13 May 1994

ELSEVIER

Chemical Physics Letters 222 (1994) 197–203

CHEMICAL PHYSICS LETTERS

Electron transport reactions between pyrene and methylviologen in a model biological membrane

Masayuki Aikawa [1], Nicholas J. Turro, Katsuya Ishiguro [2]

Department of Chemistry, Columbia University, New York, NY 10027, USA

Received 18 February 1994; in final form 15 March 1994

Abstract

Electron transport reactions in a phospholipid vesicle solution have been investigated by time-resolved laser spectroscopy. Photoelectrons were produce by two-photon absorption of the pyrene chromophore adsorbed in a model membrane (vesicle) and were captured either by bound pyrene, which was covalently attached to the surfactant molecule anchored in the hydrophobic bilayer of the membrane, or by methylviologen which was located in the outer water phase of the vesicle solution. The lifetimes and yields of pyrene fluorescence and of the lowest pyrene triplet state were not affected by the addition of methylviologen.

1. Introduction

Photoinduced electron transfer processes in organized assemblies such as micelles, microemulsions, vesicles, zeolites, etc. have been studied extensively in the last decade because of the interesting and novel features of the reactions which occur in these systems, because of the use of these systems as mimics of biological membranes, and because of the possible application of these systems as models for the capture and storage of solar energy [1]. In micellar solution, studies have been carried out on the photoionization of pyrene as a substrate and the behavior of photoelectrons produced has been investigated [2–5]; photoionization of pyrene adsorbed in the phospholipid bilayer vesicles has been studied [6]. It has also been reported that pyrene cation and hydrated electrons are produced by laser pulse irradiation of dipalmitoyl vesicle solution where the pyrene chromophore was located in the hydrocarbon region of the vesicle [6]. These two species resulted from two-photon absorption of the pyrene.

Although there are many investigations of electron transport via membranes with various donor–acceptor arrangements [7], it is not well established how long a distance an electron is able to move from donors to acceptors which are separated by the hydrophobic layers of the membranes. One of the authors [8–11] has investigated an electron transfer mechanism from hydrated electrons (e^-_{aq}) to methylviologen (MV^{2+}) in the presence of a series of phosphatidylcholine vesicles by means of pulse radiolysis. For the vesicle solutions, hydrated electrons were generated exclusively in the outer bulk aqueous phase and methylviologen was dissolved only in the inner aqueous compartments of the vesicles. In such systems where the electron donor (e^-_{aq}) and the electron acceptor (MV^{2+}) were separated about 36–50 Å by

[1] Present address: Hokkai-Gakuen University, Asahimach Toyohiraku, Sapporo 062, Japan.

[2] Present address: Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan.

SSDI 0009-2614(94)00340-V

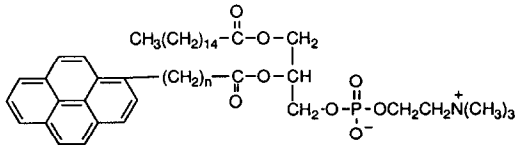
the membranes, and when the bilayer possesses no electron mediator, electron transfer across the bilayer membranes is prevented [8–11]. It has also been demonstrated that an electron transfer only occurs from e_{aq}^- in the outer water phase to MV^{2+} in the inner compartment via a membrane according to the following scheme, when the pyrene chromophore acting as an electron mediator was anchored by an alkyl chain (Py–Ves.) in the hydrophobic moiety of the vesicles [11]:

$$e_{aq}^- + \text{Py–Ves.} \rightarrow n H_2O + \text{Py}^- \text{–Ves.}$$

$$\text{Py}^- \text{–Ves.} + MV^{2+} \rightarrow \text{Py–Ves.} + MV^{+\cdot}$$

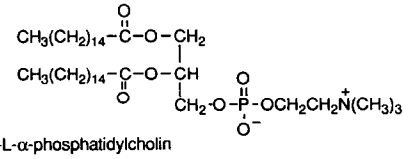
Although observation of the pyrene anion (Py^-) through its absorption spectrum would provide direct proof for the mechanism of electron transport which takes place from electron donor to acceptor via an electron mediator (Py–Ves.), this observation was not reported.

In the present work, an electron transport reaction from pyrene labeled phosphatidylcholine in a phosphatidylcholine vesicle to MV^{2+} dissolved in the outer water phase of the vesicle has been studied. Electrons were produced by two-photon absorption of the pyrene chromophore by laser pulse excitation of the vesicle solution.



n=5; β-(pyren-1-yl)hexanoyl-γ-palmitoyl-L-α-phosphatidylcholine

n=9; β-(pyren-1-yl)decanoyl-γ-palmitoyl-L-α-phosphatidylcholine



Dipalmitoyl-L-α-phosphatidylcholin

An anion band in the absorption spectrum was observed in addition to the pyrene cation band, which provides important information concerning the mechanism of the electron transfer via membranes. It is also found that there are two different pathways for the reduction of methylviologen in a membrane.

2. Experimental

β-(pyren-1-yl)hexanoyl-γ-palmitoyl-L-α-phosphatidylcholine (Py–PPC, $n=5$), β-(pyren-1-yl)-decanoyl-γ-palmitoyl-L-α-phosphatidylcholine (Py–PPC, Å $n=10$), and dipalmitoyl-L-α-phosphatidylcholin (DPPC) (Sigma) were used as received. Typically, 50–100 mg of phospholipid and 1–2 mg of Py–PPC, dissolved uniformly in 5 ml of ethanol were placed in a vial (2.5 cm diameter and 5.2 cm length) in a water bath at 60°C and the solvent was evaporated under argon bubbling. 4 ml of trizma buffer was then added to the vial and the sample was sonicated using the microtip of a sonifier (Heat Systems-Ultrasonics, Inc., model W-385, 20 kHz, 40 W) under a stream of argon gas for 60–90 min at 60°C (above the transition temperature, T_c of DPPC). The tube was then sealed with parafilm and cooled down slowly (within 2–3 h) to a temperature lower than T_c (41°C). The vesicle solution was centrifuged and purified by gel-filtration using Sephadex G-25 (medium, Pharmacia Fine Chemicals).

The concentration of methylviologen (MV^{2+}) was changed by dissolving different stoichiometric solute concentrations into the outer water phase of the phospholipid vesicle solution.

The amount of Py–PPC was changed by varying the ratio of pyrene-labeled and unlabeled phospholipids (Py–PPC/DPPC).

Sample solutions which were kept below T_c through a series of experiments where argon was bubbled prior to the observation of the transient spectra.

The excitation wavelength employed was the third harmonic (355 nm) of a Nd:YAG laser. A conventional optical detection system of transients using a Nd:YAG laser combined with a microcomputer has been described elsewhere [12].

3. Results and discussion

3.1. Monomer and excimer fluorescence

The monomer fluorescence of a Py–PPC in DPPC vesicle solution after laser excitation shows a decay consisting of two components. The fast (k_f) and slow (k_s) decays are consistent with the existence of two different and unaveraged sites for the excited singlet

state of pyrene chromophore in the vesicle. The monomer fluorescence intensities, $I_{mf}(t)$, after pulse excitation were fit to a double exponential decay with constants C_1 and C_2 as follows:

$$I_{mf}(t) = C_1 \exp(-k_f t) + C_2 \exp(-k_s t) .$$

The slow and fast decay rates without MV^{2+} are 1.4×10^7 and 0.8×10^8 s^{-1} for $n=5$ (Py–PPC/DPPC=$\frac{1}{25}$), and 0.76×10^7 and 1.0×10^8 s^{-1} for $n=9$ (Py–PPC/DPPC=$\frac{1}{29}$) of Py–PPC. The monomer fluorescence intensities and decay rates were not affected by the addition of MV^{2+} in the outer bulk aqueous phase in the concentration range from 10^{-4} to 10^{-3} M (Table 1).

Strong excimer fluorescence was observed for the vesicle solutions. However, the excimer fluorescence disappeared after destruction of the vesicles upon addition of ethanol to the solutions. The results show that formation of the excimer is due to the high local concentrations of Py–PPC in the vesicle hydrophobic layers, although such stoichiometric concentrations of pyrene ($[Py]_{stoic}=3.0\times10^{-5}$ mol/ℓ) are too low to form an excimer in a hydrocarbon solvent. The results also show that methylviologen, in such concentrations, does not quench the pyrene fluorescence in these systems.

Time dependence of the excimer fluorescence $I_{ef}(t)$ shows a build-up and a single exponential decay. Observation of excimer fluorescence and build-up of the emission suggest that a pyrene chromophor in the proximity of another pyrene which is anchored by a hydrocarbon chain can change its position slightly during the singlet's lifetime and form an excimer. The excimer fluorescence intensity after laser pulse excitation was analyzed by the following equation:

$$I_{ef}(t) = C_3 [\exp(-k_{ex} t) - \exp(-k_r t)] ,$$

where C_3 is a constant with respect to time, t. The rate constants correspond to growin (k_r) and decay (k_{ex}) components and are summarized in Table 1 as a function of $[MV^{2+}]$. Both excimer fluorescence intensities and the rise and decay rates were also not affected by changing the concentration of MV^{2+} added in the outer water phase of the vesicle solution.

3.2. Characteristics of transient absorption spectra of Py-PPC

3.2.1. Transient absorption for the vesicle solutions with relatively high values of Py–PPC/DPPC

Fig. 1 shows a typical transient absorption spectrum obtained for the vesicle solution with the ratio

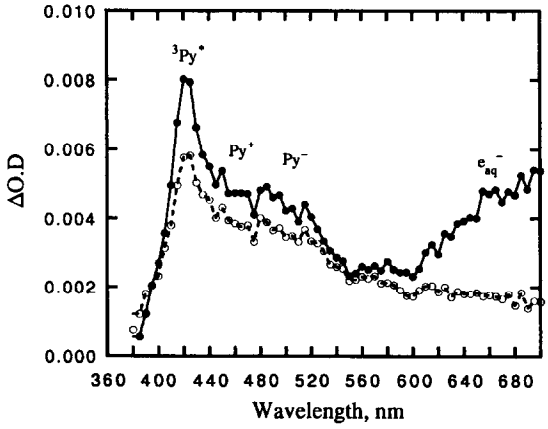


Fig. 1. Transient absorption spectra obtained for Py–PPC ($n=9$) in a DPPC vesicle solution in the absence of methylviologen at two times after laser excitation. Py–PPC:DPPC=1:29. (●, ——) 0.82 μs; (○, - - -) 8.1 μs.

Table 1
Rise and decay rates obtained from monomer and excimer fluorescence for Py–PPC($n=5$)/DPPC=1/25 vesicle solution at 20°C

$[MV^{2+}]$ (M)	Monomer		Excimer	
	k_f ($\times10^7$ s^{-1})	k_s ($\times10^7$ s^{-1})	k_r ($\times10^7$ s^{-1})	k_{ex} ($\times10^7$ s^{-1})
0	8.0	1.4	8.3	1.5
1.0×10^{-4}	7.0	1.4	5.6	1.5
2.5×10^{-4}	9.3	1.6	8.2	1.3
5.0×10^{-4}	8.3	1.5	-	1.3
1.0×10^{-3}	9.4	1.5	6.3	1.3

of Py–PPC($n=9$)/DPPC=$\frac{1}{29}$ after laser pulse excitation at an early time (0.8 μs) and a later time (8.1 μs). Based on literature precedent, the following transients are identified in the spectrum: (1) pyrene triplet ($^3Py^*$) at 420 nm; (2) pyrene cation (Py^+) at 460 nm; (3) pyrene anion (Py^-) at ≈500 nm; (4) the hydrated electron (e_{aq}^-), a broad absorption band in a longer wavelength region (600–720 nm). Thus, the spectrum is rich in potential information concerning neutral, cationic and anionic species of mechanistic importance in the electron transfer process. This spectral information, of course, complements that obtained from the fluorescence measurements.

The absorption band of $^3Py^*$ grows within the fluorescence lifetime and shows an exponential decay. The lifetimes of the pyrene triplet in the vesicle solution are 30 μs for $n=9$ and 5.4 μs for $n=5$ of Py–PPC, respectively.

Electrons are produced by photoionization of the pyrene chromophore in the membrane and a fraction of the electrons escape from the hydrophobic phase to the water phase to form the hydrated electrons. Fig. 2 shows the power dependence of the excitation pulse on the optical density of the hydrated electrons. The plot of the optical density of e_{aq}^- immediately after pulse excitation versus the *square* of excitation intensity yields a straight line. This result demonstrates that the ionization of pyrene in the vesicle is caused by two-photon absorption. The decay of e_{aq}^- does not obey simple first-order kinetics but rather second-order kinetics with a half lifetime of about 400–600 ns.

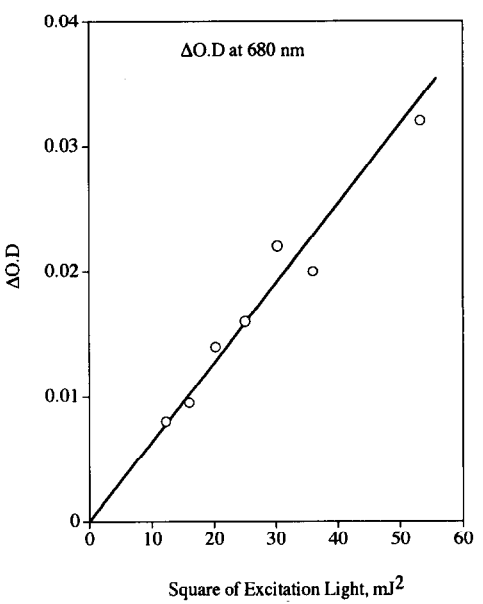


Fig. 2. Dependence of laser intensity on the yield of hydrated electrons. Optical densities of hydrated electrons immediately after the laser pulse excitation was plotted as a function of the square of laser light intensity.

The transient absorption of the pyrene cation is formed within the duration of the excitation pulse and decays in a complicated manner which is probably caused by the overlap of other transient spectra which are superimposed on the cation spectrum or by complex recombination processes involving the cation and the anion, electrons or other species. The half lifetime of the cation is about ≈8 μs.

For the vesicle solution with relatively high values (≈$\frac{1}{25}$) of Py–PPC/DPPC, the absorption band corresponding to the pyrene anion is also observed. A fraction of electrons produced in the hydrophobic layer may be captured by ground state (neutral) pyrene molecules which are located near the ionized pyrene (pyrene cation) which is produced by laser irradiation. This mechanism, generation of pyrene anion, occurs in the hydrophobic layer of the membrane and the processes may be represented by

$$Py^{**}\text{–}PPC \rightarrow Py^+\text{–}PPC + e^-, \quad (1)$$

$$Py\text{–}PPC + e^- \rightarrow Py^-\text{–}PPC, \quad (2)$$

where Py^{**}–PPC represents a higher excited state of the pyrene chromophore.

DPPC vesicles are destroyed by the addition of ethanol. In the transient absorption spectrum obtained for the vesicle solution after the destruction by EtOH, the band of pyrene anion is decreased drastically compared with the vesicle solution before the addition of EtOH. This means that the yield of Py^- is decreased by changing the high local concentration of pyrene chromophore in the vesicle bilayer into the low concentration of Py–PPC in homogeneous solution. This fact is also evidence that Eqs. (1) and (2) represent processes which occur in the vesicle bilayer but not in homogeneous fluid solutions.

3.2.2. Transient absorption for the vesicle solution with low values of Py–PPC/DPPC

Fig. 3 shows the change in the absorption spectra at short and long times with varying ratios of Py–PPC/DPPC. For the transient absorption spectra of relatively low values of the ratio ($\approx \frac{1}{100}$), the absorption band characteristic of pyrene anion at around 500 nm was drastically decreased or was absent (Fig. 3b). Thus, if one pyrene chromophore is separated far from any other pyrene by phosphatidylcholin molecules, an electron produced by photionization of one pyrene is not able to be captured efficiently by another pyrene to produce the pyrene anion. Even in this case, the bands originating from the pyrene triplet, pyrene cation, and hydrated electrons were observable in the spectrum similar to those obtained for the high value of the ratio ($\approx \frac{1}{25}$, Fig. 3a). This result provides further support that the pyrene anion is formed in the hydrophobic bilayer by electron migration of a photoelectron.

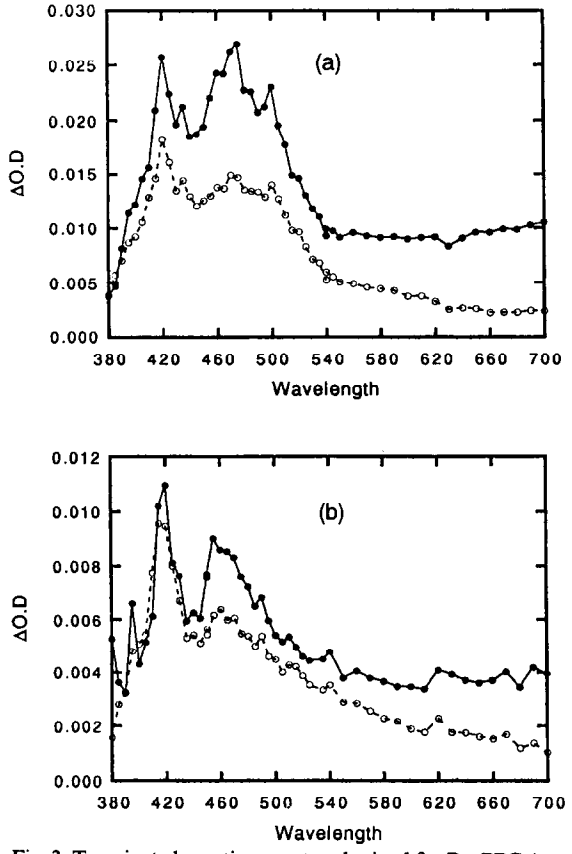


Fig. 3. Transient absorption spectra obtained for Py–PPC ($n=5$) in a DPPC vesicle solution for labeled Py–PPC/DPPC=1/25 (a), and for labeled Py–PPC/DPPC=1/100 (b). (a) (●,——) 0.55 μs; (○,– – –) 4.3 μs. (b) (●,——) 0.51 μs, (○,– – –) 4.2 μs.

3.3. Dynamics of the transients and MV^{2+} dissolved in outer water phase.

Fig. 4 shows the transient absorption spectra obtained for Py–PPC ($n=9$) in DPPC vesicle solution in which methylviologen (1×10^{-3} M) was solubilized in the external bulk aqueous phase. A comparison of Fig. 4 with Fig. 5 clearly shows that the absorption bands originating from both hydrated electrons and pyrene anion are absent and that new bands are present. The new features are a sharp peak at 395 nm and broad bands at around 600 nm, which are confidently assigned to the methylviologen cation radical ($MV^{+\cdot}$). These spectral changes suggest that at least two different pathways exist for the electron transport from Py** to MV^{2+}.

The first pathway is an electron transport process from the pyrene anion which is located inside of the bilayer membrane to MV^{2+} which may be sited near the surface of the vesicles,

$$Py^{-}\text{–}PPC+MV^{2+}\rightarrow Py^{**}\text{–}PPC+MV^{+\cdot}. \qquad (3)$$

For both Py–PPC vesicles having a chain length of

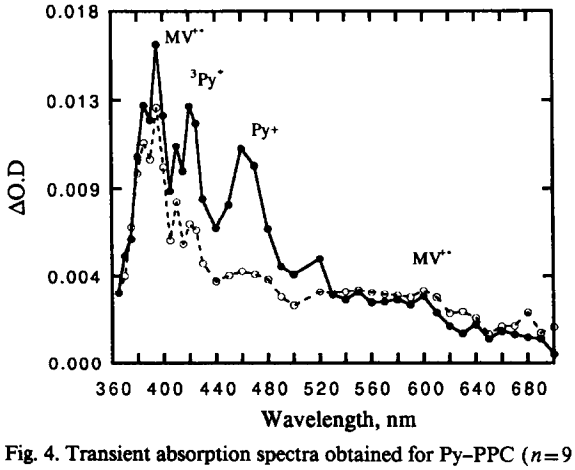


Fig. 4. Transient absorption spectra obtained for Py–PPC ($n=9$) in a DPPC vesicle solution in the presence of methyl-viologen (1×10^{-3} M). Py–PPC:DPPC=1:29. (●,——) 0.78 μs; (○,– – –) 15.0 μs.

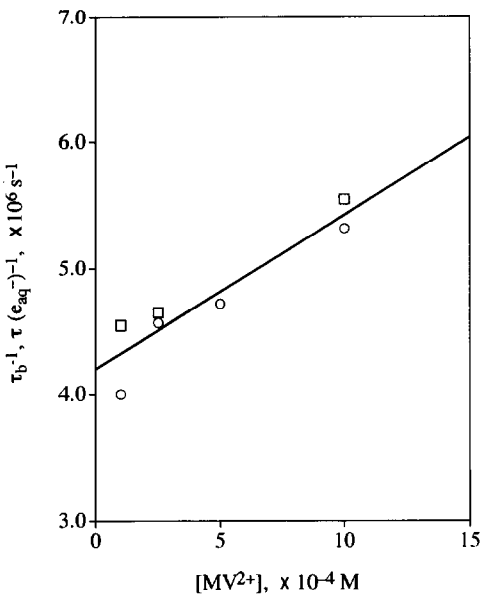


Fig. 5. Dependence of [MV^{2+}] on the decay rate constant of e_{aq}^-, $\tau(e_{aq}^-)^{-1}$ (□), and growin rate constant of $MV^{+\cdot}$, τ_b^{-1} (○).

$n=5$ and $n=9$, the yield of a pyrene anion is diminished at 500 ns after pulse excitation of the vesicle solution in the presence of MV^{2+}. If the location of the pyrene chromophore of a $n=9$ sample is taken into consideration, electron transfer may take place across an aliphatic bilayer between Py^- and MV^{2+} which are separated by ≈ 18 Å.

The second pathway is a reduction of MV^{2+} by hydrated electrons in the outer water phase of the vesicle solution. From pulse radiolysis studies it is reported that the reduced form of methylviologen is also generated by the reaction between MV^{2+} and e_{aq}^- [13,14],

$$e_{aq}^- + MV^{2+} \rightarrow MV^{+\cdot} + nH_2O\,. \qquad (4)$$

The rate constant for reaction (4) was reported as 7×10^{10} M^{-1} s^{-1} or 8.3×10^{10} M^{-1} s^{-1} in water solution [8–11]. The reaction between hydrated electrons and methylviologen across a membrane has also been studied previously by means of pulse radiolysis [8–11]. The apparent pseudo-first-order rate constant for the reaction between e_{aq}^- and MV^{2+} bounded in the vesicle membrane was obtained to be $\approx 4.1\times10^{-9}$ M^{-1} s^{-1} which is an order of magnitude smaller than the rate constant corresponding to Eq. (4). In our systems electron transfer takes place from e_{aq}^- to MV^{2+} in the outer bulk aqueous phase after hydration of photoelectrons has been produced.

The growin curves of $MV^{+\cdot}$ absorption could be observed after pulsed excitation of the vesicle solution with MV^{2+} in the outer water phase. The rise time of $MV^{+\cdot}$ decreased from 250 to 190 ns by increasing the concentration of methylviologen from 1.0×10^{-4} to 1.0×10^{-3} M for the Py–PPC vesicle solution with $n=5$.

The decay curve of the hydrated electron changed from second-order kinetics for the vesicle solution in the absence of MV^{2+} to (pseudo-) first-order kinetics in the presence of MV^{2+}. The lifetime of e_{aq}^- (for the sample with $n=5$) also decreased from 220 ns for [MV^{2+}] $=1.0\times10^{-4}$ M to 180 ns for 1.0×10^{-3} M.

The values for the rise time of $MV^{+\cdot}$ agree very closely with the value of the lifetime of e_{aq}^-, suggesting that the main process for the generation of $MV^{+\cdot}$ formation is electron transfer from e_{aq}^- to MV^{2+}. Pseudo-first-order rate constants obtained both from growin of the absorption of the methylviologen cation radical and the decay of the hydrated electrons are plotted in Fig. 5 as a function of the concentration of MV^{2+}. The second-order rate constant is available from the slope of the straight line shown in Fig. 5, and a value of 1.2×10^9 M^{-1} s^{-1} is obtained, which is at least an order of magnitude smaller than that observed for the process of the encounter between methylviologen and hydrated electrons in the aqueous solution, and is comparable to the value of the rate constant for the reaction between e_{aq}^- produced in bulk aqueous phase and MV^{2+} bound in membranes obtained by pulse radiolysis. These small values of rate constant between e_{aq}^- and MV^{2+} in vesicle membrane systems suggest that e_{aq}^- is not free in the bulk aqueous phase. Further discussion on the reaction rate will be given in another investigation.

Neither the lifetime of the pyrene triplet nor the fluorescence lifetime of pyrene change with variation of the concentration of MV^{2+} (Table 2).

Since the addition of MV^{2+} in bulk aqueous phase does not affect the lifetimes of fluorescence or of the lowest triplet state of pyrene, we conclude that a direct electron transport reaction or any other kind of interaction between the excited states of both singlet and triplet pyrene and MV^{2+} does not occur within the membrane.

Table 2
Lifetimes of pyrene triplet in vesicle solution as a function of $[MV^{2+}]$

$[MV^{2+}]$ (M)	$\tau_t(\mu s)$
1.0×10^{-3}	5.41
5.0×10^{-4}	5.27
2.5×10^{-4}	5.38
1.0×10^{-4}	5.06
0	5.36

From the above observations, the following reaction scheme for photoinduced electron transport reaction from the electron donor in the membrane to electron acceptor dissolved in the outer water phase is proposed:

$$[Py^{**}] \rightarrow [Py^{+}] + e^{-}, \quad \text{in membrane}, \tag{5}$$

$$[Py^{**} \overset{e^-}{\vdots} Py] \rightarrow [Py^{+}] + [Py^{-}], \quad \text{in membrane}, \tag{6}$$

$$e^{-} + nH_2O \rightarrow e^{-}_{aq}, \quad \text{in bulk aqueous phase}, \tag{7}$$

$$e^{-}_{aq} + MV^{2+} \rightarrow nH_2O + MV^{+\cdot}, \quad \text{in bulk aqueous phase}, \tag{8}$$

$$[Py^{-}] + MV^{2+} \rightarrow [Py] + MV^{+\cdot}, \quad \text{via membrane}, \tag{9}$$

$$MV^{+\cdot} \rightarrow \ldots . \tag{10}$$

Eq. (10) refers to any of a number of mechanisms by which $MV^{+\cdot}$ can be removed from the system. The scheme proposes that the electrons produced by two-photon absorption can result in the reduction of methyl viologen in the aqueous phase by two mechanisms: (1) through the hydrated electron (Eq. (8)) and (2) through a pyrene anion (Eq. (9)).

It is noted that after irradiation of the vesicle solution by a laser pulse, a blue color attributable to the mehylviologen cation radical was observed. Although the color of the solution persisted more than a day, the fate of the $MV^{+\cdot}$ was not established.

Acknowledgement

The authors thank the National Science Foundation and the Air Force Office of Scientific Research for their generous support of this research. KI also thanks the Japan–US Cooperative Program in Photoconversion and Photosynthesis for support.

References

[1] K. Kalyanasundaram, Photochemistry in microheterogeneous systems (Academic Press, New York, 1987).
[2] S.C. Wallace, M. Grätzel and J.K. Thomas, Chem. Phys. Letters 23 (1973) 359.
[3] M. Grätzel and J.K. Thomas, J. Phys. Chem. 78 (1974) 2248.
[4] J.K. Thomas and P. Piciulo, J. Am. Chem. Soc. 100 (1978) 3239.
[5] S. Hashimoto and J.K. Thomas, J. Phys. Chem. 89 (1985) 2771.
[6] D.J.W. Barber, D.A.N. Morris and J.K. Thomas, Chem. Phys. Letters 37 (1976) 481.
[7] S.V. Lyner, V.N. Parmon and K.I. Zamaraev, Topics in current chemistry, Vol. 159. Photoinduced electron transfer III, ed. J. Mattay (Springer, Berlin, 1991).
[8] M. Aikawa, N. Miura, T. Sumiyoshi and M. Katayama, Proceedings of 7th ICRR (Martinus Nijhoff, Dordrecht, 1983) p. A4-01.
[9] M. Aikawa and N. Minura, J. Hokkai-Gakuen Univ. No. 45 (1983) pp. 17–43.
[10] M. Aikawa and Y. Harada, J. Hokkai-Gakuen Univ. No. 52 (1985) pp. 21–33.
[11] M. Aikawa, J. Hokkai-Gakuen Univ. No. 60 (1988) pp. 31–50.
[12] P.F. McGarry, C.E. Doubleday Jr., C.-H. Wu, H.A. Staab and N.J. Turro, Photochem. Photobiol. A 77 (1994) 109.
[13] L.K. Patterson, R.D. Small Jr. and J.D. Saino, Radiat. Res. 72 (1977) 218.
[14] J.A. Farrington, M. Ebert, E.J. Land and K. Fletcher, Biochem. Biophys. Acta 314 (1973) 372.