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Functionality of Polypeptide by Induction of Specific Tertiary Structure

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ABSTRACT: Two different approaches for arranging functional groups regularly in space are presented. One way is based on the covalently bonding chemistry as exemplified by proteins. Here, a helix-bundle structure was elaborately prepared by connecting two or three helices to a template molecule. The peptide carrying three helices in the molecule formed ion channel at lower concentration than the peptides of the helical part alone or carrying two helices in the molecule. The other way is based on the self-assembling system. The amphiphiles carrying a chromophore-containing amino acid formed a bilayer membrane in water. The membrane showed efficient photo-energy migration between chromophores and effectively reduced cytochrome c by photo-irradiation.

Introduction

Functionality of proteins is exhibited on the basis of the specific tertiary structure because the efficacy of the protein function is generally impaired by denaturation. Therefore, construction of a well-defined structure should be important in terms of developing novel functional polymers where the functional groups are fixed in space to realize efficient cooperation between them. However, the problem is that we do not know the mechanism by which a polypeptide chain folds into a corresponding tertiary structure. In the present report, two ways to dissolve the difficulty are presented for the regulation of the topological arrangement of the functional amino acids.

One way is to synthesize polypeptides with graft chains connected to a template molecule, so-called template-assisted synthetic proteins (TASPs).[1] In the TASP, the spatial arrangement of the graft chains is regulated entropically and energetically by connecting it to the template molecule. Here this method is applied to the synthesis of ion-channel-forming peptides which take a bundle structure of α-helices.

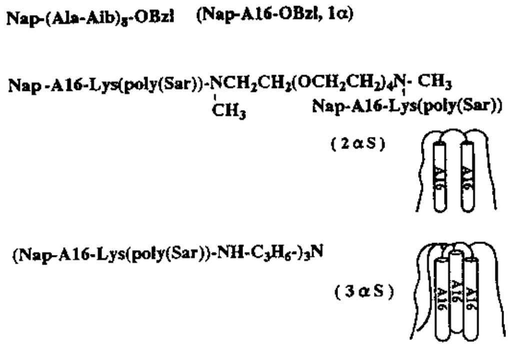
The other way is to fix functional amino acid residues in a molecular assembly. Recently, the number of reports on molecular assembly is increasing. It has been shown that amphiphilic molecules construct a bilayer membrane in a shape of a regular spherical vesicle in water.[2] Functional amino acid residues will be fixed regularly in the bilayer membrane by incorporation of the amino acid residue into the amphiphile.

Experimental

Materials. Peptides were synthesized by a conventional liquid-phase method. The synthesis of one of the amphiphiles is previously reported,[3] and the other was prepared similarly.

Methods. CD, fluorescence, and transition absorption measurements were carried out on a JASCO J600 spectropolarimeter, a Hitachi MPF-4 fluorophotometer, and an Otsuka Electronics IMUC-7000 using a XeF excimer laser (351 nm, 54 mJ).

Ion-channel formation of the peptides was examined by BLM measurement A thin Teflon film (0.25 mm thick) with an aperture of 0.2~0.3 mm diameter was clamped between two halves of a Teflon trough. The hole was precoated with hexadecane/hexane (6/4 v/v). The soybean-lectin mem-



Nap, OBzl, and A16 represent 2-naphthaleneacetyl, benzyl ester, and -(Ala-Aib)$_8$-, respectively.

Figure 1. Molecular structure or the helical peptides.

brane was formed by the method reported by Montal and Muller.[4] Both water phases contain 1 MKCl. The peptide was added to both water phases.

DMPC liposome was prepared by a sonication of the lipid dispersion in a Tris buffer solution. The liposomes of the amphiphiles were prepared by a extruder method[5] or a sonication method.

Results and Discussion

Template-Assisted Synthetic Proteins.

Molecular Design for the Ion-Channel-Forming Peptides: Cell cytoplasm is partitioned from the outside by the lipid membrane, which is essentially impermeable for ions and hydrophilic compounds. Ion-channel proteins and some antibiotics have been found to transport ions across the lipid membrane. The molecular structure of the ion-channel forming compounds has been elucidated and a helix-bundle structure has been advocated to be essential.[6] For example, fully hydrophobic helical peptides have been shown to act as a voltage dependent ion channel which span across the lipid membrane and associate together to form the helix-bundle structure.[7,8] However, the number and molecular orientation of the helices in the bundle structure are, not as yet, clear. Parallel orientation of the helices where the macrodipole moments align in the same direction has been assumed to be an active form of the ion channel. In the present study, two or three chains of hydrophobic helices were combined together into a complex molecule taking a parallel orientation (Figure 1). In these compounds, formation of the helix bundle in parallel orientation should be promoted, and consequently they will function efficiently as an ion channel.

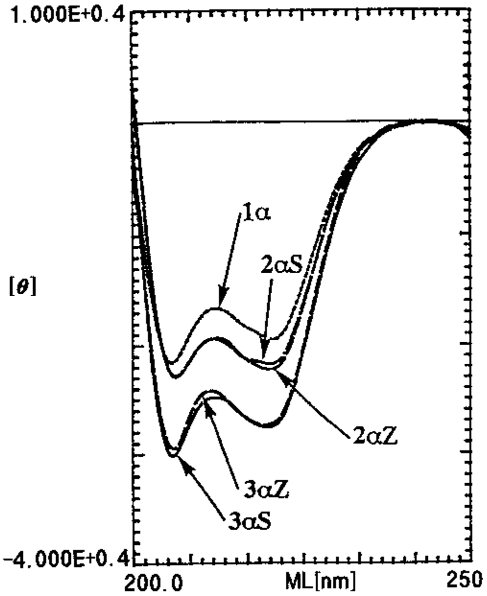


Figure 2. CD spectra of the peptides in methanol.

Conformation: The helix part of the peptides is composed of alanine and 2-aminoisobutyric acid (Aib), which are helix-promoting residues. In a solid state, x-ray analysis revealed that Boc-(Ala-Aib)$_8$-OMe takes o-helical structure.[9] Conformation of the peptides in solution was investigated by a CD spectroscopy (Figure 2). All the peptides in methanol show the double-minimum spectra supporting the G-helical structure. The poly(Sar) part of 2αS and 3αS do not influence the helical conformation of the peptides because CD spectra of 2αZ and 2αS or 3αZ and 3αS are similar. However, the helix content changes with the number of helical rods in the peptides, and notably increases in the order of $1\alpha<2\alpha Z\approx 2\alpha S<3\alpha S$. Since the bundle formation will be promoted in this order, the helical conformation is considered to be stabilized by the formation of the bundle structure despite the fact that the orientation of the helix peptides is parallel in the bundle.

The conformation of the peptides in phospholipid membrane was studied by a CD spectroscopy in

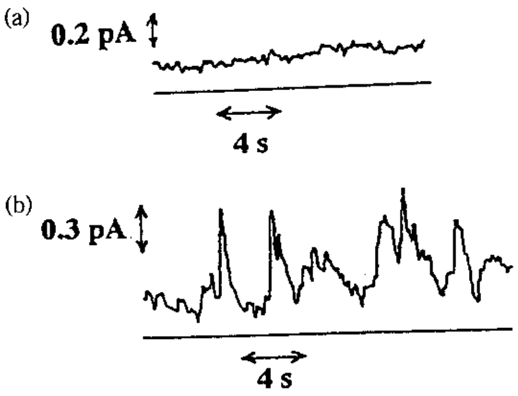


Figure 3. Current fluctuation at applied voltage of 190 mV across the soybean-lectin membrane in the presence of a) 1α(0.029 μM and b) 3αS (0.026 μM).

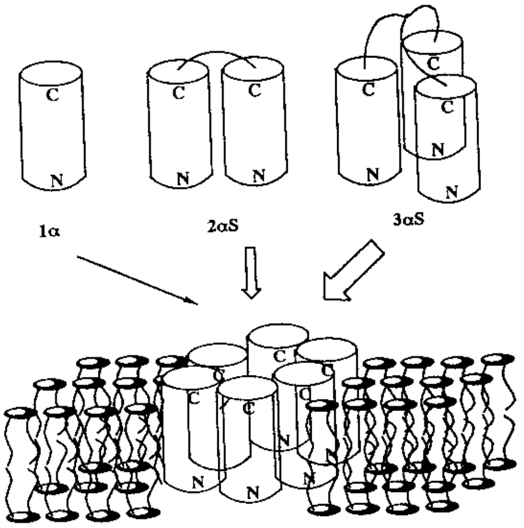


Figure 4. Schematic presentation of partition of the peptides to phospholipid bilayer membrane. The helix bundle structure in the parallel orientation is formed in the membrane easily by 3αS because three helices are already arranged in the bundle structure.

the presence of dimyristoylphosphatidylcholine (DMPC) liposome. The double-minimum spectra of 2αS and 3αS were observed, but the negative intensity at 235 nm was stronger than that at 208 nm,suggesting that the helices associate tightly together in the membrane.[10] Therefore, the helix-bundle structure of these peptides should be formed in the membrane. On the other hand, the conformation of 2αZ and 3αZ could not be determined due to the aggregation of liposome. The aggregation should be induced because the partition of the hydrophobic peptides to the membrane changes the membrane surface hydrophobic and disturbs the membrane structure. The poly(Sar) chains of 2αS and 3αS are very effective in suppressing the aggregation of liposome upon the peptide partition to the liposome because the peptides possess hydrophilic property due to the chains.

Ion-Channel Formation: Ion-channel formation in lipid membrane is generally detected by current fluctuations across the membrane. Current across BLM are measured with changing applied voltage and concentrations of the peptides. 3αS produced the current fluctuation at lower concentrations than 1α (Figure 3) or 2αS, suggesting that 3αS efficiency forms an ion channel. Interestingly, the conductance of the 3αS channel formed in the membrane was not clearly dependent on the applied voltage across the lipid membrane. These findings support the view that a bundle structure or α-helical rods in parallel orientation gives rise to ion channel, and that the TASP 3αS promotes this type of association of helices to form ion channel without the help of applied voltage as illustrated in Figure 4.

Self-Assembly of Amino-Acid Containing Amphiphile.

Molecular Design for the Light Harvesting Amphiphile: Dialkylammonium-type amphiphiles are reported to form a vesicular assembly in water.[2] When a functional group is incorporated in the amphiphile, it will be arranged regularly in the bilayer membrane to function for specific purposes. For example, a bilayer membrane composed of amphiphiles having a chromophore is expected to be an excellent model for a photoenergy-harvesting system because of the high density of the chromophore in two-dimensional membrane. In the membrane, the probability of photoenergy transfer to an acceptor molecule is large due to the efficient photoenergy migration among the chromophores.

A dialkylammonium-type amphiphile (5Cz18 and 5Cz18Z) carrying L-3-(3-*N*-ethylcarbazolyl)-alanine (Figure 5) was designed, because the dialkylammonium-type amphiphile was reported to

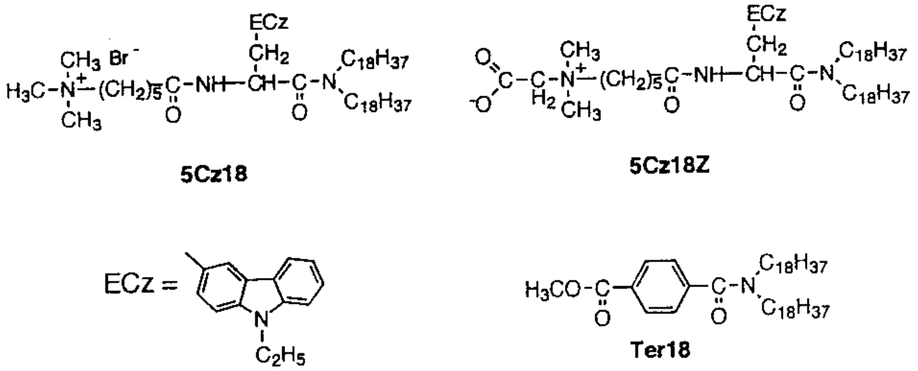


Figure 5. Molecular structure of the amphiphiles carrying the ECz group (5Cz18 and 5Cz18Z) and the quencher.

form a bilayer membranes and the *N*-ethylcarbazolyl (ECz) group is difficult to form excimer which will become a photoenergy trapping site in the membrane.[11] For the light harvesting system, the membrane should have the following properties, i) efficient photoenergy migration between chromophores, ii) high yield of electron tansfer and iii) stabilization of the charge-separated state. In the present study, the photoenergy migration was estimated by the quenching experiment in which methyl terephthaloyldialkylamide (Ter18, Figure 5) is mixed into the membrane. The stability of the radical cation produced by laser irradiation was evaluated by measuring the transient absorption spectra of the radical cation. In addition, the photoreduction of cytochrome c by the membrane was examined.[2]

(2) Characterization of the Molecular Assembly of 5Cz18: The aqueous dispersion of 5Cz18 after a brief sonication contained small vesicles of diameters between 200 and 800 Å by TEM observation.[3] Though a thermal analysis of the vesicles an endothermic peak at 25.4 °C in the DSC thermogram, which corresponds to a gel-liquid-crystal phase transition of bilayer membrane was observed. 5Cz18Z, which is a zwitter-ion type amphiphile, similarly, formed vesicles in water, and the phase-transition temperature was observed at 26.7°C.

Photoenergy Migration in the Membrane: Fluorescence spectra or 5Cz18 in water were measured with varing concentrations of Ter18 and at two different temperatures. The fluorescence quenching rate at 18 °C, which is lower than the phase-transition temperature of 25.4 °C, is larger than that at 40 °C. The quenching rate will become large in the bilayer membrane where energy migration occurs efficiendy. Therefore, the high quenching rate at a gel-state membrane may be due to the efficient excited energy migration among chromophores in the membrane.

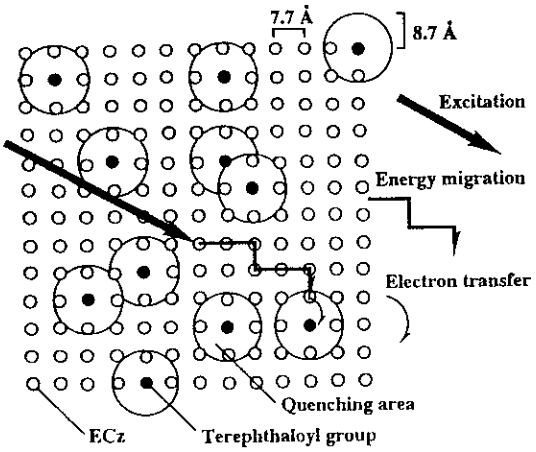


Figure 6. Simulation of the fluorescence quenching process in the mixed membrane of 5Cz18 and Ter18. ECz groups and terephthaloyl groups are placed on a square lattice with a spacing of 7.7 Å. The interaction distance between an excited ECz group and a terephthaloyl group is 8.7 Å.

Simulation of the quenching in the mixed membrane of 5Cz18 and Ter18 was carried out under the assumption that ECz groups and terephthaloyl groups are arranged in a square lattice with a spacing of 7.7 Å(Figure 6), which is taken from the x-ray data of the crystalline structure of dioctadecyldimethylammonium bromide in the bilayer

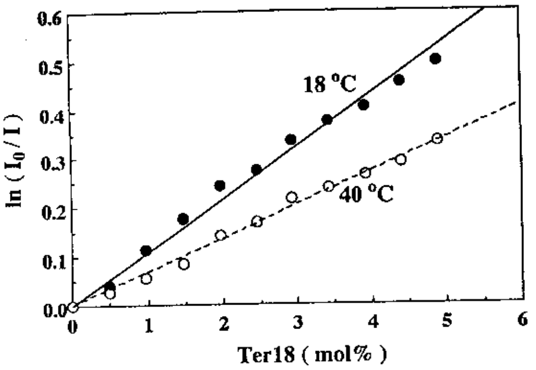


Figure 7. Perrin plot of fluorescence quenching of 5Cz18 bilayer with Ter18. The experimental data at 18 ℃ (●) and 40 ℃ (○) are shown together with the result of computer simulation taking W/k_f to be 23 (—) and 9 (···).

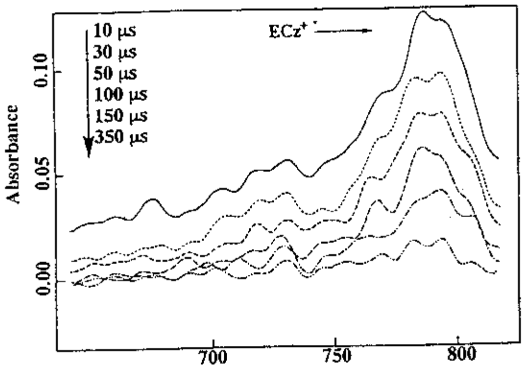


Figure 8. Transient absorption spectra of 5Cz18 bilayer membrane at 18 ℃ at different time lapses after laser irradiation.

membrane.[12] Since the interaction distance between the excited ECz group and the terephthaloyl group is 8.7 Å,[13] the excited ECz group is quenched when one of the four nearest sites from the ECz group is occupied by the terephthaloyl group. The simulation results are shown in Figure 7, which are determined uniquely by setting the values of W/k_f (W and k_f represent the rates of energy migration and emission, respectively) and the quantum yield of 5Cz18 in the membrane. The experimental results were consistently fitted to the simulation curves by taking the values of W/k_f at 18 ℃ and at 40 ℃ to be 23 and 7, respectively Thus, the number of energy migration in the life time are 5.0 times at 18 ℃ and 1.9 times at 40 ℃.

Hole Transfer in the Membrane: When the bilayer system is irradiated by an excimer-laser beam, a biphotonic process will occur to yield the radical cation (ECz+.) by ejecting an electron. The produced hole hops among the ECz groups under coulombic force of a radical anion which is produced by trapping the electron ejected from the ECz group. The distribution of the geminate electron is reported to be maximum about 20 Å apart from the parent radical cation.[14]

Figure 8 shows transeient absorption spectra of 5Cz18 bilayer membrane at 18 ℃ and the change of absorbance at 780 nm with time. The time course was fitted by a single exponential curve to obtain a life time of 130 μs of ECz+. This is relatively along life time compared with a few μs or the ECz group in micelle reported by Nakamura *et al.*[15] One explanation may be the existence of carbonyl groups in our molecular system. The ejected electron from the ECz group may be produced initially as a solvated anion, which is stabilized by trapping it in the carbonyl group.

On the other hand, the life time of ECz+. was 40 μs at 40 ℃. The shorter life time above T_c may be due to the lateral diffusion of the amphiphiles in the bilayer membrane, which should facilitate encounter of ECz+. with the radical anion. Therefore, the rate of hole transfer, occurs below T_c, it is comparable to the lateral diffusion of the amphiphiles (10^{-8} cm^2/s). The rate of hole transfer in the poly (vinylcarbazole) film is reported to be 10^{-5} cm^2/s.[16] Thus, the rate of the hole transfer in the bilayer is considered to be remarkably low, suggesting a stabilization of the radical cation in the membrane.

Photoelectron Transfer to Cytochrome c: Photoreduction of cytochrome c by the ECz containing membranes was examined.

Cytochrome c partitioned to the 5Cz18Z membrane more favorably than to the 5Cz18 membrane from the binding experiment. Since cytochrome c bears positive charges at neutral pH region, it will have higher affinity for the electrically neutral membrane (5Cz18Z) than the positively charged membrane (5Cz18).

Triethanolamine was used for electron donor. Cytochrome c was not nearly reduced by 5Cz18Z membrane when the dispersion was kept in the dark. However, upon irradiation the efficient reduc-

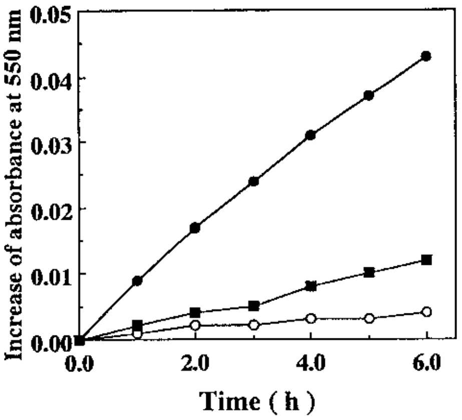


Figure 9. Reduction or cytochrome c monitored by the increase of absorbance at 550 nm. The dispersion of 5Cz18Z, cytochrome c, and triethanolamine with irradiation (○); the dispersion of those without irradiation (●); the solution of cytochrome c and triethanolamine with irradiation (■).

tion of cytochrome c was observed (Figure 9). Two mechanisms for the photoreduction are possible; i) photoelectron transfer from ECz group to cytochrome c and ii)electron transfer from triethanolamine to the heme which is activated by the excited energy transfer from the ECz group. The elucidation of the mechanism is under investigation.

Conclusions

Protein engineering is still not so powerful enough to develope a novel protein with a certain tertiary structure, because the folding mechanism of proteins has not been clarified, as yet. In the present study, two or three helices are connected together by using a template molecule to construct a helix-bundle structure. The helix-bundle formation in phospholipid membrane was shown by CD spectroscopy, and the peptide functioned efficiently as ion channel. Thus, the TASP method is very useful to construct a tertiary structure.

The other way to arrange a functional group regularly in space was also presented here. The amphiphiles carrying a chromophore-containing amino acid formed a bilayer membrane. The chromophores were fixed in the membrane to realize efficient photo-energy migration and to suppress the hole transfer by stabilizing the radical cation in the membrane. The membrane reduced cytochrome c effectively by irradiation. Therefore, molecular-assembly system is also useful for realizing the regular arrangement of functional groups as the case of the tertiary structure of proteins.

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