CHEMISTRY LETTERS, pp. 1813-1816, 1992.

Design of a Hybrid of Two α-Helix Peptides and Ruthenium Trisbipyridine Complex for Photo-induced Electron Transfer System in Bilayer Membrane

Hisakazu MIHARA, Norikazu NISHINO,* Ryuji HASEGAWA, Tsutomu FUJIMOTO, Satoshi USUI,† Hitoshi ISHIDA,† and Katsutoshi OHKUBO†

Department of Applied Chemistry, Faculty of Engineering, Kyushu Institute of Technology, Tobata, Kitakyushu 804

†Department of Applied Chemistry, Faculty of Engineering, Kumamoto University, Kurokami, Kumamoto 860

Two strands of an amphiphilic α-helical 22-peptide were anchored on ruthenium trisbipyridine complex. The hybrid was equipped with the triad of the ruthenium complex, anthraquinone, and viologen as photo-induced electron transfer system embedded in bilayer membrane.

Three-dimensional structures of polypeptides play decisively important roles for electron transfer reactions in biological system. There are finely oriented functional groups such as chlorophylls, pheophytins and quinones in the α-helical bundle of membrane proteins.[1] These orientations are thought to be for the efficiency of the electron transfer. On the other hand, the *de novo* design of artificial proteins has been intensively studied by trying to construct the three-dimensional structures of polypeptides.[2] For example, α-helix bundle structures have been successfully constructed by using various tricks.[3] In the next step, functional groups should be arranged to design artificial functional proteins. Recently, Lieberman and Sasaki[4] and Ghadiri *et al.*[5] reported the construction of three α-helix bundle structure in aqueous solution with metal complex of trisbipyridine in which each bipyridine bore a single 15-peptide chain with the amphiphilic α-helix motif.[6] More recently, Mecklenburg *et al.*[7] demonstrated photo-induced electron transfer in a redox triad molecule consisting of phenothiazine, ruthenium trisbipyridine and viologen based on Lys. For more sophisticated design of the peptide with photo-induced electron transfer, functional moieties should be arranged on a row of the electron path and the peptide-module must be assembled in bilayer membrane or in the solid state.

We independently designed a transmembrane molecular device which will furnish a photo-induced electron path (Fig. 1). The triad, which we have employed, was ruthenium complex of trisbipyridine ($Ru(bpy)_3^{2+}$), anthraquinone (AQ) and propylviologen (prV^{2+}) (Fig. 1A). The redox potentials of them were estimated as -0.88 (Ru^{2+}/Ru^{3+}), -0.72 and -0.42 V vs. SCE, respectively. The photosensitizer and the electron acceptor were placed on the both sides of the 22-peptide segment with length in α-helix to lie across the bilayer membrane (Fig. 1A, B). Between the key moieties was placed an AQ group anchored on an ornithine (Orn) side chain (Fig. 1C). For the design of the peptide segment, various cares were taken. The heptapeptide was adopted as a unit amphiphilic α-helical segment (Fig. 1B, D). At the center was positioned α-aminoisobutyric acid (Aib)[8] to reinforce the stability of the helix structure. The four Leu in seven residues were incorporated to

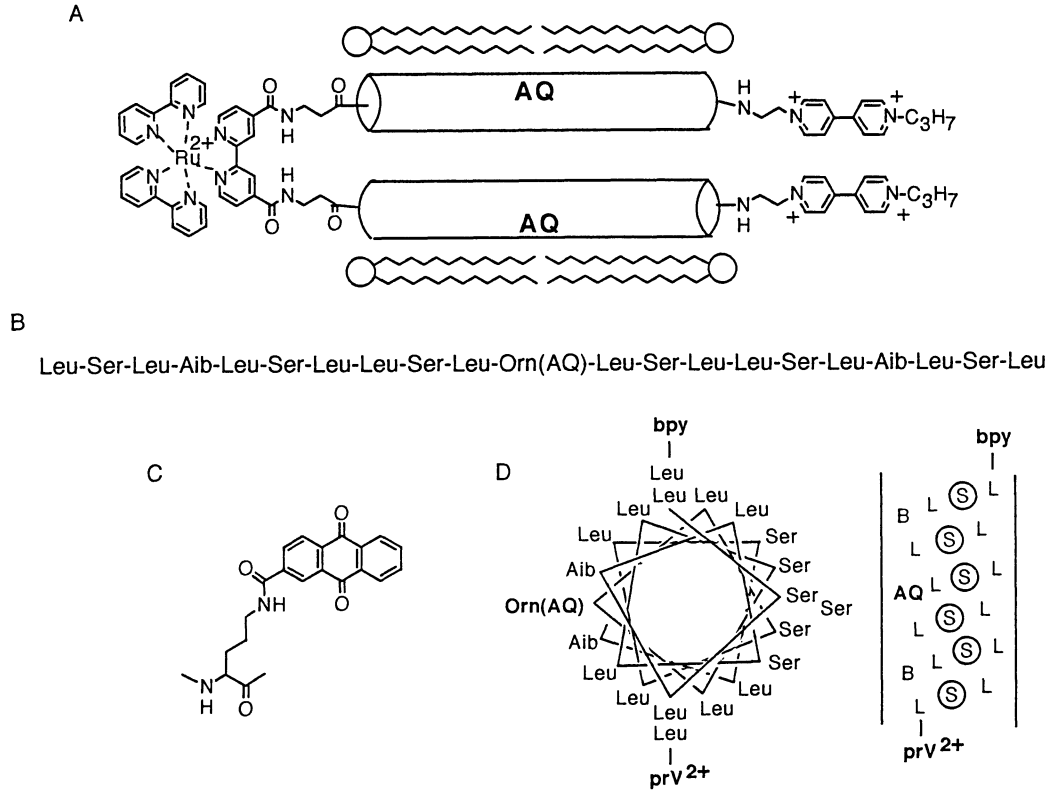

Fig. 1. (A) The designed structure of the hybrid peptide with a redox triad, $Ru(bpy)_3^{2+}$, anthraquinone (AQ) and propylviologen (prV^{2+}) presumed to be embedded in bilayer membrane. Cylinders represent the α-helical 21-peptides. (B) The amino acid sequence of the 21-peptide. (C) Structure of Orn(AQ). (D) The wheel and net presentations of the α-helical 21-peptide. B denotes Aib.

increase the lipophilicity of the whole segment. The hydrophilic and hydrogen bonding hooks between two segments are allotted to two Ser in seven residues. Two 22-peptide segments are expected to take a twisted form with two α-helical conformation so as to stabilize the whole structure and to be embedded in the hydrocarbon region in membrane.

The 21-peptide was synthesized by the conventional method with *t*-butyloxycarbonyl (Boc) for the protection of α-amino group and without the protection of the side chain of Ser. $Ru(bpy)_3^{2+}$ combining two β-Ala residues was prepared by the complexation between ruthenium bisbipyridine[9] and 2,2'-bipyridine-4,4'-dicarbonyl-β-Ala-OMe followed by saponification of the methyl ester. The Boc-21-peptide was coupled with *N*-2-aminoethyl-*N'*-1-propyl-4,4'-bipyridine with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)/1-hydroxybenzotriazole (HOBt).[10] The resulted Boc-21-peptide-prV^{2+} was deprotected and condensed with $Ru(bpy)_3^{2+}$-β-Ala by using the benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP)[11] to give the desired hybrid **1**. The intermediates and the final hybrid peptide were purified by gel filtration chromatography with Sephadex LH-60 after each coupling. They showed the corresponding molecular weights (6200 for **1**, calcd. 6454 and 3200 for Boc-21-peptide-prV^{2+}, calcd. 2947) on gel-filtration HPLC (TSK-GEL G3000HXL, 7.8 mm x 300 mm, with polystyrene standard in dimethylformamide). Amino acid analysis, absorption and fluorescence spectra[12] were consistent with the structure of the hybrid **1**.

The circular dichroism (CD) spectra of **1** showed that the peptide segments in **1** took α-helical conformation in MeOH (45% α-helicity) and aqueous MeOH (40% α-helicity) (Fig. 2). The increase in H_2O content gradually unwinded the α-helical conformation. The hybrid **1** in the presence of sodium dodecylsulfate (SDS) micelles (10 mM, 1 M = 1 mol·dm^{-3}) showed a similar α-helical pattern in CD spectrum, but significant increase in α-helicity (63 %)[13] was observed (Fig. 2). These results indicate that the peptide segments in **1** fold more highly in α-helical structure under hydrophobic conditions than hydrophilic ones. In aqueous solution, two α-helical segments should come together in close proximity by placing the hydrophobic faces of the helices inside, while under hydrophobic environment they gather by placing the hydrophilic faces inside. Thus, the hybrid should take a three-dimensional structure in which two α-helices are assembled with each other even under the different conditions due to the amphiphilic nature of the α-helix. The induced CD was also observed at the absorption region of $Ru(bpy)_3^{2+}$ and AQ (255 and 280 nm, respectively). The profiles of CD at this region were influenced significantly by various MeOH contents and the micellar conditions. This fact suggests that two chromophores are exposed to the different circumstances depending on the solution conditions. These CD profiles must be related to the orientation of these functional groups on the varying three-dimensional structures of the α-helical peptides.

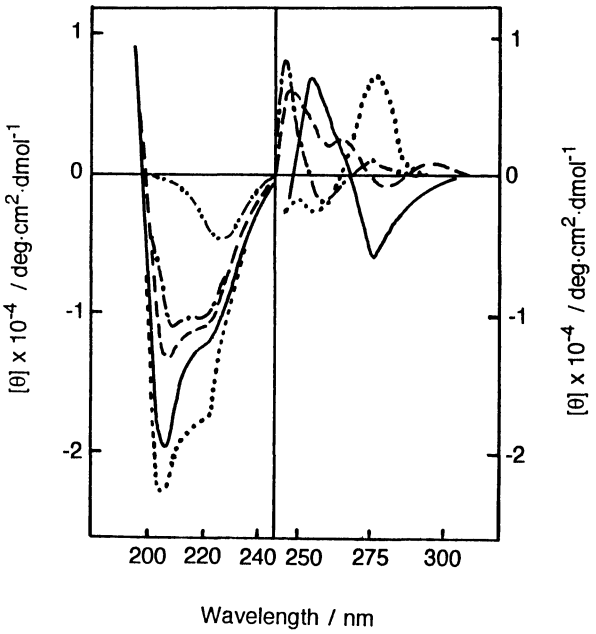

Fig. 2. CD spectra of the hybrid peptide in MeOH (———), 60% MeOH (– – –), 40% MeOH (–·–·–), in the presence of SDS micelles (10 mM) (·····), and in the egg yolk lecithin vesicles (–··–··) at peptide concentration of 0.2-1.0 x 10^{-4} M.

The hybrid **1** was incorporated into egg yolk lecithin vesicles and the vesicle solution was subjected to gel filtration with Sephadex G-75. The hybrid was quantitatively (82% from the absorbance at 454 nm) recovered in the fractions containing vesicles, indicating that the peptide **1** was stably embedded into the membrane. However, **1** in the vesicles (1.0 mM) showed peculiar CD spectrum (Fig. 2), which suggested that the conformation of the peptide was different from α-helix but undefined at present. Supposing that **1** takes β-sheet structure, the negative peak at 225 nm is at too longer wavelength than usual (216 nm). Though we expected the supercoil of the α-helices as shown in Fig. 1, **1** might have formed double β-helices such as a conformer of gramicidin A, a natural membrane-channel peptide, whose CD profile resembles that of **1** in vesicles.[14]

Since the hybrid **1** was successfully placed in the egg yolk lecithin vesicles, we attempted to examine the efficiency of the photo-induced electron transfer reaction across the phospholipid bilayer. The lecithin vesicles contained the peptide (20 μM) with EDTA (0.2 M) inside and methylviologen (5 mM) outside. The increase of absorption at 395 and 605 nm derived from the methylviologen cation radical outside the vesicles was observed

by the prolonged irradiation (>420 nm) under anaerobic conditions according to the reported procedure[15] (8.0 μM after 10 h irradiation). The slow electron transfer may be related to the undefined conformation of **1** in bilayer membrane. Photochemical studies in detail are being carried out to evidence the electron transfer *via* the AQ group.

The photo-functional groups were successfully deployed on the peptide segments. This hybrid peptide could afford the electron transfer reaction across the lipid bilayer. Though further study on the conformation of the hybrid in membrane is remained, the combined strategy of the *de novo* design of polypeptides and photochemical studies will allow to construct the efficient electron transfer system in artificial proteins.

The present research was partly supported by a Grant-in-Aid for Scientific Research on Priority Area from the Ministry of Education, Science and Culture, Japan (N. N. and H. M.).

References

1) J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, *Nature*, **318**, 618 (1985); G. Feher, J. P. Allen, M. Y. Okamura, and D. C. Rees, *ibid.*, **339**, 111 (1989).
2) W. F. DeGrado, Z. R. Wasserman, and J. D. Lear, *Science*, **243**, 622 (1989); M. Mutter and S. Vuilleumier, *Angew. Chem., Int. Ed. Engl.*, **28**, 535 (1989).
3) L. Regan and W. F. DeGrado, *Science*, **241**, 976 (1988); M. Mutter and G. Tuchscherer, *Makromol. Chem., Rapid Commun.*, **9**, 437 (1988); T. Sasaki and E. T. Kaiser, *J. Am. Chem. Soc.*, **111**, 380 (1989); H. Morii, K. Ichimura, and H. Uedaira, *Chem. Lett.*, **1990**, 1987; K. W. Hahn, W. A. Klis, and J. M. Stewart, *Science*, **248**, 1544 (1990); N. Nishino, H. Mihara, Y. Tanaka, and T. Fujimoto, *Tetrahedron Lett.*, in press.
4) M. Lieberman and T. Sasaki, *J. Am. Chem. Soc.*, **113**, 1470 (1991).
5) M. R. Ghadiri, C. Soares, and C. Choi, *J. Am. Chem. Soc.*, **114**, 825 (1992).
6) E. T. Kaiser and F. J. Kézdy, *Science*, **223**, 249 (1984).
7) S. L. Mecklenburg, B. M. Peek, B. W. Erickson, and T. J. Meyer, *J. Am. Chem. Soc.*, **113**, 8540 (1991).
8) I. L. Karle and P. Balaram, *Biochemistry*, **29**, 6747 (1990) and references cited therein.
9) B. P. Sullivan, D. J. Salmon, and T. J. Meyer, *Inorg. Chem.*, **17**, 3334 (1978).
10) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
11) B. Castro, J.-R. Doromoy, G. Evin, and C. Selve, *Tetrahedron Lett.*, **1975**, 1219.
12) Absorption spectra in MeOH; λmax(ε), 258 nm (148000), 281 nm (93000), 454 nm (13000). Fluorescence spectrum in MeOH (2.0×10^{-5} M); λex 450 nm, λem 530 nm and 625 nm.
13) P. C. Lyu, J. C. Sherman, A. Chen, and N. R. Kallenbach, *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 5317 (1991).
14) S. V. Sychev, N. A. Nevskaya, St. Jordanov, E. N. Shepel, A. I. Miroshnikov, and V. T. Ivanov, *Bioorg. Chem.*, **9**, 121 (1980).
15) M. S. Tunuli and J. H. Fendler, *J. Am. Chem. Soc.*, **103**, 2507 (1981); T. Matsuo, *J. Photochem.*, **29**, 41 (1985).

(Received June 17, 1992)