Conformation-Dependent Energy Transfer between Copolypeptide Carrying L-Ornithine and L-Tyrosine and Chlorophyll in Aqueous Sodium Dodecyl Sulfate Solution

Junichi Kurawaki,* Yuriko Sameshima, and Yoshihumi Kusumoto

Chemical Institute, College of Liberal Arts, Kagoshima University, Korimoto, Kagoshima 890, Japan

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Potentiometric and spectroscopic studies of a chlorophyll *a* (Chl)−copolypeptide of L-ornithine and L-tyrosine (poly(L-Orn, L-Tyr)) system in aqueous solutions of sodium dodecyl sulfate (SDS) were carried out. The cooperative binding of SDS to poly(L-Orn, L-Tyr) studied by the potentiometric measurement of the binding isotherm showed that coil to α-helix transition of the poly(L-Orn, L-Tyr)−SDS complex takes place with an increase in SDS concentration. An increase in the helix content of poly(L-Orn, L-Tyr)−SDS complex can be well interpreted in terms of the hydrophobic interaction among bound SDS ions. On the basis of theoretical analysis of the cooperative binding isotherm, it was concluded that the formation of a micelle-like cluster consisting of at least seven SDS ions is required for the stabilization of a surfactant-induced helical structure. Singlet excitation energy transfer between L-Tyr-containing copolypeptide and Chl was investigated with steady-state fluorescence spectroscopy, and the efficiency of energy transfer from L-Tyr to Chl was evaluated as a function of SDS concentration or the degree of binding of SDS ion to poly(L-Orn, L-Tyr). We also determined energy-transfer parameters such as critical transfer distance and effective mean distance between L-Tyr and Chl, based on the Förster theory. It was shown that the effective mean distance varies from 49 to 34 Å with an increse in SDS concentration or the degree of binding, and the effective local concentration of Chl acceptor is about 350 times larger than the analytical concentration (1.0×10^{-5} M); thus it can be concluded that Chl molecules are highly concentrated in poly(L-Orn, L-Tyr)−SDS complexes and Chl and L-Tyr are located close to each other. Resonance Raman spectra of the Chl−poly(L-Orn, L-Tyr)−SDS system indicated that the keto-carbonyl at the 9-position of Chl interacts with phenol side chain of L-Tyr in poly(L-Orn, L-Tyr) via hydrogen bond. Therefore, it is reasonable to assume the formation of Chl−poly(L-Orn, L-Tyr) complexes in the presence of SDS. The present results on quantum yields of Chl fluorescence and energy transfer indicate that Chl molecules are incorporated into poly(L-Orn, L-Tyr)−surfactant complexes accompanying conformational change from the random coil to α-helix, and then the conformation-dependent energy transfer effectively occurs from L-tyrosine residue to Chl in the Chl−copolypeptide−surfactant system.

Introduction

Chlorophyll *a* (Chl) is the ubiquitous pigment involved in light-harvesting and photochemistry in the photosynthetic energy conversion processes of green plants and related organisms. It is well-known that all the in vivo Chl are bound to protein in thylakoid membrane and the resultant several types of Chl−protein complexes play an important role in the primary process of photosynthesis.[1−3] Green plant thylakoid membranes, which contain a number of peripheral antenna complexes belonging to the same family of proteins, are associated with photochemical reaction centers of the photosystems.[4,5] A polypeptide existing in the light-harvesitng chlorophyll−protein complexes of photosystem II has three membrane-spanning α-helixes, which are highly conserved in many organisms.[6] Chlorophyll molecules are localized in two layers parallel to the membrane surface. Thus, such an arrangement of chlorophyll molecules is considered favorable for the absorption and transmission of light energy. In addition, the conformation changes of the light-harvesting complexes of photosystem II are considered to control energy transfer by changing the interaction of pigments with the surrounding protein and altering the relative distances and orientation between chromophores. Although most pigments such as chlorophyll and bacteriochlorophyll in photosynthetic organisms are associated with proteins, little information is available on the consequence of this association for the primary processes of photosynthesis such as energy and electron transfer.

From this point of view, we have used some types of random copolypeptides carrying L-glutamate and L-tyrosine (poly(L-Glu, L-Tyr)), and L-ornithine and L-tyrosine (poly(L-Orn, L-Tyr)) as model proteins, and investigated photochemical and photophysical properties of Chl−copolypeptide complexes in aqueous surfactant solutions.[7−9] A purpose of this research is to clarify the effects of proteins containing aromatic amino acids such as L-Tyr and L-tryptophan on the photochemistry and photophysics of Chl in the artificial Chl−protein model systems. This model allows us to control the conformation of polypeptide induced by electrostatic interation with surfactant and hydrophobic interaction. The study of the interaction of Chl with the aromatic amino acid also should contribute to a better understanding of the nature of Chl−protein complex in green plants.

This paper deals with the potentiometric and spectroscopic properties of Chl−poly(L-Orn, L-Tyr)−surfactant system. We report here about the formation of Chl−copolypeptide complexes in the presence of an anionic surfactant, sodium dodecyl sulfate (SDS), by means of absorption, circular dichroism (CD), resonance Raman (RR), and steady-state fluorescence spectroscopies and using a potentiometric technique. In addition, energy transfer between L-Tyr residue and Chl in the copolypeptide−SDS complexes accompanying conformational transition from the random coil to α-helix is also described in detail.

Experimental Section

Materials. Chl (>99.8%) was purchased from Wako Chemical Industries Ltd. (Wako, Japan) and used as received. Poly-

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(L-Orn·hydrobromide, L-Tyr) with a molar ratio of 4:1 (Sigma, MW 23000) was dissolved in 0.1 M hydrochloride and dialyzed against distilled water until no chloride was detected by silver nitrate. The concentration of stock solution of poly(L-Orn, L-Tyr) was determined from absorbance measurement of L-Tyr ($\lambda_{max} = 275$ nm, $\epsilon_{max} = 1340$ M^{-1} cm^{-1}). The concentration of poly(L-Orn, L-Tyr), based on the mean residue concentration of L-Tyr, and that of Chl were fixed at 1.0×10^{-4} and 1.0×10^{-5} M, respectively, throughout the experiment. SDS was purified by recrystallization from ethanol three times. Laboratory deionized water was distilled twice. The pH of the sample solutions was not controlled but was found to be around 6.5.

Spectroscopic Measurements. CD spectra were measured with a JASCO J-720 spectropolarimeter and digitized data were transferred to a microcomputer and processed. Absorption spectra were recorded on a Shimadzu MPS-2000 spectrophotometer using 5 mm path legnth cell equipped with water-circulating jacket. Raman spectra were recorded on a Jasco R-800 laser-Raman spectrophotometer, using the 441.6 nm line of a He−Cd laser (Kimmon Electric Co., Ltd., CD4601R) as an excitation source. Steady-state fluorescence spectra measurements were carried out with a Hitachi 850 spectrofluorometer with a cell-holder in the front-face configuration. Fluorescence quantum yields of Chl and L-Tyr in poly(L-Orn, L-Tyr) were, respectively, determined by using Chl in ethanol (quantum yield = 0.30)[10] and L-Tyr amino acid in water (quantum yield = 0.14)[11] as references. All spectroscopic measurements were made at 25 °C for aerated solutions.

Binding Isotherm Measurements. The binding isotherm of surfactant ion to copolypeptide was determined potentiometrically at 25 °C, using a poly(vinyl chloride) (PVC) plastic membrane electrode which had been shown to respond to SDS ions.[12] This potentiometric technique is a powerful tool for the purpose of investigating the binding processes of SDS ion to poly(L-Orn, L-Tyr) in aqueous solution. The surfactant-selective membrane electrodes used in this work were fabricated using procedures which have been described by Maeda et al.[13] and Hayakawa and Kwak.[14] The cell constructed was as in the following:

Reference electrode
(Ag−AgCl) | 3.3 M KCl Agar bridge | Reference solution (SDS,
1×10^{-3} M) | PVC membrane | Sample solution | 3.3 M KCl Agar bridge | Reference electrode (Ag−AgCl)

The plastic PVC membrane electrode was prepared by dissolving 0.6 g of PVC and 1 mg of carrier complex composed of dimethyldioctadecylammonium chloride and SDS into mixed solution of 2.4 mL of tricresyl phosphate and 15 mL of tetrahydrofuran. The solution was poured onto a covered glass dish and allowed tetrahydrofuran to evaporate slowly. A resulting film 0.30 mm thick was fixed to a PVC tube of 10 mm diameter with the use of tetrahydrofuran solution. The electromotive force of the cell was measured with a Denki Kagaku Keiki Co., Ltd. Model COM8 pH meter with an accuracy of ±0.5 mV. Prior to the measurements on copolypeptide solutions, the PVC plastic membrane was calibrated with SDS solutions. It was checked that this PVC membrane electrode excellently responds to SDS ion in the concentration range studied.

Results and Discussion

Conformation of Poly(L-Orn, L-Tyr)−SDS System. Figure 1 shows typical CD spectra of poly(L-Orn, L-Tyr) in aqueous solutions of varying SDS concentration. No significant change in CD spectra occurred on addition of Chl to poly(L-Orn, L-Tyr)−SDS system The ellipticities between 190 and 250 nm have been shown to be a sensitive indicator of the conformations of α-helix, β-sheet, and random coil of copolypeptides as well as homopolypeptides.[15] The CD spectrum in Figure 1a represents the random coil conformation of positively charged poly(L-Orn, L-Tyr) in aqueous solution. As shown in Figure 1f the CD spectrum varies from a characteristic spectrum of the random coil to a typical spectrum which has a double minimum at 208 and 222 nm characteristic of α-helical conformation with increasing SDS concentration. This behavior of CD spectra suggests that the environment produced by the bound SDS ions affects the rotatory strengths of n−π* and π−π* transitions. The absolute value of the ellipticity at 222 nm observed for this system is smaller than that for homopolypeptide (poly(L-Orn))−SDS system reported previously.[16] This result suggests a transient conformation from the random coil to α-helix and indicates that the content of the random coil is relatively larger than that of α-helix in the case of this system. Chou and Fasman[17] have computed the helix and β-sheet conformational parameters and formulated a set of empirical rules govering the folding of the secondary structural regions in proteins. According to them, L-Tyr is assigned to be a helix breaker.

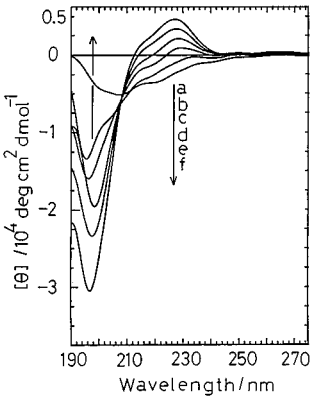

Figure 1. Circular dichroism (CD) spectra of poly(L-Orn, L-Tyr) with varying SDS concentration. (a) 0 M, (b) 0.05×10^{-3} M, (c) 0.10×10^{-3} M, (d) 0.15×10^{-3} M, (e) 0.20×10^{-3} M, and (f) 0.25×10^{-3} M.

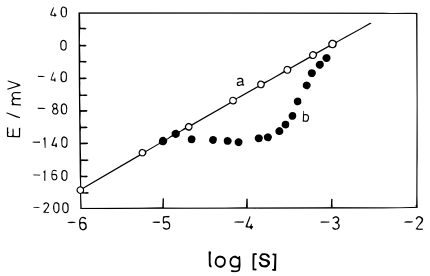

Figure 2. Plots of electromotive force (*E*) versus the logarithm of SDS concentration (log [S]) in the absence (a) and presence (b) of poly(L-Orn, L-Tyr).

Binding Isotherm for Poly(L-Orn, L-Tyr) and SDS. To aid the interpretation of CD spectral data, we examined the interaction between poly(L-Orn, L-Tyr) and SDS potentiometrically using surfactant-selective membrane electrodes. Figure 2 shows typical semilogarithmic plots of electromotive force (*E*) against SDS concentration ([S]) in the absence and presence of poly(L-Orn, L-Tyr). The straight calibration line (open circles in Figure 2a) indicates excellent performance with nearly Nernstian response over the concentration of SDS in this work. Upon addition of poly(L-Orn, L-Tyr) the observed *E* deviates from the calibration curve with an increase in [S], as shown by

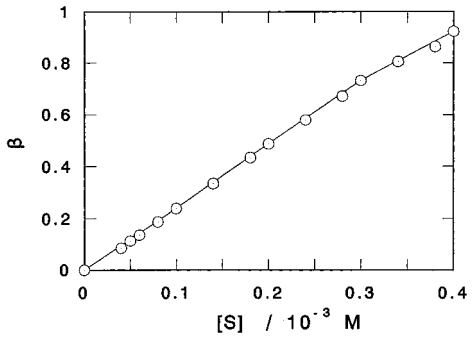

Figure 3. Plot of β versus SDS concentration ([S]) at 25 °C.

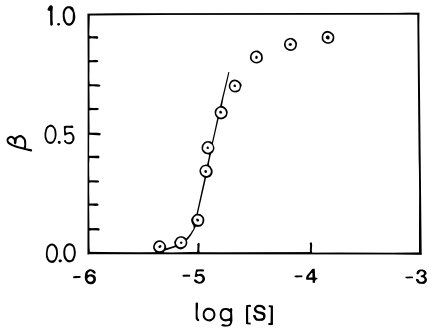

Figure 4. Binding isotherm (plot of β vs log $[S]_f$) for poly(L-Orn, L-Tyr)−SDS system. Solid line: calculated curve from eq 2 with $u = 15$. [poly(L-Orn, L-Tyr)] $= 1 \times 10^{-4}$ residue M.

the full circles in Figure 2b. The first addition of SDS does not lead to binding, and all surfactant remains free (full circles overlap with calibration curve in Figure 2). At a certain value of [S] binding occurs and free SDS concentration ($[S]_f$) remains nealy constant, where β increases linearly. By comparison with the calibration curve, $[S]_f$ is obtained and the degree of binding (β) of SDS ion to poly(L-Orn, L-Tyr) in aqueous solution can be estimated by using a following relation

$$\beta = \frac{[S] - [S]_f}{[P]} = \frac{[S]_b}{[P]} \qquad (1)$$

where [S] is the total concentration of SDS, $[S]_f$ the equilibrium concentration of free (unbound) SDS ions, $[S]_b$ the concentration of bound SDS ions, and [P] the residual concentration of poly(L-Orn, L-Tyr) ([P] $= 1 \times 10^{-4}$ residue M). In Figure 3, β of SDS ion to poly(L-Orn, L-Tyr) in aqueous solution obtained from Figure 2 is plotted against [S]. β first increases almost linearly with increasing [S] and then increases gradually with further increase in [S]. This result indicates that since the amino groups of L-Orn in poly(L-Orn, L-Tyr) are completely ionized at pH 6.5, SDS ions can interact electrostatically with amino groups of L-Orn, giving rise to an ion pair.

The plot of β vs log $[S]_f$, i.e., the binding isotherm of SDS ion to poly(L-Orn, L-Tyr), is shown in Figure 4. The resulting binding isotherm shows a steep rise at a critical binding concentration; this result suggests that a cooperative binding of SDS ion to poly(L-Orn, L-Tyr) occurs in the range of SDS concentration far below the critical micellar concentration (cmc) (cmc $= 8.1 \times 10^{-3}$ M). As was pointed out by Satake and Yang,[18] the binding process of surfactant ion to polypeptide ion becomes cooperative because of an additional hydrophobic interaction among bound surfactant ions. In these circumstances, the bound surfactant ions cluster side by side onto the polypeptide chain even in a small degree of binding and cause the binding isotherm to rise steeply in narrow region of $[S]_f$.

TABLE 1: Cooperative Binding Parameters *u* and *K* for Poly(L-Orn, L-Tyr)−SDS System

u	*K*
15	4.5×10^3

TABLE 2: Calculated Value of $\bar{m}$ as a Function of β

	β								
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
$\bar{m}$	1.9	2.6	3.3	4.0	4.9	6.0	7.7	10.5	17.4

The β of the SDS ion to poly(L-Orn, L-Tyr) based on the theory of Satake and Yang[18] is given by

$$2\beta - 1 = (uK[S]_f - 1)/\{(1 - uK[S]_f)^2 + 4K[S]_f\}^{1/2} \qquad (2)$$

where u is a cooperative parameter and measures the cooperativity of surfactant (SDS) binding: $u > 1$ for cooperative binding, $u = 1$ for noncooperative binding, and $u < 1$ for anticooperative binding. K is the intrinsic binding constant between a surfactant and an isolated copolypeptide ion site. We can characterize the copolypeptide−surfactant interaction by a cooperative parameter (u); this parameter is determined by hydrophobic interactions among the bound surfactant ions. The solid line in Figure 4 is the best fit of the experimental data at lower β to eq 2. The parameters u and K obtained for the best fit are listed in Table 1. The binding isotherm obtained for this system was not so sharp as for poly(L-Orn).[18] As shown in Table 1, the most reliable values of u was found to be 15 in the case of the poly(L-Orn, L-Tyr)−SDS system. As would be anticipated from the present results of CD spectra in Figure 1, the value of u was considerably smaller as compared with that of 77 for the poly(L-Orn)−sodium decyl sulfate system.[18] This may reasonably be ascribed to a change in hydrophobic interaction between neighboring groups. In addition, the average cluster size ($\bar{m}$) of the bound SDS ions, i.e., the average number of bound SDS ions which constitute a one-dimensional micellar cluster on the copolypeptide chain, is given as

$$\bar{m} = 2\beta(u - 1)/\{[4(1 - \beta)(u - 1) + 1]^{1/2} - 1\} \qquad (3)$$

The calculated values of $\bar{m}$ from eq 3 with $u = 15$ are given as a function of β and are summarized in Table 2. The values of $\bar{m}$ at $\beta = 0.6$ and 0.7 are estimated to be 6.0 and 7.7, respectively. This corresponds to the number of residues included in two turns of α-helical structure. From these findings, we could find an interrelation of β or $\bar{m}$ and the conformational change induced by SDS ion. Thus, the present results of CD spectra and the potentiometric measurement can lead us to conclude that the anionic surfactant SDS ion binds cooperatively to the positively charged amino group of L-Orn of poly(L-Orn, L-Tyr) to give rise to a micelle-like cluster due to an additional hydrophobic interaction among bound SDS ions. The resulting clusters are anticipated to solubilize the hydrophobic molecules such as Chl, because the environment of poly(L-Orn, L-Tyr)−SDS complexes formed by virtue of electrostatic and hydrophobic interactions is very hydrophobic.

Quantum Yields of Chl and L-Tyr Fluorescence. Absorption and fluorescence spectra of Chl in aqueous surfactant solutions have been investigated.[19−21] Kusumoto et al.[20,21] have shown the formation of solute-rich induced micelles at surfactant concentration below the cmc. Upon addition of poly(L-Orn, L-Tyr) to Chl−SDS system, the absorbance of a shoulder band around 700 nm of Chl decreases slightly. The absorption band around 700 nm may be attributed to the hydrated Chl aggregates and/or Chl in colloidal states. The fluorescence quantum yield of Chl excited at 435 nm in the absence and presence of poly-

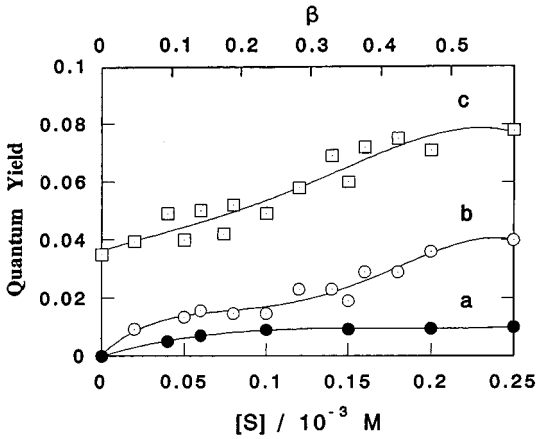

Figure 5. Changes in fluorescence quantum yields of Chl (a, b) and L-Tyr (c) with [S] or β in (a) Chl−SDS, (b) Chl−poly(L-Orn, L-Tyr)-SDS, and (c) poly(L-Orn, L-Tyr)−SDS systems.

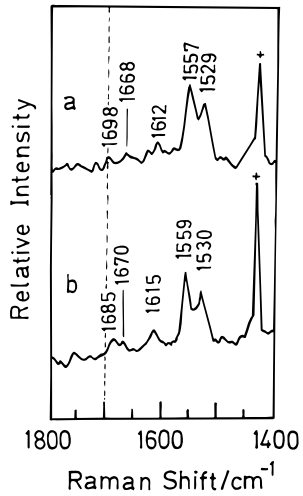

Figure 6. Resonance Raman spectra of (a) Chl−SDS and (b) Chl−poly(L-Orn, L-Tyr)−SDS systems. [S] = 0.20 mM.

(L-Orn, L-Tyr), and that of L-Tyr residue excited at 225 nm are shown in Figure 5 as a function of [S] or β. The quantum yield of Chl increases with increasing [S] or β, and is larger in the presence than in the absence of poly(L-Orn, L-Tyr). Similar results were also obtained for poly(L-Glu, L-Tyr) systems.[7] The hydrated Chl and Chl in colloidal states are less fluorescent. An increase of quantum yield of Chl fluorescence in the presence of poly(L-Orn, L-Tyr) shown in Figure 5, therefore, suggests that Chl exists as a monomeric state in these complexes and becomes more fluorescent, and Chl molecules are incorporated into micelle-like clusters formed by the cooperative binding of SDS ion to poly(L-Orn, L-Tyr) ion to give rise to Chl−poly(L-Orn, L-Tyr) complexes.

Specific Interaction of Chl with Poly(L-Orn, L-Tyr). For the purpose of getting information regarding interaction of Chl with poly(L-Orn, L-Tyr)−SDS complexes, we measured RR spectra of Chl in the absence and presence of poly(L-Orn, L-Tyr) in aqueous SDS solutions, using resonance enhancement in the respective Soret band. RR spectroscopy is a powerful tool for studying Chl association in vivo and in vitro.[22−24] We have so far investigated association states of Chl with an amphiphilic molecule such as *N*-methylmyristamide (NMMA), which bear amide group characteristic of proteins, in aqueous surfactant solutions and proposed a basic model for Chl−NMMA association on the basis of RR studies.[25,26] RR spectra of Chl in the absence and presence of poly(L-Orn, L-Tyr) at [S] = 0.20 mM are shown in Figure 6. In this paper, we focus our attention on Raman bands in the higher frequency region of 1750−1400 cm^{-1}. RR spectra of Chl in the absence and presence of poly(L-Orn, L-Tyr) showed that the central Mg atom is assgined to five coordinates becasue of marker bands of the 16-membered-ring vibration at 1615−1612, 1559−1557, and 1530−1529 cm^{-1} proposed by Fujiwara and Tasumi,[23] and Chl exists as a monomeric state in both systems, because the intensity of 1559−1557 cm^{-1} band is stronger than that of 1529−1527 cm^{-1} band.[24] The presence of a carbonyl stretching mode at 1698 cm^{-1} (Figure 6a) indicates that the keto carbonyl group at the 9-position (C_9=O) is free in the case of Chl−SDS system. Upon addition of poly(L-Orn, L-Tyr) to the Chl−SDS system, however, the frequency of this Raman band exhibited a downshift in frequency by 13 cm^{-1}, as shown in Figure 6b. Such a frequency downshift could be observed only when Chl−poly(L-Orn, L-Tyr)−SDS system adopted α-helical conformation state. Moreover, we also measured RR spectra of Chl−poly(L-Orn)−SDS and Chl−copolypeptide of L-Orn and L-phenylalanine (poly(L-Orn, L-Phe))−SDS systems, for the comparison with those obtained in this work. RR spectra for both Chl−poly(L-Orn)−SDS and Chl−poly(L-Orn, L-Phe)−SDS systems were almost similar to that of Chl−SDS system in Figure 6a. Thus, the frequency downshift of 1698−1685 cm^{-1} observed in this work can be ascribed to the interaction of Chl with L-Tyr residue in poly(L-Orn, L-Tyr). Judging from these results, it can be concluded that the C_9=O of Chl, incorporated into poly(L-Orn, L-Tyr)−SDS complexes, interacts with phenol side chain of L-Tyr residue in poly(L-Orn, L-Tyr) via hydrogen bond in the case of this system.

Energy Transfer Efficiency for Chl−Poly(L-Orn, L-Tyr)−SDS System. To make it clear that Chl molecules are associated with poly(L-Orn, L-Tyr)−SDS complexes through the micelle-like clusters, we also examined energy transfer from L-Tyr as a donor to Chl as an acceptor. We have measured the fluorescence spectra of L-Tyr and Chl when the sample solution was excited at 225 nm and determined the efficiency of energy transfer as a function of [S] or β. This excitation wavelength was selected to excite only L-Tyr in the sample. Energy transfer is accompanied by quenching of the donor fluorescence, and by appearance of sensitized fluorescence of the acceptor. Therefore, the energy-transfer efficiency can be described by the quantity, e_{ET}, defined by[27]

$$e_{ET} = (I_A/I_D)(q_D/q_A)/[1 + (I_A/I_D)(q_D/q_A)] \quad (4)$$

where I_D and I_A are the fluorescene intensities of the donor (L-Tyr) and the acceptor (Chl) observed in the sample solutions, and q_D and q_A are the fluorescence quantum yields of the L-Tyr and Chl, respectively. The result is shown in Figure 7 as a function of [S] or β. The efficiency of energy transfer can also be described by the energy loss (e_{EL}), the fractional loss of donor fluorescence, defined by

$$e_{EL} = (I_0 - I_D)/I_0 \quad (5)$$

where I_0 and I_D are the fluorescence intensities of L-Tyr in the absence and presence of Chl. The estimated value of e_{EL} is also shown in Figure 7. As shown in Figure 7 the values of e_{ET} and e_{EL} are almost the same without experimental uncertainties and increase with an increase in [S] or β. It should be noted that since $e_{ET} \approx e_{EL}$, the efficient energy transfer between L-Tyr and Chl occurs in Chl−poly(L-Orn, L-Tyr)−SDS system. Similar results were also obtained for poly(L-Glu, L-Tyr) systems.[7] The enhancement of e_{ET} with an increase in [S] corresponds to the behavior of the CD spectral change described above. These facts indicate that the cooperative coil-to-helix

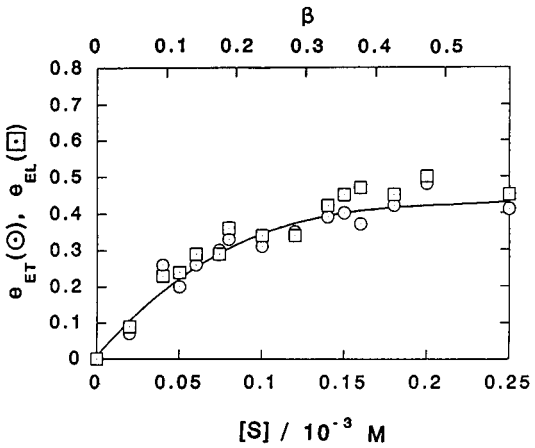

Figure 7. Plots of e_{ET} and e_{EL} versus [S] or β for Chl−poly(L-Orn, L-Tyr)−SDS system.

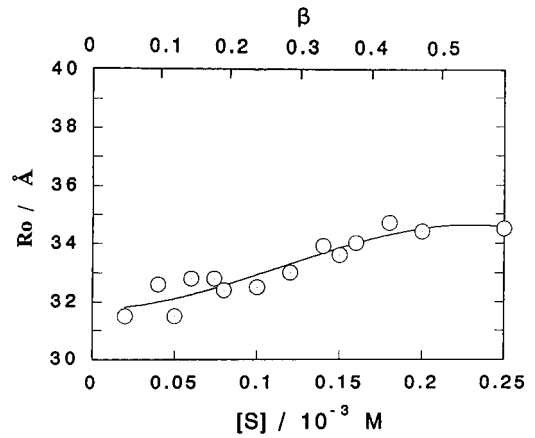

Figure 8. Change in R_0 with [S] or β for Chl−poly(L-Orn, L-Tyr)−SDS system.

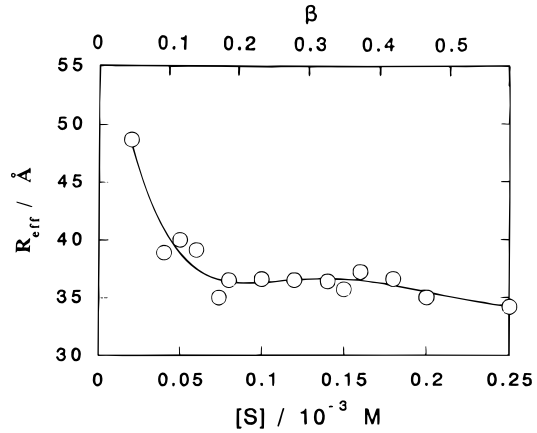

Figure 9. Change in R_{eff} with [S] or β for Chl−poly(L-Orn, L-Tyr)−SDS system.

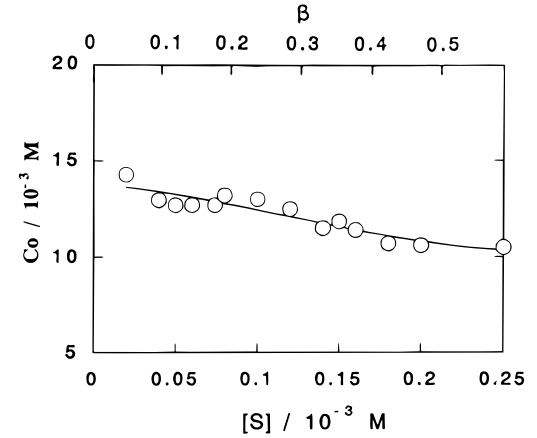

Figure 10. Change in C_0 with [S] or β for Chl−poly(L-Orn, L-Tyr)−SDS system.

conformational change of poly(L-Orn, L-Tyr)−SDS complexes contribute to the enhancement of energy transfer efficiencies from L-Tyr to Chl in the present system. We can conclude that poly(L-Orn, L-Tyr)−SDS complexes in the α-helical conformation provide a highly hydrophobic environment which solubilizes Chl and the resultant Chl−poly(L-Orn, L-Tyr) complexs are formed.

Förster Energy-Transfer Parameters. According to the Förster theory,[28] the critical transfer distance (R_0), the interchromophore separation such that the efficiency of energy transfer is 50%, can be evaluated from the overlap of fluoresence spectrum of L-Tyr and absorption spectrum of Chl in aqueous SDS solutions. The orientation factor κ^2 depends on the relative orientation of Chl and L-Tyr in poly(L-Orn, L-Tyr). In principle it can vary from zero to four. In practice an assumption of random relative orientation is made which results in $\kappa^2 = 2/3$. The quantum yield of the donor (L-Tyr) shown in Figure 5c was also used for the determination of R_0 on the basis of this theory. Figure 8 shows the dependence of R_0 on [S] or β. R_0 slightly increases from 31.5 to 34.5 Å with an increase in [S] or β. The energy-transfer efficiency, e_{ET} can be also expressed by a following relation

$$e_{ET} = {R_0}^6({R_0}^6 + {R_{eff}}^6) \quad (6)$$

A parameter R_{eff} in this equation is the effective mean distance between L-Tyr and Chl. In Figure 9, the estimated R_{eff} is plotted against [S] or β. The value of R_{eff} varies from 49 to 34 Å with increasing [S] or β; this fact indicates that Chl and L-Tyr molecules are located closely with each other. Thus, it is reasonable to assume that Chl molecules are also incorporated into SDS-induced micelle-like clusters (poly(L-Orn, L-Tyr)−SDS complex) to give rise to Chl−poly(L-Orn, L-Tyr) complexes.

In addition, the critical concentration at $e_{ET} = 0.76$ (C_0) can be estimated from $C_0 = (7.65/R_0)^3$. In Figure 10 is shown the dependence of C_0 on [S] or β. With increasing [S], C_0 decreases gradually and then becomces almost constant. The enhanced energy transfer in such a comparatively diluted Chl concentration as 1.0×10^{-5} M cannot be expected if L-Tyr and Chl molecules uniformly distributed in the homogeneous solution. Figure 11 shows the effective local concentration (A_{eff}) plotted against [S] or β. Above [S] = 1.2×10^{-3} M the value of A_{eff} increases gradually and then becomes almost constant; its value is about 3.5×10^{-3} M. This concentration is larger about 350 times than the analytical concentration (1.0×10^{-5} M). Therefore, this indicates that Chl molecules are highly concentrated in poly(L-Orn, L-Tyr)−SDS complexes formed by the cooperative binding of SDS ion to poly(L-Orn, L-Tyr) in aqueoous solution. Since the environment of the resulting clusters are very hydrophobic, and Chl is nonionic but have long hydrophobic chains, the hydrophobic interaction among Chl and copolypeptide−surfactant complex plays an important role in the formation of Chl−poly(L-Orn, L-Tyr) complexes in the presence of surfactant molecule.

Judging from such energy-transfer parameters and potentiometric results in this work, we can conclude that Chl molecules are incorporated into micelle-like clusters through strong hydrophobic interaction to give rise to Chl−poly(L-Orn, L-Tyr) complexes, and as a result Chl and L-Tyr molecules are located

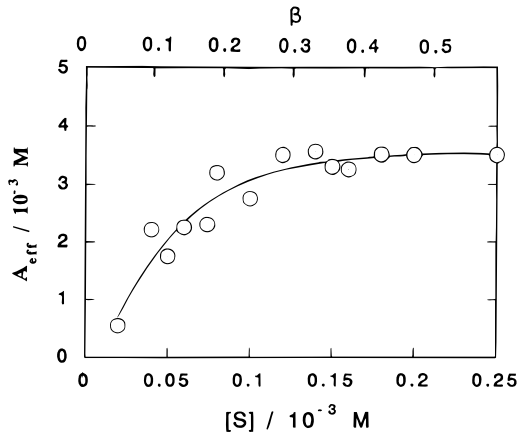

Figure 11. Change in A_{eff} with [S] or β for Chl−poly(L-Orn, L-Tyr)−SDS system.

closely in these complexes and C_9═O of Chl interacts with phenol side chain of L-Tyr in poly(L-Orn, L-Tyr). Thus, the conformation-dependent energy transfer from L-Tyr residue to Chl occurs effectively. The present results on energy transfer are of great interest in relation to in vivo peridinin−Chl−protein complex[29] and may point out the validity as a model for photosynthetic antenna pigments.

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