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Chemistry

Photosensitized electron transport across lipid vesicle walls: Enhancement of quantum yield by ionophores and transmembrane potentials

(solar energy/membrane/ruthenium complex/ionophore/transmembrane potential)

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ABSTRACT **The photosensitized reduction of heptylviologen in the bulk aqueous phase of phosphatidylcholine vesicles containing EDTA inside and a membrane-bound tris(2,2′-bipyridine)ruthenium(2+) derivative is enhanced by a factor of 6.5 by the addition of valinomycin in the presence of K^+. A 3-fold stimulation by gramicidin and carbonyl cyanide *m*-chlorophenylhydrazone is observed. The results suggest that, under these conditions, the rate of photoinduced electron transfer across vesicle walls in the absence of ion carriers is limited by cotransport of cations. The rate of electron transfer across vesicle walls could be influenced further by generating transmembrane potentials with K^+ gradients in the presence of valinomycin. When vesicles are made with transmembrane potentials, interior more negative, the quantum yield of heptylviologen reduction is doubled, and, conversely, when vesicles are made with transmembrane potentials, interior more positive, the quantum yield is decreased and approaches the value found in the absence of valinomycin.**

In recent years, light-induced electron-transfer processes have been extensively investigated with the aims of understanding the mechanism of natural photosynthesis and of designing artificial systems that will decompose water by sunlight to produce chemical energy in the form of H_2 (1–3). A basic concept in the design of such systems is the use of dyes to photosensitize electron-transfer reactions that produce chemical species capable of oxidizing and reducing water. A major problem accompanying the dissociation of water by sunlight involves the back reactions of the intermediary redox species, whereby the potential energy of the photochemical process is degraded. One way to control the forward and backward reactions is to separate the photooxidized and photoreduced species by a phospholipid vesicle wall (4–9).

As a model for studying photosensitized electron transfer across vesicle walls, we have used the system described earlier (9, 10). An amphiphilic Ru^{2+} complex incorporated in the membrane mediates vectorial electron transfer from EDTA trapped in the inner compartment of the vesicle suspension to heptylviologen added to the bulk aqueous phase. Recent evidence suggests that electron transfer through the vesicle wall can be accomplished by an electron-exchange mechanism between Ru^{2+} and Ru^{3+} at opposing sides of the membrane (10). Although this mechanism allows electron transport across the vesicle wall, the quantum yield is rather low (3.8×10^{-4}). Transmembrane electron transfer was found to be the rate-limiting step in the reduction of heptylviologen.

In solar energy devices that contain membranes, the quantum yield should be increased for practical reasons. In the literature, several ways are described to facilitate photoinduced transmembrane electron transport. (*i*) In chlorophyll-containing liposomes, the photoreduction of $Fe(CN)_6^{3-}$ is reported to be enhanced by the addition of proton carriers (11). (*ii*) In Zn–porphyrin-containing vesicles, the photoreduction of 9, 10-anthraquinone 2,6-disulfonate is stimulated by the addition of electron mediators such as 1,3-dibutylalloxazine and 1,3-didodecylalloxazine (12). (*iii*) In chlorophyll-containing bilayer lipid membranes (13) or in cyanine dye-containing monolayer assemblies (14), vectorial electron transport across the lipid barrier is enhanced by applying transmembrane electric fields by means of electrodes. (*iv*) In monolayer assemblies, a chainlike π-electron system facilitates transmembrane electron transport (14). In this paper we report the effects of several ionophores and K^+-diffusion potentials on the quantum yield of heptylviologen reduction in the model system described above.

MATERIALS AND METHODS

Materials. Phosphatidylcholine from hen egg yolks was purified by the method of Singleton *et al.* (15). As sensitizer, the Ru^{2+} complex [*N*,*N*′-di(1-hexadecyl)-2,2′-bipyridine-4,4-dicarboxamide]-bis(2,2′-bipyridine)ruthenium(2+) was used (10). 1,1′-Diheptyl-4,4′-bipyridinium dibromide (heptylviologen, C_7VBr_2) was purchased from Aldrich, and EDTA was from Mallinckrodt. The ionophores valinomycin and gramicidin were obtained from Sigma and Calbiochem, respectively. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) was a generous gift of W. Hubbell.

Vesicle Preparation. Vesicle dispersions containing phosphatidylcholine and the Ru^{2+} complex at a molar ratio of 200:10 were prepared by the injection method (16) according to Ford *et al.* (10). Vesicle suspensions were freshly prepared before gel filtration and illumination. The vesicle concentration was estimated to be ≈ 0.13 μM, assuming a mean vesicle diameter of 700 Å (17). For the generation of transmembrane potentials, vesicles were prepared in 0.3 M EDTA/50 mM sodium glycine (pH 8.5) containing a high concentration of either K^+ or Na^+.

Generation of Transmembrane Potentials. The procedure used to obtain vesicles having a potential difference across their membrane was analogous to that described by Cafiso *et al.* (18). Vesicles having transmembrane K^+ gradients with ratios K^+_{in}/K^+_{out} of 1:1, 3:1, 10:1, 45:1, and 90:1, or vice versa, and EDTA trapped inside were obtained by passing vesicles prepared by the injection method through a Sephadex G-25 column. The column was equilibrated with a buffer containing the desired concentration of $(K_2SO_4)_{out}$ and sufficient Na_2SO_4 to make the ionic strength and the osmotic pressure of the continuous

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Abbreviations: C_7V^{2+}, 1,1′-diheptyl-4,4′-bipyridinium(2+); Ru^{2+} complex, [*N*,*N*-di(hexadecyl)-2,2′-bipyridine-4,4′-dicarboxamide]-bis(2,2′-bipyridine)ruthenium; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; ϕ_m, initial time slope quantum yield.

aqueous phase equal to that of the internal vesicle solution. $MgSO_4$ (2 mM) was added to the outside medium to ensure that any EDTA leaking from inside to outside the vesicle was not the source of electrons for the C_7V^{2+} reduction. For example, to obtain K^+_{in}/K^+_{out} = 10:1, vesicles containing 0.9 M K^+/0.3 M EDTA/50 mM sodium glycine buffer were passed through the column, which was equilibrated with 90 mM K^+/0.81 M Na^+/2 mM Mg^{2+}/0.452 M SO_4^{2-}/50 mM sodium glycine buffer. After the vesicles had passed the column, transmembrane potentials were developed by the addition of valinomycin. The time between the addition of valinomycin and illumination was at least 45 min (18). In this way, vesicles for which $K^+_{in}/K^+_{out} > 1$ establish a more negative potential inside and vesicles for which $K^+_{in}/K^+_{out} < 1$ establish a more positive potential inside. Gramicidin and CCCP were added only to vesicles for which $K^+_{in}/K^+_{out} = 1$.

Transmembrane equilibrium potentials were measured as described (18).

Illumination. After addition of C_7V^{2+} to a final concentration of 1 mM, the vesicle suspension was transferred to a gas-tight cuvette and deaerated with scrubbed argon. The cuvette was then irradiated with blue light (440–550 nm) using a 1000-W xenon arc lamp, according to Ford *et al.* (10). The temperature was 26.0 ± 0.20°C, and the incident photon flux was (1.67 ± 0.10) × 10^{-5} einstein min^{-1} cm^{-2}, as determined by Reinecke salt actinometry (19). The formation of viologen radical (C_7V^{+}) was monitored at 602 nm after intervals of illumination. The concentration of C_7V^{+} was calculated by assuming the extinction coefficient of the radical to be the same as that for methylviologen radical, 12,400 M^{-1} cm^{-1} (20). The initial time slope quantum yield (ϕ_m) was calculated by dividing the maximal rate of C_7V^{+} formation by the rate of quanta absorbed.

RESULTS

Effect of Ionophores on Quantum Yield. In vesicle suspensions containing equimolar concentrations of K^+, Na^+, and H^+ on both sides of the membrane, the quantum yield of heptylviologen reduction was enhanced by the addition of CCCP, gramicidin, and valinomycin (Fig. 1.). A common feature of these compounds is that they make the membrane more permeable to certain cations (21). However, the transport mechanisms and the selectivity for cations are different. CCCP carries only H^+ and valinomycin carries mainly K^+ from one side of the membrane to the other. The pore-forming ionophore gramicidin has, in contrast to CCCP and valinomycin, a fixed position in the membrane and facilitates the movement of several univalent cations (e.g., H^+, K^+, and Na^+).

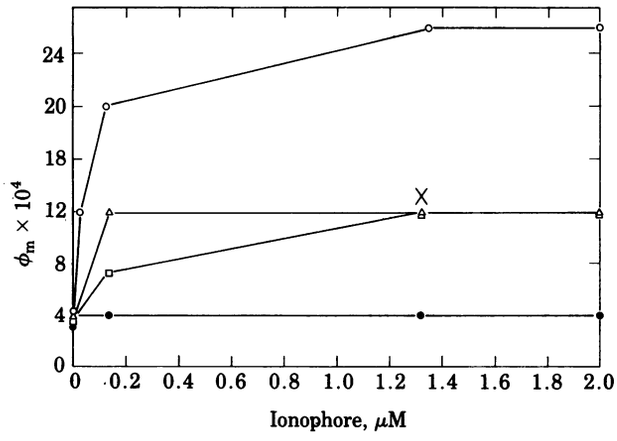

FIG. 1. Effect of ionophores on quantum yield of heptylviologen reduction. Vesicle suspensions were prepared and illuminated with blue light as described in *Materials and Methods*. ○, Valinomycin plus K^+; ●, valinomycin plus Na^+; △, CCCP; □, gramicidin; ×, CCCP plus gramicidin.

When the CCCP concentration in the vesicle suspension was increased, the quantum yield increased from 4 × 10^{-4} to a constant level of 1.2 × 10^{-3}. Maximal stimulation was already observed at a CCCP concentration of 0.13 μM; i.e., approximately one CCCP molecule per vesicle. Recently, comparable effects of CCCP on the rate of $Fe(CN)_6^{3-}$ reduction in chlorophyll-containing liposomes were observed by Kurihara *et al.* (11). Our results, therefore, substantiate their conclusion that transmembrane electron transfer is facilitated by cation carriers when it is coupled to cation transport in the same direction.

Although less pronounced at relatively low concentrations, the enhancing effect of gramicidin on the quantum yield was similar to that of CCCP. In the case of gramicidin, about 10 molecules per vesicle were necessary to obtain maximal stimulation. When, gramicidin and CCCP together were added in excess to the vesicle suspension, the quantum yield hardly increased further. This suggests that the ion-carrying capacity of either gramicidin or CCCP alone is sufficient to allow for charge neutrality during transmembrane electron transfer. Furthermore, the nature of the cation does not seem to be important.

With valinomycin, the quantum yield could be increased even more (6.5-fold). Valinomycin appeared to be very active; one valinomycin per 10 vesicles (13 nM) was sufficient to stimulate transmembrane electron transport to the same extent as found for CCCP and gramicidin at much higher concentrations. This result is in agreement with the observation that one valinomycin per 30 vesicles is sufficient to make all vesicles permeable to K^+ (22). Apparently, valinomycin can hop from one vesicle to another. Addition of excess CCCP or gramicidin did not further affect the quantum yield. The presence of K^+ appeared to be necessary for the action of valinomycin. In vesicle suspensions in which K^+ was replaced by Na^+, valinomycin did not influence the quantum yield, a result consistent with the fact that the permeability of Na^+ is hardly affected by valinomycin (23).

Effect of Transmembrane Potentials on Quantum Yield. The fact that electrons can cross vesicle walls implies that the rate of electron transfer should be influenced by a transmembrane electric field. Fig. 2 shows that the quantum yield of heptylviologen reduction responds strongly to changes in the magnitude and direction of an applied transmembrane electric field. Long-lasting transmembrane potentials were developed by the addition of valinomycin to vesicles having K^+ gradient across their phospholipid wall. K^+-diffusion potentials were estimated by measuring the distribution of a hydrophobic nitroxide cation between aqueous and membrane phases (18). The values determined by this method were in good agreement with those calculated by using the Nernst equation. For example, the experimentally determined potential difference generated in vesicles having a K^+_{in}/K^+_{out} of 90 is 115 mV interior more negative, and the calculated value is 117 mV. At a valinomycin concentration of 10 molecules per vesicle (1.3 μM), the quantum yield was approximately doubled by increasing the transmembrane potential to −115 mV. Similar results were found at a much lower valinomycin concentration (one molecule per 10 vesicles). Conversely, the quantum yield could be decreased by reversing the direction of the electric field. With both the high and low valinomycin concentrations, a limiting quantum yield was reached at 4 × 10^{-4}, which is similar to values obtained in the absence of valinomycin. A quantum yield of 4 × 10^{-4} represents the minimum rate of transmembrane electron transfer, that can-

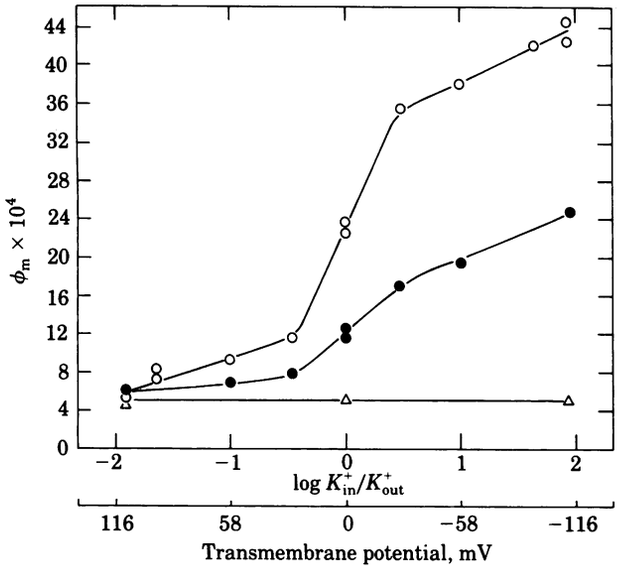

FIG. 2. Effect of transmembrane potentials on quantum yield of heptylviologen reduction in the presence of different concentrations of valinomycin. Experiments were performed as described in *Materials and Methods*. ○, Valinomycin at 1.3 μM; ●, valinomycin at 13 nM; △, no valinomycin. It should be noted that the transmembrane potential scale does not apply for the data obtained without valinomycin.

not be decreased further by potential gradients. This minimum seems to be determined by the intrinsic ability of the membrane to transport cations.

The highest quantum yield obtained in our experiments was 4.4×10^{-3}. The combined effect of valinomycin (see Fig. 1) and a transmembrane electric field (see Fig. 2) on photoinduced electron transfer, therefore, resulted in an 11-fold increase of the quantum yield.

DISCUSSION

Transmembrane Transport of Cations and Electrons. The results obtained with ionophores extend an earlier study (11) on the coupling between ion transport and photoinduced electron transfer across lipid bilayers. The charge imbalance is shown to be counteracted effectively by enhancing the cation permeability of the membrane by ionophores (see Fig. 1). For valinomycin in the presence of K^+, it has been shown that charge imbalances induced by a voltage jump are relaxed with a time constant of 1–8 μsec (24). Similar values are reported for H^+ conductors (25). For the pore-forming ionophore gramicidin, the observed ion fluxes are at the upper limit of what could reasonably be expected from a carrier (21). This implies that gramicidin should be at least as effective as CCCP and valinomycin in relaxing an applied charge imbalance. Yet valinomycin promotes transmembrane electron transfer better than CCCP and gramicidin. It therefore seems likely that valinomycin exhibits another function in addition to acting as a K^+ carrier. One possibility is that valinomycin with K^+ bridges the Ru complexes across the membrane by lateral diffusion and thereby lowers the activation barrier for transmembrane electron transfer. Long-distance electron transfer mediated by ion-containing macrocyclic compounds has already been suggested by Mazur *et al.* (26).

Transmembrane Potentials and Electron Transfer. Our results show that photoinduced electron transfer can be influenced by applying an electric field across the membrane. For vesicles having $K^+_{in}/K^+_{out} > 1$, transmembrane electron transfer is enhanced and, conversely, for vesicles having $K^+_{in}/K^+_{out} < 1$, electron transfer is inhibited. In a previous paper (10), evidence was presented suggesting that the most likely mechanism for transmembrane electron transfer is electron exchange between the Ru^{2+} and Ru^{3+} complexes at opposing sides of the lipid bilayer. The electrons probably cross the potential barrier of the hydrocarbon portion of the membrane by tunneling. Our results are consistent with an electron-exchange mechanism for electron transfer because transmembrane electric fields are known to affect the tunneling rate by changing the barrier height of the membrane (14).

The highest quantum yield for the reduction of heptylviologen that could be obtained in our model system was 4.4×10^{-3}. In a comparable homogeneous system with $Ru(bipy)^{2+}_3$ as sensitizer, the overall quantum yield for the reduction of viologen was 5×10^{-2}. It appeared, however, that the luminescence quantum yield in the vesicle system was about half that of the homogeneous system. Furthermore, in the vesicle system, only 20% of the photoexcited Ru-complex could be quenched by 1 mM viologen although, in the comparable homogeneous system, 50% of the photoexcited $Ru(bipy)^{2+}_3$ was quenched. Thus, the highest quantum yield that can be expected under these circumstances for our vesicle system is about 1×10^{-2}. Fig. 2 (highest $\phi_m = 4.4 \times 10^{-3}$) shows that this limit is approached by enhancing the cation permeability of the membrane and by applying a transmembrane electric field of 115 mV, interior more negative.

Attempts to increase the quantum yield further either by decreasing the back reaction of the initial photoproduct by using excess methylviologen as a sink for the electron or by adding EDTA to the aqueous bulk phase as an electron source were unsuccessful. Apparently a substantial fraction of the absorbed light is thermally degraded and has no opportunity to produce photoreduction. Parts of the overall photochemical reaction are now being investigated by flash photolysis. These studies may lead to greater insight into the photochemical reactions that take place in a vesicle system.

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1. Bolton, J. R. (1978) *Science*, **202**, 705.
2. Harriman, A. & Barber, J. (1979) in *Topics in Photosynthesis–Photosynthesis in Relation to Model Systems*, ed. Barber, J. (Elsevier/North Holland, Amsterdam), Vol. 3, pp. 243–280.
3. Calvin, M. (1979) *Int. J. Energy Res.* **3**, 73–87.
4. Berns, D. S. (1976) *Photochem. Photobiol.* **24**, 117–139.
5. Tien, H. Ti. (1979) in *Topics in Photosynthesis–Photosynthesis in Relation to Model Systems*, ed. Barber, J. (Elsevier/North Holland, Amsterdam), Vol. 3, pp. 116–173.
6. Kuhn, H. (1979) *J. Photochem.* **10**, 111–132.
7. Kurihara, K., Sukigara, M. & Yoyoshima, Y. (1979) *Biochim. Biophys. Acta* **547**, 117–126.
8. Sudo, Y. & Toda, F. (1979) *Nature (London)* **279**, 808.
9. Ford, W. E., Otvos, J. W. & Calvin, M. (1978) *Nature (London)* **274**, 507–508.
10. Ford, W. E., Otvos, J. W. & Calvin, M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3590–3593.
11. Kurihara, K., Toyoshima, Y. & Sukigara, M. (1979) *Biochem. Biophys. Res. Commun.* **88**, 320–326.
12. Matsuo, T., Itoh, K., Takuma, K., Hashimoto, K. & Nagamura, T. (1980) *Chem. Lett.* **8**, 1009–1012.
13. Tien, H. Ti. (1974) *Bilayer Lipid Membranes–Theory and Practice* (Dekker, New York).
14. Polymeropoulos, E. E., Möbius, D. & Kuhn, H. (1978) *J. Chem. Phys.* **68**, 3918–3931.

15. Singleton, W. S., Gray, M. S., Brown, M. L. & White, J. L. (1965) *J. Am. Oil Chem. Soc.* **42**, 53–56.
16. Batzri, S. & Korn, E. D. (1973) *Biochim. Biophys. Acta* **298**, 1015–1019.
17. Ford, W. E. (1980) Dissertation (Univ. California, Berkeley, CA).
18. Cafiso, D. S. & Hubbell, W. L. (1978) *Biochemistry* **17**, 187–195.
19. Wegner, E. E. & Adamson, A. W. (1966) *J. Am. Chem. Soc.* **88**, 394–404.
20. Steckhan, E. & Kuwana, T. (1974) *Ber. Bunsenges. Phys. Chem.* **78**, 253–259.
21. Haydon, D. A. & Hladky, S. B. (1972) *Q. Rev. Biophys.* **5**, 187–282.
22. Johnson, S. J. & Bangham, A. D. (1969) *Biochim. Biophys. Acta* **193**, 82–91.
23. Nichols, J. W. & Deamer, D. W. (1978) in *Frontiers of Biological Energetics*, eds. Dutton, P. L., Leigh, J. S. & Scarpa, A. (Academic, New York), Vol. 2, pp. 1273–1283.
24. Laprada, R., Ciani, S., Eisenman, G. & Szabo, G. (1975) in *Membranes*, ed. Eisenman, G. (Dekker, New York), Vol. 3, pp. 127–214.
25. Neumcke, B. & Bamberg, E. (1975) in *Membranes* ed. Eisenman, G. (Dekker, New York), Vol. 3, pp. 215–253.
26. Mazur, S., Dixit, V. M. & Gerson, F. (1980) *J. Am. Chem. Soc.* **102**, 5343–5350.