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Energy Transfer from Zinc-Myoglobin to a Cyanine Dye Facilitated by Their Simultaneous Binding to a Phosphate Bilayer Membrane[1)]

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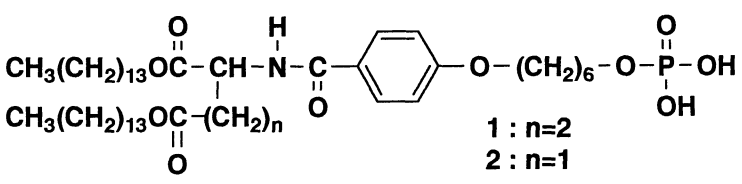
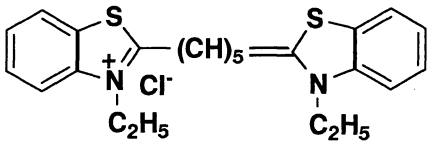
Zinc-substituted myoglobin (Zn-Mb) and a cationic cyanine dye were bound simultaneously to an aqueous dispersion or a cast film of a phosphate bilayer membrane. The energy transfer from Zn-Mb to the cyanine was more efficient in the cast film than in aqueous dispersion.

Artificial organization of proteins and other biological macromolecules is an important target in the current chemical research.[2)] We recently found that functional conversion of myoglobin was induced by its designed assembly together with cofactors such as dihydronicotinamide adenine dinucleotide (NADH) and flavin mononucleotide (FMN) on the surface of synthetic bilayer membranes.[3)] This finding strongly suggests that synthetic bilayers are useful to organize proteins and other functional molecules in controlled manners.[4)] We describe herein that energy transfer from zinc-substituted myoglobin (Zn-Mb) to a cyanine dye proceeds efficiently by their simultaneous binding to a phosphate bilayer membrane.



1 : n=2
2 : n=1



NK-1146

Phosphate-bearing amphiphile **1** which has been shown to interact with myoglobin in a specific manner,[3-6)] was used as a matrix membrane. Fluorescent Zn-Mb as energy donor was prepared according to the reported method.[7,8)] Protoheme was extracted from the holoprotein, met-myoglobin (horse heart, Sigma Chemical Co.), with 2-butanone at pH 2.2. To the resultant apo-myoglobin (apo-Mb), zinc-protoporphyrin IX (Aldrich Chem.) in 10% aqueous ethanolamine was added (pH 7.0, 50 mM phosphate buffer), and incubated for 4 h at 4 °C. Crude Zn-Mb was purified by gel filtration (Sephadex G-25), followed by ion exchange column chromatography (CM 52). Pow-

dered amphiphile **1** was sonicated in 10 mM Tris-HCl buffer (pH 7.5), into which given amounts of Zn-Mb and cyanine dye NK-1146 were added for spectral measurements.

Figure 1 shows absorption and fluorescence spectra of Zn-Mb and NK-1146 that are separately dispersed in aqueous phosphate bilayer **1**. Absorption maxima of Zn-Mb at 428 (Soret-band), 552 and 595 nm (Q-bands) and fluorescence maxima at 600 and 650 nm are essentially identical with the literature value of the reconstituted Zn-Mb.[6] Denaturation of Zn-Mb is not detected under the present conditions. The absorption maximum of NK-1146 shows a large bathochromic shift (λ_{max} = 675 nm) relative to that of the monomer species in ethanol (λ_{max} = 650 nm). This shift can be ascribed to the formation of the head-to-tail dye aggregate (so-called J-aggregate) on the membrane surface.[9] An emission peak of the J-aggregate is located at 710 nm. The emission band of Zn-Mb overlaps with the absorption band of the membrane-bound NK-1146. This satisfies the conditions for the Foerster type energy transfer.

Figure 2 displays fluorescence spectra of the aqueous bilayer dispersion which contains both Zn-Mb and NK-1146. Upon excitation at 428 nm (Soret-band of Zn-Mb), an emission peak (710 nm) from the J-aggregate of NK-1146 is observed in addition to the emission from Zn-Mb (600 and 650 nm). The emission from NK-1146 is not observed without Zn-Mb. The emission from NK-1146 by direct excitation is negligible at

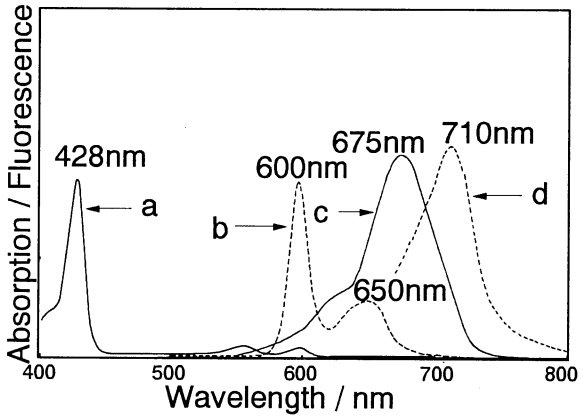


Fig. 1. Absorption/fluorescence spectra of Zn-Mb, NK-1146 in bilayer **1**.
a, absorption of Zn-Mb
b, fluorescence of Zn-Mb
c, absorption of NK-1146
d, fluorescence of NK-1146
[**1**] = 1.7×10^{-4} M
[NK-1146] = 4.3×10^{-6} M
[Zn-Mb] = 7.0×10^{-6} M
pH 7.5 (Tris-HCl buffer), 20 ℃
excitation wavelength 428 nm.

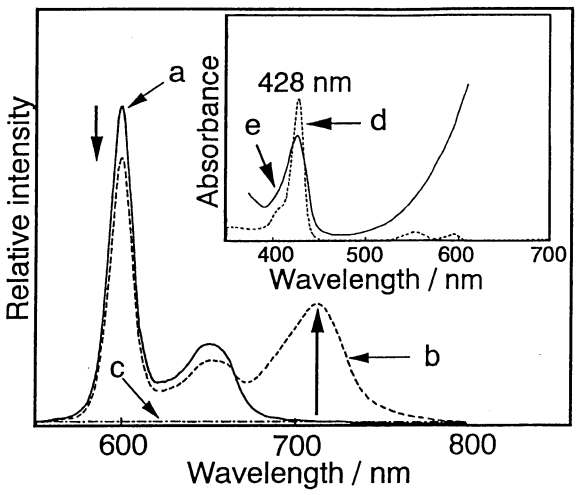


Fig. 2. Fluorescence and absorption spectra(inset) of Zn-Mb, NK-1146 in bilayer **1**. [**1**] = 1.6×10^{-4} M,
a, **1** : Zn-Mb = 40 : 1
b, **1** : Zn-Mb : NK-1146 = 40 : 1 : 1
c, **1** : NK-1146 = 40 : 1
d, absorption Zn-Mb
e, excitation spectra of Zn-Mb
pH 7.5 (Tris-HCl buffer), 20 ℃
excitation wavelength 428 nm.

this excitation wavelength. The excitation spectrum monitored at 710 nm practically coincides with the absorption spectrum of Zn-Mb. With increasing amounts of added cyanine, its emission is gradually enhanced and the emission from Zn-Mb is lessened. These data indicate that the energy transfer occurs from Zn-Mb to the cyanine on the surface of the phosphate bilayer membrane. On the other hand, the energy transfer from Zn-Mb to the cyanine did not occur, when the phosphate bilayer was replaced with the bilayer of zwitterionic distearoylphosphatidylcholine.

A self-supporting film was obtainable by casting the aqueous bilayer on a glass plate, as is the case with other bilayer membranes.[10] Emission maxima of both Zn-Mb and NK-1146 immobilized in the film (1.25 x 10^{-8} mol of Zn-Mb and 1.25-12.5 x 10^{-9} mol of NK-1146 against 4.2 mg (5 x 10^{-6} mol) of amphiphile **1**, room temperature) were identical with those in the aqueous dispersion ([**1**] = 1.7 x 10^{-3} M, [Zn-Mb] = 4.2 x 10^{-6} M and [NK-1146] = 4.2-42 x 10^{-7} M, 20 °C). Therefore, the efficiency of the energy transfer in both of the aqueous and film systems may be compared simply by the emission intensity ratio of NK-1146 and Zn-Mb (i.e., I(710 nm)/I(600 nm)). As shown in Fig. 3, the emission ratio increases linearly with increasing amounts of immobilized NK-1146. The slope of the emission ratio in the cast film is two times greater than that in the aqueous dispersion, indicating more efficient energy transfer in the cast film. This may be explained by assuming that Zn-Mb and NK-1146 were concentrated in the hydrophilic interbilayer region. However, when the bilayer component **1** was replaced with a closely-related bilayer of **2**, the energy transfer became less efficient both in the aqueous diepersion and in the cast film: see Fig. 3. It is clear that the efficiency depends not only on the polyanionic nature of the interbilayer but also on its subtle molecular organization. This presumption is supported by examining the energy transfer in an isotropic sodium alginate matrix (a conventional anionic polymer). A flexible sodium alginate film (contents: 1.25 x 10^{-8} mol of Zn-Mb, 1.25-12.5 x 10^{-9} mol of NK-1146 against 4.2 mg (2.4 x 10^{-5} unit mol) of sodium alginate) was obtainable by simple casting. The cyanine dye gave a broad absorption at around 500 nm in this film. Although the emission of Zn-Mb overlaps with the absorption of NK-1146, fluorescence emission from the cyanine was not

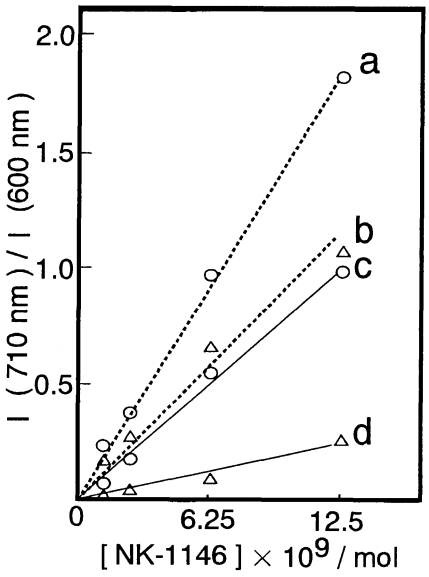


Fig. 3. Emission intensity ratio of NK-1146 and Zn-Mb.
a, in cast film of **1**
b, in cast film of **2**
c, in aqueous dispersion of **1**
d, in aqueous dispersion of **2**.

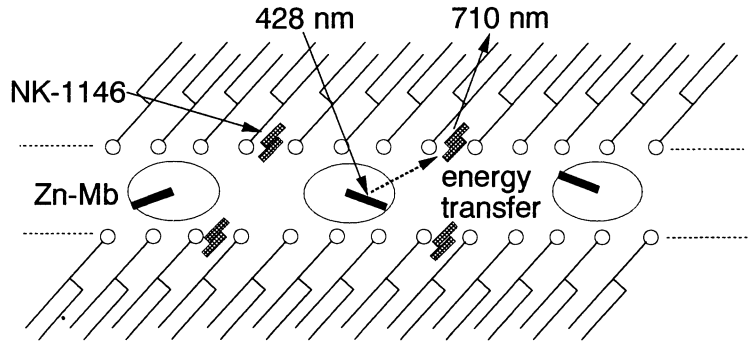


Fig. 4. Schematic illustration of specific binding of Zn-Mb and NK-1146 and the consequent energy transfer.

observed by excitation of Zn-Mb at 428 nm. Clearly simple concentration of positively-charged Zn-Mb and NK-1146 in an anionic matrix is not sufficient to induce efficient energy transfer.

We thus conclude that a highly organized matrix composed of the synthetic phosphate bilayer uniquely promote the photophysical interaction between Zn-Mb and a cyanine dye both in aqueous dispersion and in cast film (Fig. 4). Designed organization of protein molecules together with other functional groups is a key concept for protein-based molecular devices. The present results demonstrate that synthetic bilayer membranes are superior materials for this purpose.

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10) For example, see: S. Asakuma, H. Okada, and T. Kunitake, J. Am. Chem. Soc., **113**, 1749 (1991) and references cited therein.

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