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Cyclic Peptides as Scaffold for Organization of Functional Groups on Surface

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We herein propose a novel method for surface modification with using cyclic peptides as scaffold for choromophores. Cyclic peptides have been designed and synthesized to function specifically: for example, cyclic hexa- and octapeptides can be designed to arrange carbonyl groups for complexation selectively with cation. It is also possible to design cyclic peptides having a chromophore with specific orientation against the cyclic plane. In the present study, Disperse Red-1 (DR-1) was conjugated with a cyclic dipeptide or a cyclic octapeptide, which was designed to act as scaffold for immobilization of DR-1 specifically on surface. The immobilization of DR-1 on substrate was studied by polarization modulation FT-IR reflection-absorption spectroscopy (PM-IRRAS) and optical second harmonic generation (SHG) measurement. The results indicate that both cyclic peptides with DR-1 are useful as scaffold of functional groups on surface. Surface modification is one of the key technologies for nano science, which may open up possible applications in biomaterials, molecular electronics, and optomaterials, etc.
Key words: cyclic peptide, monolayer, second harmonic generation, nonlinear optics, molecular orientation

1. INTRODUCTION

The synthesis of new molecules with a unique architecture and useful function is an area that keeps chemists stimulating their imagination ever. Among a large number of molecules, cyclic peptide has attracted much attention with respect of various functions and molecular assembling property. Naturally occurring cyclic depsipeptides and cyclic peptides of typical examples of valinomycin and gramicidin A of antibiotics have been intensively studied together with their model compounds to elucidate the structure-activity relationship. Valinomycin complexes selectively with potassium ion to transport it through cell membrane. Utilization of valinomycin to ion-selective electrode is also successful[1-4]. On the other hand, gramicidin A is known as ion selective channel[5,6], and the molecular mechanism of ion-channel formation in lipid membrane has been extensively studies. Aside of them, cyclic peptides show a good self-assembling property to form nanotubes on the basis of intermolecular hydrogen bondings. Ghadiri and co-workers made a new class of organic nanotubes based on cyclic peptides of alternating D,L-α-amino acids[7-9]. Various cyclic peptides have been reported so far to construct tubular assemblies, where alternating D- and L-amino acids in the primary sequence is a key designing for the tubular form. It is therefore possible to design cyclic peptides that adopt a low-energy ring-shaped flat conformation where all amide bonds in the peptide backbone lie approximately perpendicular to the plane of the ring structure. The nanotubes composed of cyclic peptides are already shown to work as a transmembrane channel for transport of ions and biomolecules[10].

Nanotechnology contributes tremendously to modern industry, and the bottom-up technique such as preparation of self-assembled monolayers[11,12] (SAMs) is considered to become more important when fine structures in a nano scale are required. For immobilization of organic compounds to surface, metal-sulfar[13,14] bonding on metal substrate or Si-O bonding on transparent substrates such as quartz and glass are generally used. On the other hand, we propose here to use cyclic peptides as scaffold of functional groups. Cyclic peptides of smaller ring size, less than decapeptide, tend to take a flat-ring conformation. Besides, one surface of the ring structure can be designed to be endowed with complexation ability with a cation. Using these properties of cyclic peptides, cyclic peptides should be a good candidate for immobilization of functional groups on surface by secondary interactions such as complexation. This is our first attempt to apply cyclic peptides as scaffold of functional groups for surface modification.

A chromophore for nonlinear optics[15] (NLO) was here conjugated with cyclic peptides, and quartz surface was modified by the chromophore with using the cyclic peptide as scaffold. The nonlinear optical property of the modified surface was evaluated by a measurement of second harmonic generation[15] (SHG), which is very sensitive to the orientation of the chromophore on the surface. The spatial arrangement of the chromophore is

necessary to be noncentrosymmetric for SHG observation. The ability of cyclic peptides as scaffold of the chromophore is therefore evaluated precisely by the SHG observation.

We synthesized two kinds of cyclic peptides. One is composed of two amino acid residues (the smallest cyclic peptide) and an NLO active dye, and the other is composed of eight amino acid residues and the dye. Disperse Red-1 (DR-1), which is an azobenzene derivative, was chosen for the NLO active dye. DR-1 is characterized by its large hyperpolarizability. The primary sequence of the cyclic octapeptide contains four repetitions of Gly-Pro sequence but a Tyr residue at one of the Pro residues. It is reported that cyclic octapeptide cyclo(Gly-Pro)$_4$ formed complex with alkali metal ions and alkaline-earth metal ions such as sodium ion, calcium ion, and magnesium ion[16]. DR-1 was bound to the cyclic peptide through ether linkage with the side chain of the Tyr residue. A part of the sequence, Gly-Tyr-Gly is the same as the key sequence of yellow fluorescent protein (YFP), which is a mutant of green fluorescent protein (GFP) having the key sequence of Ser-Tyr-Gly sequence[17]. The cyclic peptide designed here is expected to possess complexation ability with cations and to show a similar property to the GFP. The latter point is under investigation in our laboratory.

2. EXPERIMENTAL SECTION

2.1 Materials

Two kinds of cyclic peptides were synthesized (Fig.1). Cyclic octapeptide with DR-1 (cyclo[8+DR-1]) is designed to complex with cations. On the other hand, cyclic dipeptide with DR-1 (cyclo[2+DR-1]) has the smallest ring size. The peptides were synthesized by the conventional liquid phase method. DR-1 was introduced to the side chain of the Tyr residue by Mitsunobu reaction[18]. These two compounds were identified by ^{1}H NMR spectroscopy and FAB MASS measurement.

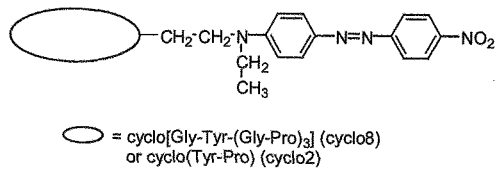


Fig. 1 Molecular structures of cyclo[8+DR-1] and cyclo[2+DR-1]. The primary sequence of cyclo8 is –[GYGPGPGP]- and that of cyclo2 is –[YP]-.

2.2 Preparation of substrates and monolayers

Two kinds of substrates, gold and quartz, were used. The former substrate was prepared by subsequent vapor deposition of chromium (100 nm thickness) and then gold (1000 nm thickness) onto a slide glass. The gold surface was modified by the SAM formation, which was converted to a cationic surface. The gold substrate was incubated in an aminoethanethiol solution followed by treatment with a hydrochloric acid solution to afford ammonioum salts on the surface. The quartz substrate was used after washing according to an RCA cleaning procedure [immersion of substrates in a mixture of $NH_3 \cdot H_2O$:H_2O_2:H_2O (1:1:5) for 20 min at 80 °C]. The quartz surface was also modified by siliconization to become a cationic surface. The quartz substrate was immersed in a toluene solution of trimethoxyaminopropylsilane for several minutes at 60 °C. After thorough washing, the substrate was incubated in a hydrochloric acid solution to obtain ammonium salts on the surface.

The cyclic peptides were spread on water subphase to obtain monolayers. Two kinds of surfaces, bare substrate and modified surface by ammonium salts, were prepared as described above and subjected to the conventional Langmuir-Blodgett technique to be covered by the monolayers. The transfer was carried out at the surface pressure of 10 mN/cm.

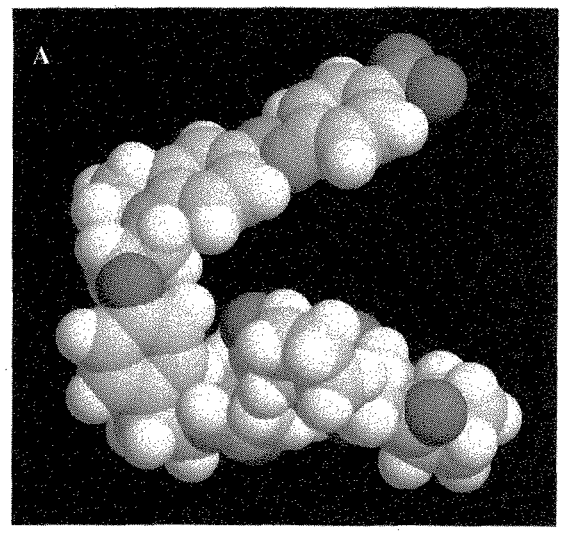
2.3 Measurements

The surface pressure-area isotherms were obtained by a USI FSD-110. Polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS) measurements were recorded on a Nicolet Magna 850 Fourier transform infrared spectrophotometer. SHG intensity was monitored by a transmission mode with rotating the quartz substrate against the incident light.

3. RESULTS AND DISCUSSION

3.1 Surface pressure-area isothems

Monolayer balance measurements provide valuable information on the molecular alignment of the peptides at air/water interface. Cyclic peptides in a chloroform solution were spread on water subphase. The π-A curves of both peptides were very similar to the result of cyclic peptide reported before[19], indicating formation of stable monolayers. The molecular area calculated from extrapolation of π-A curves to zero surface pressure is agreeable with the area of cross section of the cyclic peptide ring. The cyclic peptide moiety is considered to take horizontal orientation, and is facing the hydrophilic surface to water. Notably, DR-1 alone as well as cyclic peptides without DR-1, cyclo8 and cyclo2, could not form stable monolayer at the air/water interface. Probably the balance of hydrophobic DR-1 and relatively hydrophilic cyclic peptide is important for monolayer formation.



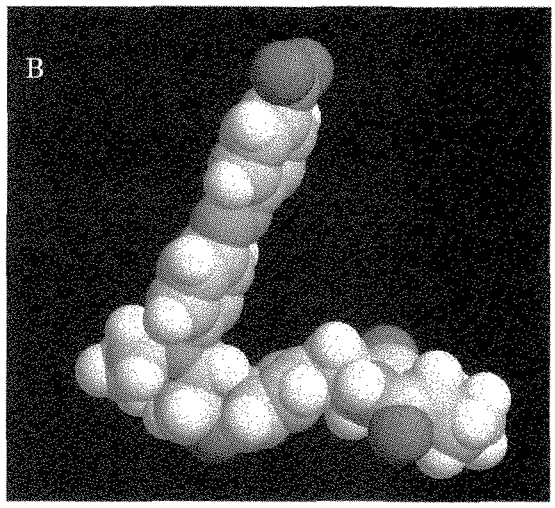


Fig. 2 CPK models of cyclic peptides with DR-1 at the side chain. The cyclic peptide moiety takes horizontal orientation at the air/water interface, thereby protruding the hydrophobic DR-1 moiety over the water subphase. Cyclo[8+DR-1] (A) and cyclo[2+DR-1] (B)[20].

3.2 Polarization modulation IRRAS

The PM-IRRAS technique was used to analyze the molecular structure of eight- or two-residue cyclic peptide with DR-1 monolayer. It is well known that infrared spectroscopy used in the external reflection absorption (IRRAS) is a remarkably eminent method for characterization of monolayers[21,22]. Polarization modulation IRRAS technique can provide sensitively details of molecular orientation and molecular packing in the monolayer. This technique uses a photoelastic modulator (PEM), which modulates the polarization state of the incident electric field[23,24] repeatedly between horizontal and vertical directions. The advantage over the usual IRRAS mode is that modulated reflectivity of gas or bulk water is independent of the direction of the incident electric field because of isotropic absorption, resulting that the interrupting effects of water vapor and carbon dioxide can fairly be eliminated[23,24].

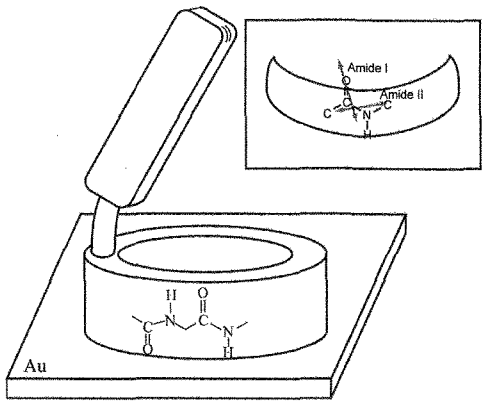


Fig. 3 Schematic illustration of configuration of cyclo[8+DR-1] deposited on gold. PM-IRRAS measurement is effective to estimate the orientation of the peptides with DR-1 on gold because of the virtually orthogonal relationship between amide I and amide II bands. The doughnut-shaped ring on gold and the flat stick attached to the ring represent cyclo8 and DR-1 unit, respectively. The inset shows the transition moments of amide I and amide II bands in the cyclic structure[25].

Amide I absorption assigned to mainly carbonyl (C=O) stretching vibration is observed around 1650 cm^{-1}, and amide II assigned to mainly N-H bending and C-N stretching modes are observed around 1540 cm^{-1}. Absorption with a transition moment perpendicular to the surface is strong, whilst that parallel to the surface is weak[26,27]. As cyclo[8+DR-1] doesn't have an alternate D,L-amino acid sequence, the transtion moment of the amide I band is not strictly perpendicular to the peptide ring as cyclic peptides with alternating D,L-amino acids[9,19,26,27]. On the other hand, the transition moment of amide II band is nearly parallel to the cyclic skeleton (Fig.3). From the large value of amide I/II ratio, it is shown that the amide I band is strongly absorbed and amide II, weakly. It therefore indicates that the cyclic skeleton is laid on gold substrate. Cyclo[2+DR-1] takes cis configuration about the two amide bonds because of steric constraint. IRRAS is insufficient for evaluation of cis amide, but the cyclic peptide moiety is also considered to be laid down on gold.

PM-IRRAS measurement was carried out on two kinds of substrates, bare gold and ammonium-attached gold. The IRRAS spectra of cyclo[8+DR-1] on both substrates are nearly the same in the amide I and amide II region. Therefore, ammonium groups on substrate do not affect configuration of the cyclic peptide on it.

3.3 Second harmonic generation

Second harmonic generation (SHG) was used to determine the nonlinear optical efficiency of the Langmuir monolayer. The polarized SHG measurement was carried out in the transmission mode using the 1064 nm output of a Nd:YAG laser. Fig. 4 shows the SHG intensity as a function of incident angle from -60° to 60° of the cyclo[8+DR-1] monolayer. The Maker fringes[28] arise from the interference of the second harmonic signal generated from the monolayers that are transferred on both sides of the quartz substrate[29].

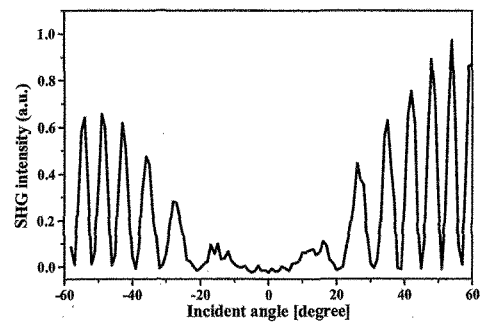


Fig. 4 SHG intensity (arbitrary units) as a function of p-polarized fundamental beam incident angle from the quartz substrate having a cyclo[8+DR-1] monolayer on both sides. The fringe pattern derives from the interaction between the SH waves generated from the

monolayers on both sides of the quartz substrate.

The minimum of Maker fringe generated by the monolayers on both sides appears at nearly zero degree, indicating that the monolayer is nearly identical on both sides of the substrate. Interestingly, SHG intensity from cyclo[2+DR-1] is stronger than that from cyclo[8+DR-1]. The surface density of cyclo[2+DR-1] is higher than cyclo[8+DR-1]. In addition, DR-1 of cyclo[2+DR-1] should orient more perpendicularly on surface than cyclo[8+DR-1]. These two factors should be the reasons for the intense SHG from cyclo[2+DR-1] monolayer.

SHG intensity of cyclo[2+DR-1] on bare quartz was stronger that that on ammonium-attached quartz. DR-1 is known to exhibit an absorption peak around 480 nm[30,31]. UV/Vis spectrum of cyclo[2+DR-1] on bare quartz was nearly the same as that on ammonium-attached quartz. Therefore, the DR-1 densities on the both substrates are the same. In addition, ammonium groups on the substrate do not affect the configuration of the monolayer. Taken together, the ammonium positive charges should affect the polarizability of DR-1 on the surface to change the SHG intensity from the monolayer with the same configuration of the peptide.

We synthesized two kinds of cyclic peptides having DR-1. The cyclic peptides formed stable monolayers on water subphase, and were transferred successfully on substrates. Cyclic peptide moieties were laid on water subphase with protruding DR-1 moiety over water phase. The monolayers on substrate showed nonlinear optical activity, suggesting that DR-1 moieties oriented on the surface. Cyclic peptides are thus regarded as a good scaffold for surface modification with chromophores. This method has a high potential for wide application of materials science in nano scale.

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