9. T. Arnason, C.K. Wat, K. Downum, E. Yamamoto, E.A. Graham, and G.H.N. Towers, *Can. J. Microbiol.*, **26**, 698 (1980).
10. A. David and Ben-Efraim, *Tetrahedron Lett.*, **11**, 957 (1967).
11. D. Demoulin, *Chem. Phys.*, **11**, 329 (1975).
12. A. Weller in "Progress in Reaction Kinetics", Vol 1, G. Porter, Ed., Pergamon Press, New York, N.Y., (1961), Chapter 7.
13. E.J. Corey and J.O. Albright, *J. Org. Chem.*, **48**, 2114 (1983).
14. D.D. Perrin, W.L.F. Armarego, and B.R. Perrrin, "Purification of Laboratory Chemicals," Pergamon Press, Oxford, (1980).
15. G. Eglington and W. McCrae, *J. Chem. Soc.*, 2295 (1963).
16. J.B. Armitage, E.R.H. Jones, and M.C. Whiting, *J. Chem. Soc.*, **44** (1951).
17. A.S. Hay, *J. Org. Chem.*, **27**, 3320 (1962).
18. George Zweifer and Shyamala Rajagopalan, *J. Am. Chem. Soc.*, **107**, 700 (1985).

Photoelectron Transport Across Phospholipid Liposomes Pigmented by Anthracene and Naphthalene Derivatives

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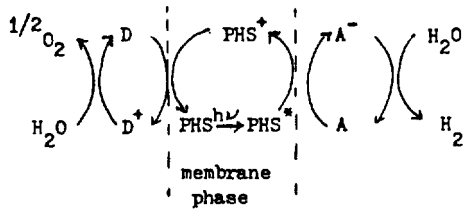
In order to investigate effective solar energy conversion system, the light-induced electron transfer reactions have been examined across single-lamellar liposomes incorporated organic photosensitizers such as anthracene and naphthalene derivatives. We have observed photosensitized reduction of methyl viologen (1,1'-dimethyl-4,4'-bipyridinium^{2+}) dissolved in the exterior aqueous phase of the pigmented phospholipid liposomes when EDTA, as electron donor, is dissolved in the enclosed aqueous phase of the liposomes. The anthroyl stearic acid incorporated in the hydrophobic bilayer of liposomes leads to much less quantum yield for the photosensitized reduction of MV^{2+} than the anthracene carboxylate incorporated in the outer hydrophilic layer. However, β-carotene with anthroyl stearic acid incorporated into the bilayer enhances the quantum yield significantly (Φ=0.2-0.3), preventing the reverse reaction of electron transfer ($MV^{+} \rightarrow MV^{2+}$) so that it might be useful for solar energy conversion into chemical energy. A naphthalene derivative, octadecyl naphthylamine sulfonic acid incorporated into the outer layer of liposomes results in less efficiency of MV^{2+} reduction than anthroyl stearic acid. These results have been also tested with respect to lipid components of liposomes.

Introduction

Scientists have focussed their attention to photobiological solar energy conversion to estimate the possibility of obtaining electrical and chemical energy from sunshine. The green plant is the typical photobiological system which converts quanta into chemical energy with high degree of efficiency and store them long enough to do some chemistry.[1-8] The photosynthetic systems(PS) I and II of green plant reside in the green chloroplast which is a membranous structure consisted of lipid bilayer. The green pigment absorbs the sunlight and separates the positive and negative across the thylakoidal membrane during the process of electron transport. The separated charges can be used for oxidizing water in the PS I or reducing carbon dioxide in the PS II through Calvin cycle.[2,5]

The natural photosynthesis has been stimulated in several laboratories by using pigmented artificial lipid membranes such as lipid vesicles (liposomes) and black lipid membranes (BLM).[9-13] Recent works have shown that BLM or liposomes containing photosensitizers(PHS) such as chlorophyll or ruthenium bipyridine($Ru(bpy)_3^{2+}$) exhibit interesting photoelectron transfer across the bilayer of membrane from electron donor (D) site to electron acceptor site(A), and subsequently generates D^+ and A^-(see scheme I).



Scheme I.

This system has been expected to show that the forward photoelectron transfer ($PHS^* + A \rightarrow PHS^+ + A^-$) is efficient and the back reaction is obviated to be useful for reduction of H_2O in the presence of a certain catalyst.[14] However, there are still some obstacles to be overcome to use that system for practical purpose. First, the efficiency of the forward reaction is relatively lower than in homogeneous solution. Even if the efficiency is increased, the back reaction is also enhanced. Thus, the artificial photosystem should be optimized by better combination of a proper photosensitizer and lipid component. Secondly, the artificial lipid membranes are not so stable, and it is necessary to develop more stable synthetic lipid-like polymer.

Hopefully to solve these problems, we have tried to develop ever more improved models for light-sensitized electron

transfer across liposomes by using new organic phtosensitizers such as anthracene and naphthalene derivatives with various kinds of phospholipid. We have observed the highly efficient photosensitized reduction of methyl viologen dissolved in the exterior aqueous phase of phospholipid vesicles incorporated with anthracene derivatives when EDTA, as electron donor, is dissolved in the interior aqueous phase of the vesicles. These results were also examined with respect to lipid components of liposomes and location of photosensitizers in liposomes.

Experimental

Materials. The organic photosensitizers such as anthroyl stearic acid(AS) and octadecyl naphthylamine sulfonic acid (ONS) were synthesized and analyzed according to the methods reported by Waggoner and Stryer.[15] Another photosensitizer, 9-anthracene carboxylate obtained from Aldrich was recrystallized from ethanol solution. Phospholipids such as dimyristoyl phosphatidylcholine (DMPC), phosphatidyl ethanolamine(PEA) (from sheep brain) and phosphatidylcholine(PC) (from egg yolk) were used as received from Sigma Chemical Co.. Methyl viologen dichloride from Fluka was recrystallized from water by addition of acetone and dried over calcium chloride anhydrous in a vacuum desiccator. Disodium ethylene diamine tetraacetate(EDTA) was used as supplied by Shinyo. Distilled water was further deionized by redistillation in the presence of acidic dichromate and alkaline permanganate.

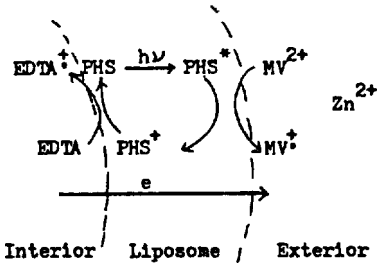
Preparation of liposomes. Small unilamellar liposomes (average diameter, 500 Å) were prepared by sonication of a phospholipid suspension according to the reported methods.[16,17] The thin films were formed by evaporating a chloroform solution of lipids and organic photosnsitizers. EDTA in phosphate buffer solution(0.5M) (pH, 7.00) was added to the thin film and stirred vigorously followed by sonication under nitrogen atmosphere in a Bransonic 221 bath for 20min. The solution changed from an opaque, milky color to a transparent opalescence. Multilamellar vesicles were removed by centrifugation at 30,000 g for 30min. The untrapped EDTA were removed by gel filtration of the suspension of liposomes over a Sepharose 4B column(1.8 × 20cm) that had been equilibrated with phosphate buffer solution(pH, 7.00). The concentration of untrapped EDTA was determined spectrofluorometrically by using Ca^{2+} complex of calcein blue as indicator.[11]

Photoreaction. Methyl viologen(MV^{2+}) solution and zinc acetate solution were added to the prepared suspension of liposomes. This mixed solution was transferred (by using a syringe) to a quartz UV cuvette capped with rubber septum and deoxygenated with helium gas before irradiation. The sample cuvette was irradiated with a collimated light from a 300 W Xenon arc lamp(Hanovia). Light with wavelengths shorter than 280nm was removed by using Pyrex filter. The fluence rate of incident light was 4.1×10^{-9} einstein/s.cm² in average as determined by chemical actinometry using ferrioxalate solution.[18] The concentration of methyl viologen radical(MV^{+}) generated by the photoreaction was determined by measuring absorbance at 602nm (ε_{602} = 12,400 $M^{-1}cm^{-1}$).[11]

Spectroscopic measurement. Absorption and fluorescence spectra were recorded on a Beckman 5240 spectrophotometer and Jovin Yvon 3 spectrofluorometer, respectively.

Results and Discussion

The system studied in the present work is described in Scheme II, including organic photosensitizer(PHS) (2.0×10^{-4} M) incorporated into liposomes, EDTA(1.5×10^{-3} M) as sacrificial electron donor and methyl viologen (MV^{2+}) (2.0×10^{-4} M) as electron acceptor.



Scheme II.

Zinc ion was added in the exterior aqueous phase of liposomes to remove trace of EDTA that was not removed by gel filtration or that may have escaped from the interior.[11]

Illumination of this system resulted in formation of MV^{+} radical cation, indicating that electron transfer across the lipid bilayer of liposomes. Figure 1 shows the quantum yield of MV^{+} production photosensitized by 9-anthracene carboxylate(AC) incorporated into different liposome systems as well as dissolved in homogeneous phosphate buffer solution(pH, 7.00). In the homogeneous solution, the quantum yield of MV^{+} production was reached to 0.96 rapidly in 3min of irradiation,

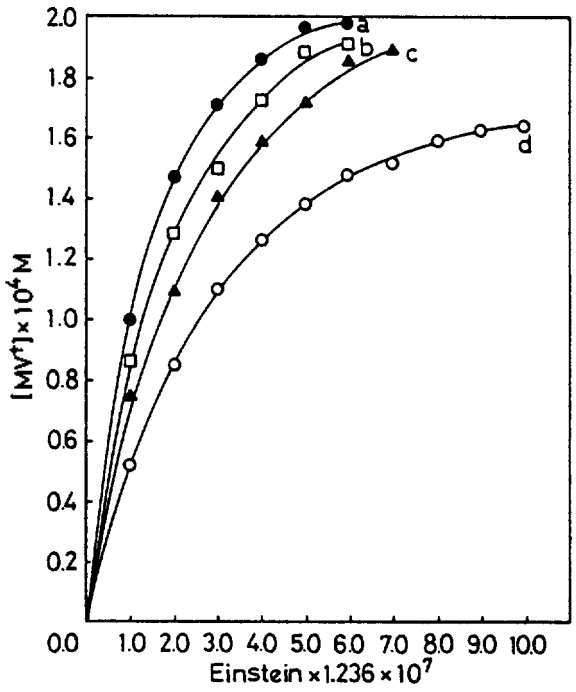


Figure 1. Quantum yield for methyl viologen radical production photosensitized by anthracene carboxylate(AC). (a).Homogeneous system; [AC]= 2.0×10^{-4} M, [MV^{2+}] = 2.0×10^{-4} M, [EDTA] = 2.0×10^{-3}M. (b). Liposome system(PEA). (c).Liposome system (DMPC). (d).Liposome system(Egg-Lecithin). [AC] = 2.0×10^{-4}M, [MV^{2+}] = 2.0×10^{-4} M, [EDTA] = 1.5×10^{-3} M, [Zn^{2+}] = 2.0×10^{-3} M in all liposome systems.

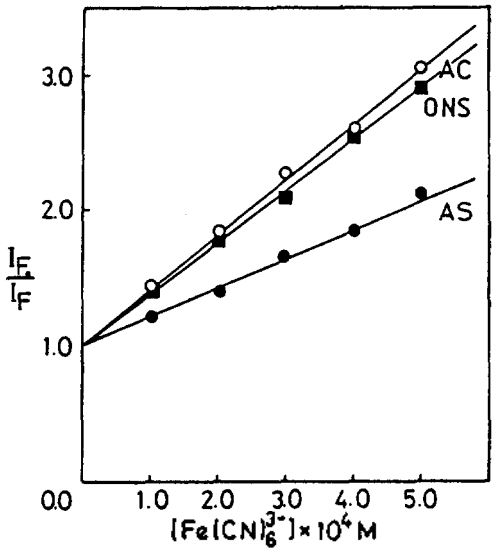


Figure 2. Stern-Volmer plot for fluorescence quenching of photosensitizer in the DMPC liposome vesicle walls by $Fe(CN)_6^{3-}$ inside and outside vesicles.

Table 1. Fluorescence Emission Max. of AS, ONS, AC in Various Solvents and DMPC Liposomes

Medium	A S[a] λmax.(nm)	Medium	A C[b] λmax.(nm)	O N S[c] λmax.(nm)
MeOH	464	Water	432	430
EtOH	460	MeOH	415	412
Benzene	445	EtOH	408	409
Hexane	440	ButOH	405	407
DMPC liposome	444	DMPC liposome	424	420

[a,b] Excitation wavelength - 360 nm.

[c] Excitation wavelength - 340 nm.

which is in good agreement with the value reported by Johansen *et al.*[19] On the other hand, the rate of electron transfer across liposomes was little slower, even though the quantum yield was relatively high(ϕ=0.42 -0.90) compared to when other conventional photosensitizers are employed. This might be because AC photoionizes very efficiently but is incorporated in both site of the hydrophilic layer of liposomes where the electron acceptor or the electron is still available with diffusion driven permeability of AC.[12] Actually, the Stern-Volmer plot of fluorescence quenching of AC in DMPC liposomes by $Fe(CN)_6^{3-}$ (Figure 2) suggests the incorporation of AC in liposome phase by showing little efficient quenching than that of AC in homogeneous aqueous solution. Also, the fluorescence emission maximum of AC in the suspension of liposomes was observed at 425nm(Table 1) which is in between the emission maxima measured in water and methanol, indicating that AC is possibly located in polar site of liposomes.

However, another anthracene derivative such as anthroyl stearic acid(AS) seems to be bound deeply in nonpolar phase of the lipid bilayer of liposome, since its fluorescence emission maximum in DMPC liposomes(444nm) was observed to be close to that measured in benzene solution(Table 1) and its fluorescence quenching constant by $Fe(CN)_6^{3-}$ was determined to be much lower than that of AC in the same DMPC liposomes. Thus, one would expect that AS has lower permeability to contact electron acceptor than AC and no acceptor is directly available in the nonpolar phase of liposomes. Therefore, the excited AS has to wait to find MV^{2+} and EDTA to hand on electron so that the electron transfer reaction is slow. As expected, AS-liposome system slowed the forward photoelectron transfer($AS^* + MV^{2+} \rightarrow AS + MV^{+}$) with much lower quantum yield of MV^{+} production ($\phi<1.0\times10^{-2}$), compared to the system of AC-liposomes. Fortunately this slow reaction could be improved to be faster with significantly high quantum yeild of MV^{+} production (ϕ= 0.23-0.36) by incorporating β-carotene as electron carrier or/and hexyl-viologen into the bilayer with AS (see Figure 3). In this case, the excited AS may hand over an electron to the β-carotene or hexylviologen, which exchange with MV^{2+} in the aqueous phase.

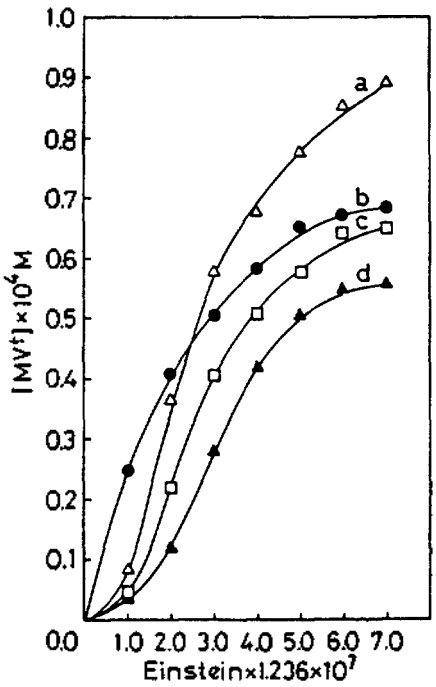


Figure 3. Quantum yield for methyl viologen radical production photosensitized by anthroyl stearic acid (AS) in liposome system (a).Liposome system (Egg-Lecithin) ; (AS,MV^{2+},HV^{2+},β-carotene, EDTA,Zn^{2+}). (b).Liposome system(Egg-Lecithin) ; (AS,MV^{2+},HV^{2+} EDTA,Zn^{2+}). (d).Liposome sustem (Egg - Lecithin) ; (AS,MV^{2+},β-carotein,EDTA,Zn^{2+}). [AS] = 2.0×10^{-4} M, [MV^{2+}] = 2.0×10^{-4} M, [HV^{2+}] = 2.0×10^{-4} M, β-carotene = 1.0 μg [EDTA] = 1.5×10^{-3} M, [Zn^{2+}] = 2.0×10^{-3} M, in all liposome systems.

Octadecyl naphthylamine sulfonic acid(ONS), a naphthalene derivative was also examined for photosensitizing production of MV^{+} in the system of DMPC liposomes. The forward photoelectron transfer was found to be slow with low quantum yield of MV^{+} production (Figure 4), even though ONS is bound to the outer layer of the liposome as AC is.[15] This might be due to low quantum yield of photoelectron formation by naphthalene of ONS, as demonstrated by low quantum yield of MV^{+} production photosensitized by another naphthalene derivative, 1,6-naphthyl-amine sulfonic acid(NAS) in homogeneous solution (Figure 4).

It is noteworthy from Figures 2 and 3 that the efficiency of forward photoelectron transfer across liposome seems to depend on lipid components of liposomes. Figure 2 shows that phosphatidylethanolamine(PEA) liposomes exhibited the greatest photoeffect in agreement with the result obtained

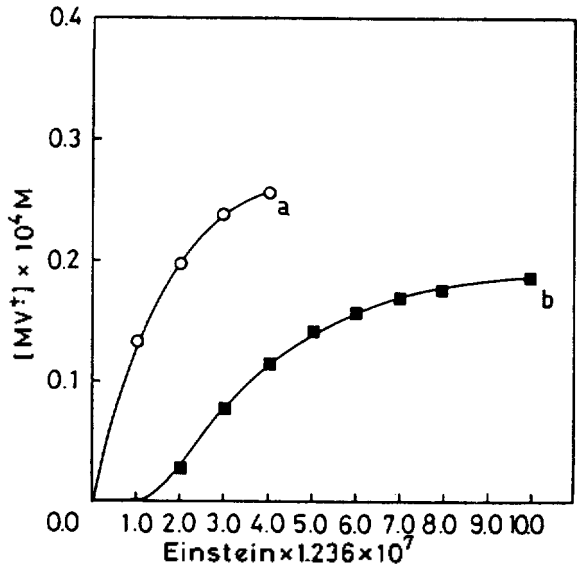


Figure 4. quantum yield for methyl viologen radical production photosensitized by naphthalene derivatives. (a).Homogeneous system ; [NAS] = 2.0 X 10^{-4} M, [MV^{2+}] = 2.0 X 10^{-4} M, [EDTA] = 2.0 X 10^{-2} M. (b).Liposome system(DMPC) ; [ONS] = 2.0 X 10^{-4} M, [MV^{2+}] = 2.0 X 10^{-4} M, [EDTA] = 1.5 X 10^{-3} M, [Zn^{2+}] = 2.0 X 10^{-3} M, β-carotene = 1.0μg.

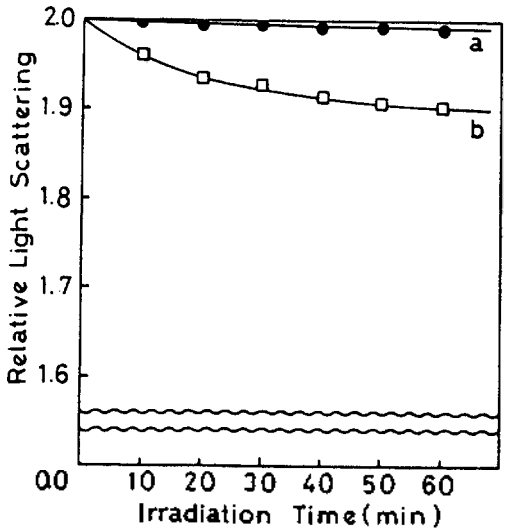


Figure 5. Photolysis of various liposome in the phosphate buffer(pH 7.0) after N_2 bubbling. (a). DMPC liposome. (b).PEA liposome.

from chlorophyll-containing black lipid membrane(BLM) system.[20] However in this experiment, no obvious correlation was established between the degree of unsaturation of phospholipid and the photoresponse, since the photoresponse was surprisingly better in saturated DMPC liposomes than in unsaturated lecithin liposomes under the same condition. Thus, there may be another particular structural factors associated with such lipids as PEA that are favorable for electron transfer through the membrane.

In spite of the high photoresponse of PEA liposomes, the PEA liposomes were observed to be less stable than DMPC liposomes as demonstrated by the result that relative light scattering of PEA liposomes measured at 750nm is significant while that of DMPC liposomes is not observed (see Figure

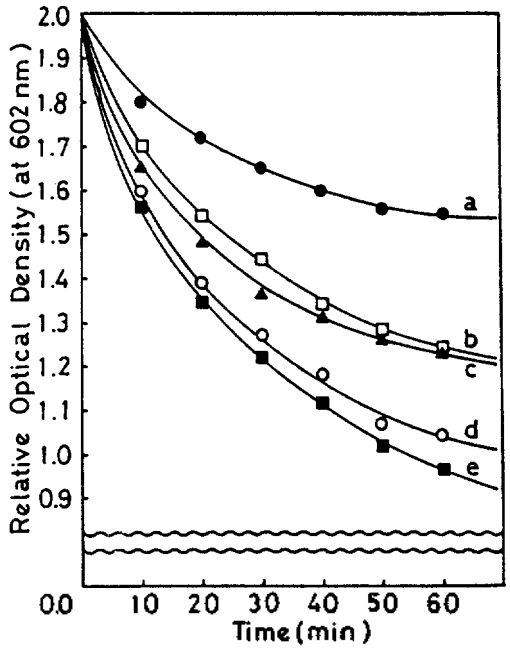


Figure 6. Rate of MV^+ decay ($MV^+ \rightarrow MV^{2+}$) in various systems. (a).Liposome system(DMPC) ; [AS] = 2.0 X 10^{-4} M, [MV^{2+}] = 2.0 X 10^{-4} M,β-carotene = 1.0μg, [EDTA] = 1.5 X 10^{-3} M, [Zn^{2+}] = 2.0 X 10^{-3} M. (b).Liposome system (Egg-Lecithin) ; [AC] = 2.0 X 10^{-4} M, [MV^{2+}] = 2.0 X 10^{-4} M, [EDTA] = 1.5 X 10^{-3} M, [Zn^{2+}] = 2.0 X 10^{-3} M. (c).Liposome system (DMPC) ; [AC] = 2.0 X 10^{-3} M. (d).Liposome system(PEA) ; [AD] = 2.0 X 10^{-4} M, [MV^{2+}] = 2.0 X 10^{-4} M, [EDTA] = 1.5 X 10^{-3} M, [Zn^{2+}] = 2.0 X 10^{-3} M. *(e).Homogeneous system;* [AC] = 2.0 X 10^{-4} M,[MV^{2+}] = 2.0 X 10^{-4} M, [EDTA] = 2.0 X 10^{-2} M.

5). This may be due to repulsion of same anionic charges of PEA which causes energetically unfavorable formation of PEA liposomes. However, the lack of stability could be overcome by formation of liposomes after mixing PEA with DMPC (1/1 ratio). These new liposomes could be kept for more than few weeks at room temperature and showed the same efficiency of photoresponse as that of PEA liposomes.

The back electron transfer from MV^+ to PHS^+ results in decay of MV^+. In order to store the quanta for long time enough to do efficient conversion of solar energy to chemical energy, such a back reaction must be prevented. The back reaction was monitored in terms of decay of MV^+ by measuring decrease in absorption of MV^+ at 602nm vs. time after light-off. Figure 6 shows the rate of decay of MV^+ in various liposome systems containing anthracene derivatives. Initial concentration of MV^+ was normalized to 2.0 x 10^{-7} M for the relative comparison of decay rate. The decay rate was observed to be greatest in homogeneous solution and the decay in AC-incorporated liposomes is relatively fast even if the forward electron transfer is fast as shown in Figure 2. However, the reverse reaction was significantly prevented when AS with β-carotene was incorporated into the hydrophobic site of DMPC bilayer which exhibited relatively high quantum yield of forward electron transfer compared to the system containing any other photosensitizers ever reported.

In conclusion, we have demonstrated new design of photosensitive membrane model containing organic photosensitizer to enhance the efficiency of charge separation and the stability of the photoproducts of electron-transfer reaction.

The AS with β-carotene incorporated into the hydrophobic bilayer of phospholipid (DMPC or DMPC/PEA) resulted in the photosensitized reduction of MV^{2+} with less efficiency than AC-incorporated into the outer hydrophilic layer, but preventing the reverse reaction of electron transfer more effectively than the latter system. Also DMPC or PEA liposomes were observed to be more stable than any other phospholipid-liposomes. Thus, application of the model system, AS-β-carotene-DMPC appears to be feasible for the reduction of water to hydrogen in the presence of a certain catalyst, because the transient photoproducts (e.g., MV^{+}) of electron-transfer reaction are stable engough to efficiently react with H_2O.

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References

1. V. Balzani, L. Moggi, M.F. Manfrin, F. Bolletts, and M. Geleria, *Science* **189**, 852 (1975).
2. M. Calvin, *Acc. Chem. Res.* **11**,369 (1978).
3. K. Kalyanasundaran and M. Gratzel, *Photochem. Photobiol.* **40**, 807 (1984).
4. J.R. Bolton, *Science*, **202**, 705 (1978).
5. M. Calvin, *Photochem. Photobiol.*, **37**, 349 (1983).
6. H.T. Tien, *Photochem. Photobiol.*, **24**, 97 (1976).
7. H.T. Tien, *J. Phys. Chem.*, **72**, 4512 (1968).
8. S.B. Donald, *Photochem. Photobiol.*, **24**, 117 (1976).
9. M. Mange, *Biochim. Biophys. Acta*, **430**, 459 (1976).
10. Y. Toyoshima, M. Marino, H. Motoki,and M. Sukigara, *Nature*, **265**, 187 (1977).
11. W.E. Ford, J.W. Otvos, and M. Calvin, *Nature*, **274**, 507 (1978).
12. J.H. Fender, "Membrane Mimetic Chemistry" John Wiley & Sons, New York, 1982, pp. 492.
13. W.E. Ford, J.W. Otvos, and M. Calvin, *Proc. Natl. Acad. Sci. USA*, **76**, 3590 (1979).
14. J.H. Fendler, *J. Phys. Chem.*, **89**, 2730 (1985).
15. A.S. Waggoner and L. Stryer, *Proc. Natl. Acad. Sci. USA*, **67**, 579 (1970).
16. M. Yoon, Ph.D. Dissertation, Texas Tech University, U.S.A. 1981.
17. C.H. Huang, *Biochem.*, **8**, 344 (1969).
18. C.G. Hatchard and C.A. Parker, *Proc. R. Soc. London Ser. A* **235**,518 (1956).
19. O. Johansen, A.W.H. Mau, and W.H.F. Sasse, *Chem. Phys. Lett.*, **94**,113 (1983).
20. D.S. Berns, *Photochem. Photobiol.*, **24**,117 (1976).

Effects of Molecular Attraction and Orientations in the Vibration-Vibration Energy Exchange

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The effects of molecular attraction and orientations for the energy mismatch variance, vibrational energy level and double-quantum transition, in the vibration-vibration energy exchange, have been considered. The contribution of molecular attraction increases the exchange rate of the purely repulsive interaction, in general, significantly, but which becomes smaller as the temperature is increased. As the energy mismatch is increased, its contribution is also increased, but which is small. However, its contribution for the double-quantum transition is very paramount. At each orientation, the exchange rate constants have been calculated and compared with the results for rotational average, and it is found that the exchange rate is a strong function of the orientation angles of colliding molecules. We have also discussed about the system having the strong interaction such as the hydrogen bond, and it is found that for this system the preferred orientation should be considered in order to calculate the exchange rates.

Introduction

The molecular attraction plays a crucial role in the vibration-vibration(VV) energy transfer.[1] In particular, if there is a strong attraction, its importance is very significant.[2-6] As colliding molecules approach each other, if they are not symmetric thus cannot freely rotate, they must interact with the preferred orientation.[7,8] But, if they are almost symmetric, there is no reason having the preferred orientation, so the colliding molecules can freely rotate during the interaction. Such motions can seriously affect the magnitude of the overall interaction potential, thus influencing the processes of intermolecular VV energy exchange. Therefore, it is important to consider the effects of molecular orientation on vibrational