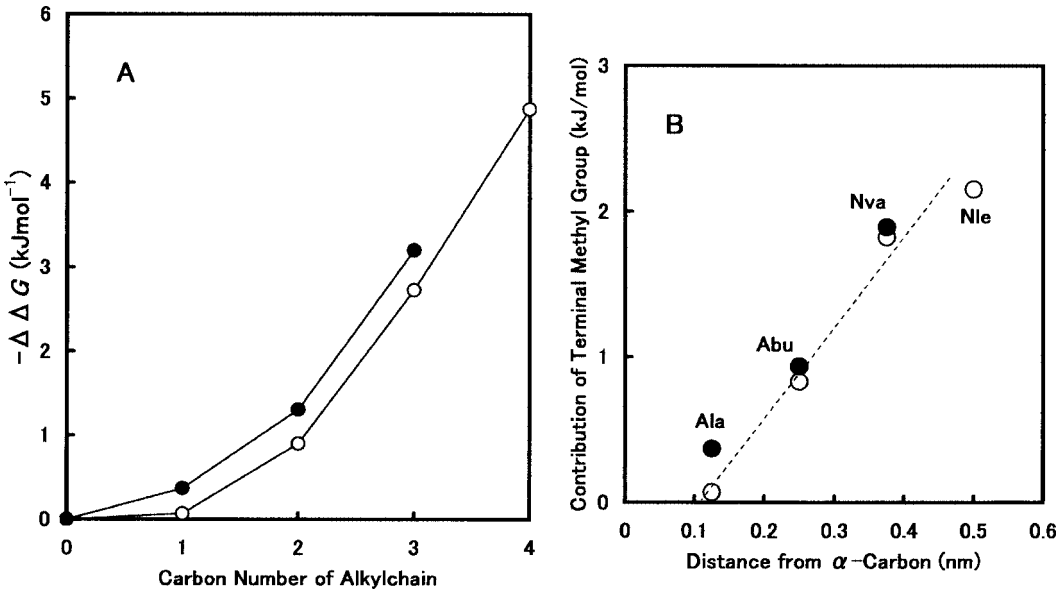

Figure 7 Contribution of alkyl groups in model oligopeptides on the free energies of transfer (A) and relationship between the contribution of terminal methyl groups of X residues in the model pentapeptides on the free energies and the perpendicular distances from the α-carbons to the methyl groups (B). The oligopeptides were Acetyl GlyXGlyGlyTrp-NH_2 (open circles), X; –H (Gly), $-CH_3$ (Ala), $-CH_2CH_3$ (Abu), $-CH_2CH_2CH_3$ (Nva) and $-CH_2CH_2CH_2CH_3$ (Nle) and acetyl GlyXLeuGlyTrp-NH_2 (closed circles) (X = Gly,Ala,Abu, and Nva). (A): Since the sample of X = Nle synthesized was only slightly soluble in the buffer, it was not used for the measurement. Free energy changes ($\Delta\Delta G$) were the differences between the free energies (open circles) for Acetyl GlyXGlyGlyTrp-NH_2 and AcetylGlyGlyGlyGlyTrp-NH_2 and those (closed circles) for Acetyl GlyXLeuGlyTrp-NH_2 and AcetylGlyGlyLeuGlyTrp-NH_2. (B) The conformation of the alkyl side chains of X residues was regarded as a *trans*-type.

X residues and the perpendicular distances from the α-carbons of the amino acid residues to the methyl groups. The relationship was approximately linear and the line intersected the horizontal line at around 0.1 nm. The micellar surface is around on 0.1 nm from the α-carbons of the X residues and, accordingly, the α-carbons are exposed to the aqueous phases. The depth of the terminal methyl group of the Nle residue is about 0.4 nm from the micellar surface. Considering the interface length mentioned above, the terminal methyl group would be on the interface region near the micellar core. Needless to say, the positions of terminal methyl groups estimated represent the average positions depending on thermal motions of all functional groups constituting the micelle–compound complexes.

Pentapeptides AcGlyXLeuGlyTrp-NH_2 were synthesized for the purpose of investigating the effect of the hydrophobic alkyl amino acid residue adjacent to X on the free energies of distribution. Although the introduction of the Leu alternative to Gly significantly elevated the affinity up to $\Delta G(AcGly_4Trp\text{-}NH_2) - \Delta G(AcGly_2LeuGlyTrp\text{-}NH_2) = 3.6$ kJ mol^{-1}, the values of $-\Delta\Delta G$ per methylene unit varied slightly, as shown in Figure 7(B). This means that the contribution of the peptide backbone, which serves as an anchor at the micelle surface, is significant and that each side chain of amino acid residue contributes independently to the distribution. Polypeptide–SDS complexes, which are formed by the micelle-like bindings of the SDS molecules to proteins, have unique secondary structures [20]. Common existence of the side chains of the amino acid residues under the environmental conditions mentioned in the preceding text would be favorable for formation of the secondary structures such as α-helixes.

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