Efficient Photoreduction of Cytochrome c in the Presence of a Bilayer Membrane of *N*-Ethylcarbazole-Containing Amphiphiles

Tomoyuki Morita, Shunsaku Kimura,* and Yukio Imanishi†
Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Yoshida Honmachi, Sakyo-ku, Kyoto 606-8501
†*Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama-cho, Ikoma, Nara 630-0101*

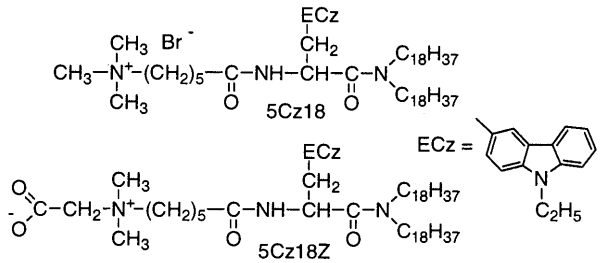
(Received September 29, 1999; CL-990839)

Photoreduction of cytochrome c (cyt.c) in the presence of a bilayer membrane of amphiphiles having an *N*-ethylcarbazolyl (ECz) group was investigated. It was found that efficient energy migration among ECz groups promoted electron transfer to cyt.c, and the photoreduction yield was thus insensitive to the concentration of cyt.c, but was determined mainly by the amount of a sacrificial donor bound to the membrane.

In a bilayer membrane composed of amphiphile molecules covalently carrying a chromophore, the chromophores are regularly and densely packed in a plane, where effective photoenergy migration may occur. When an electron acceptor is embedded in the membrane system, efficient photoinduced electron transfer will be observed. In the present study, cytochrome c (cyt.c) was mixed with the chromophoric membrane composed of amphiphiles covalently carrying *N*-ethylcarbazolyl (ECz) group, and photoreduction of cyt.c was investigated. Cyt.c belongs to a family of electron transfer proteins that play an essential role in bioenergetics and drug metabolism[1] and has been most extensively studied in this class of proteins, and its interactions with lipid membranes have been well investigated.[2-5]

In the previous investigation, we showed that effective energy migration accelerates electron transfer from an ECz group to a viologen group.[6] Thus, the membrane is an efficient photoenergy-harvesting system, and is used for photoreduction of cyt.c in the present study. Cyt.c is used in place of a viologen-containing amphiphile in the previous paper[6], because cyt.c in a reduced form is relatively stable, and can dissociate from membranes to transport an electron to the other species in solution. When the present molecular system is combined with cytochrome oxidase, transmembrane proton gradient may be formed. Such photoreduction system could be the basis for fabrication of biomimetic photoelectric devices.[7]

Two kinds of dialkylammonium-type amphiphiles carrying L-3-(3-*N*-ethylcarbazolyl)alanine (5Cz18, 5Cz18Z) are used for the preparation of bilayer membranes. The zwitterionic membrane of 5Cz18Z is expected to show higher affinity for cyt.c than the cationic membrane of 5Cz18. Sacrificial donors used were triethanolamine (TEOA) and ethylenediaminetetraacetic acid (EDTA).



5Cz18 and 5Cz18Z were synthesized by a method reported previously.[8,9] EDTA was used as a water solution which was adjusted at pH 7.0 with an aqueous $NaHCO_3$ solution. Aqueous dispersions of bilayer membrane were prepared by a method reported previously.[6] Cyt.c and a sacrificial electron donor were added individually to the dispersion after the membrane preparation.

Partitioning of cyt.c to the membrane was determined by measuring the amount of cyt.c in the filtrate of the mixture of cyt.c and the membrane using a Millipore Ultrafree-MC (100000 NMWL). Photoreduction experiment was carried out after replacement of the sample solution with argon. The excitation light was sourced from a JASCO Xe lamp (500 W), and was filtered by a colored optical glass and a solution filter to obtain a light with a narrow band width centered at 300 nm. The degree of photoreduction of cyt.c was determined by the change of absorbance at 550 nm. The concentrations of compounds were as follows: centrifugal filtration; [5Cz18] = [5Cz18Z] = 21 – 84 μM, [cyt.c] = 40 μM: quenching experiment; [5Cz18] = [5Cz18Z] = 6.5 μM, [cyt.c] = 0 – 17 μM, in the case of the mixed membrane; [5Cz18] = 6.5 μM, [dioctadecyldimethylammonium bromide] = 650 μM: photoreduction of cyt.c; [5Cz18] = [5Cz18Z] = 56 μM, [cyt.c] = 16 μM, [sacrificial electron donor] = 160 μM. All measurements were performed at 20 °C, where the bilayer membrane of 5Cz18 or 5Cz18Z was in the gel-state.

Partitioning of cyt.c to the bilayer membrane was investigated with varying concentrations of the amphiphile. Cyt.c was found to be partitioned more favorably to the 5Cz18Z membrane than to the 5Cz18 membrane. This result can be attributed obviously to the cationic nature of cyt.c at the neutral pH. About 30 molecules of 5Cz18 are calculated to be necessary for one molecule of cyt.c to be partitioned to the membrane. However, only 4.5 molecules are enough for cyt.c to bind to the 5Cz18Z membrane. Such extraordinary binding of cyt.c is also reported with the anionic membrane of dioleoyl phosphatidylglycerol.[10] The high partitioning of cyt.c to the 5Cz18Z membrane might be due to the molecular structure of 5Cz18Z where a carboxylate group is located at the terminal, thus, being exposed fully out of the membrane. This arrangement might be favorable for the electrostatic interaction of the cationic cyt.c molecule with the membrane.

Fluorescence quenching of ECz group in the membranes by electron transfer to cyt.c is shown in Figure 1 according to the Perrin plot. The quenching rate in the 5Cz18 membrane was higher than that in the bicomponent membrane of 5Cz18 and dioctadecyldimethylammonium bromide (DDAB). Since the amphiphiles of 5Cz18 and DDAB are the cationic type of having an ammonium group and dioctadecyl chain, the amount of cyt.c absorbed to the membrane surfaces is considered to be nearly the same due to the same surface-charge density between

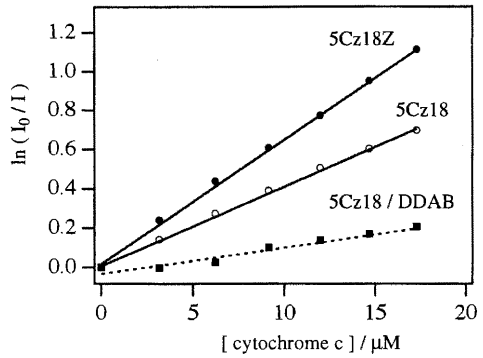


Figure 1. Perrin plots of fluorescence quenching of ECz group in the bilayer membranes. Excitation wavelength was 299 nm.

the 5Cz18 and 5Cz18/DDAB membranes. The difference in quenching between these membranes should be therefore explained mainly by efficient energy migration among ECz groups in the 5Cz18 membrane, because the energy migration in the 5Cz18/DDAB membrane is suppressed by DDAB molecules intervening in the lattice points of ECz group in the membrane. It was reported that dipole-dipole interaction is allowed between the neighboring ECz groups in the 5Cz18 membrane, but they are separated to inhibit excimer formation.[8,11] Although cyt.c is faintly partitioned to the 5Cz18 membrane, the quenching rate was relatively high. This fact suggests that energy migration occurs efficiently to activate an ECz group near a cyt.c bound to the cationic membrane.

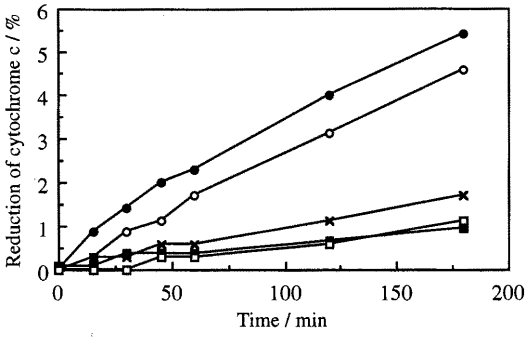


Figure 2. The effects of the 5Cz18 or 5Cz18Z membrane and photoirradiation on the photoreduction of cyt.c in the presence of TEOA; ● 5Cz18Z/cyt.c/TEOA with irradiation, ○: 5Cz18/cyt.c/TEOA with irradiation, ×: cyt.c/TEOA with irradiation, ■: 5Cz18Z/cyt.c/TEOA without irradiation, □: 5Cz18/cyt.c/TEOA without irradiation.

Photoreduction of cyt.c in the membranes was investigated in the presence of TEOA used as a sacrificial electron donor. The rate of cyt.c reduction are shown in Figure 2 together with the reference data in the absence of amphiphiles or without photoirradiation. Although cyt.c was hardly reduced in the control experiments, cyt.c reduction was promoted in the presence of membranes with photoexcitation of an ECz group. A slightly faster reduction in the presence of the 5Cz18Z membrane than the 5Cz18 membrane qualitatively agrees with the results of the quenching experiment.

Figure 3 shows the cyt.c reduction in the presence of EDTA. Cyt.c was reduced in the presence of the 5Cz18 membrane at a significantly higher rate than in the 5Cz18Z membrane and than in the TEOA systems. The quantum yields of

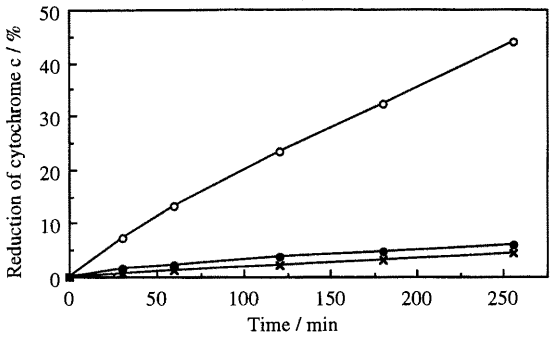


Figure 3. The effects of the 5Cz18 or 5Cz18Z membrane on the photoreduction of cyt.c in the presence of EDTA; ●: 5Cz18Z/cyt.c/EDTA, ○: 5Cz18/cyt.c/EDTA, ×: cyt.c/EDTA.

Table 1. Quantum yields for photoreduction of cyt.c in an aqueous dispersion of 5Cz18 or 5Cz18Z with two different sacrificial donors, TEOA and EDTA.

	5Cz18 / %[a]	5Cz18Z / %[a]	in solution / %[b]
TEOA	0.58	0.75	0.092
EDTA	5.1	0.36	0.33

[a]The quantum yields were calculated on the basis of the number of photon absorbed by ECz group. [b]In the absence of bilayer membranes, the number of photon absorbed by cyt.c was used for the calculation.

cyt.c photoreduction are summarized in Table 1. The efficient cyt.c photoreduction should be attributed to the concentrated anionic EDTA molecules at the cationic membrane surface. It is notable that the cationic membrane surface does not seriously influence the rate of photoreduction of cyt.c with the electrically neutral sacrificial donor, but significantly promotes the photoreduction in the presence of the anionic donor. Therefore, the rate-determining step for this photoreduction system is the photoreduction of the oxidized ECz group by the sacrificial donor. In other words, a photoexcited ECz group should easily reduce cyt.c due to the efficient photoenergy migration to an ECz group near to a cyt.c bound to the membrane.

References

1 F. S. Mathews, *Prog. Biophys. Mol. Biol.*, **45**, 1 (1985).
2 Z. Salamon and G. Tollin, *Biophys. J.*, **71**, 2 (1996).
3 S. Sui, H. Wu, J. Sheng, and Y. Guo, *J. Biochem.*, **115**, 1053 (1994).
4 J. Wang, C-J. A. Wallace, I. Clark-Lewis, and M.Caffrey, *J. Mol. Biol.*, **237**, 1 (1994).
5 H. Tanaka, K. Hayashi, T. Akatsuka, J. Toyama, K. Noda, T. Kida, Y.Ogoma, T. Fujii, and Y. Kondo, *J. Biochem.*, **117**, 1151 (1995).
6 T. Morita, S. Kimura, and Y. Imanishi, *J. Am. Chem. Soc.*, **121**, 581 (1999).
7 D. L. Zaslavsky, I. A. Smirnova, S. A. Siletsky, A. D. Kaulen, F. Millett, and A. A. Konstantinov, *FEBS Lett.*, **359**, 27 (1995).
8 K. Taku, H. Sasaki, S. Kimura, and Y.Imanishi, *Amino Acids*, **7**, 311 (1994).
9 T. Morita, S. Kimura, and Y. Imanishi, *Langmuir*, **14,** 171, (1998).
10 T. Heimburg and D. Marsh, *Biophys. J.*, **68**, 536 (1995).
11 K. Taku, H. Sasaki, S. Kimura, and Y. Imanishi, *Biophys. Chem.*, **44**, 187, (1992).