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PHOTOSYNTHESIZED ELECTRON TRANSPORT ACROSS LIPID  
VESICLE WALLS: ENHANCEMENT OF QUANTUM YIELD BY  
IONOPHORES AND TRANSMEMBRANE POTENTIALS

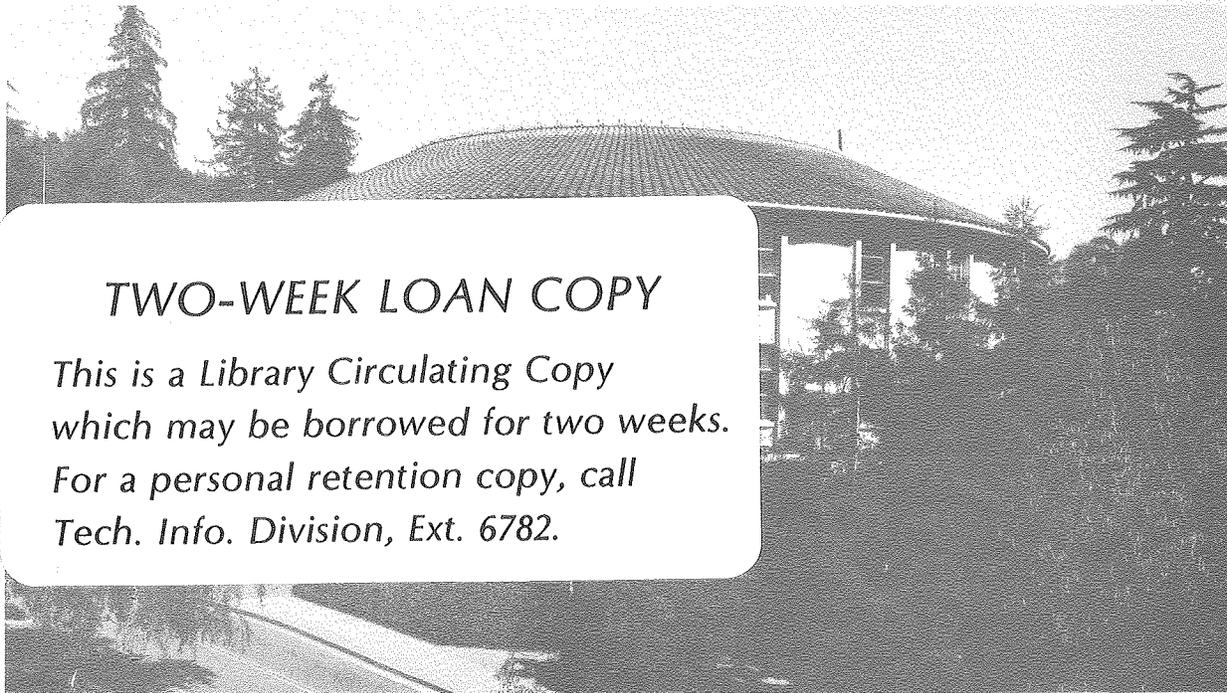
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and Melvin Calvin

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PHOTOSYNTHESIZED 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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ABBREVIATIONS:  $C_7V^{2+}$ , 1,1'-diheptyl-4,4'-bipyridinium(2+); PtdCho, egg yolk  
phosphatidylcholine;  $Ru^{2+}$ -complex[N,N-di(hexadecyl)-2,2'-bipyridine-4,4'-  
dicarboxamide]-bis(2,2'-bipyridine) ruthenium; CCCP, carbonyl cyanide  
m-chlorophenylhydrazone;  $\phi_m$ , initial time slope quantum yield.

## SUMMARY

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 indicate that under these conditions the rate of photo-induced electron transfer across vesicle walls in the absence of ion carriers is limited by co-transport 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 increased 2-fold, and conversely, when vesicles are made with transmembrane potentials, interior more positive, the quantum yield is decreased and approaches the value obtained in the absence of valinomycin.

## INTRODUCTION

In recent years, light-induced electron transfer processes have been extensively investigated, with the aim of understanding the mechanism of natural photosynthesis, and of designing artificial systems that decompose water by sunlight to produce chemical energy in the form of H<sub>2</sub> (1-3). A basic concept in the design of such devices is the use of dyes to photosynthesize 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<sup>2+</sup> and Ru<sup>3+</sup> at opposing sides of the membrane (10). Although this mechanism allows electron transport across the vesicle wall, the quantum yield is rather low ( $3.8 \times 10^{-4}$ ). 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 photo-induced transmembrane electron transport:

i) In chlorophyll-containing liposomes, the photoreduction of  $\text{Fe}(\text{CN})_6^{3-}$  was 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 was 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 was enhanced by applying transmembrane electric fields <sup>by means of</sup> / electrodes, and

iv) in monolayer assemblies a chainlike  $\pi$ -electron system facilitates transmembrane electron transport (14).

Here we investigated the effect of several ionophores and  $\text{K}^+$ -diffusion potentials on the quantum yield of heptylviologen reduction in the model system described above.

#### MATERIALS AND METHODS

Materials PtdCho, from hen egg yolks, was purified by the method of Singleton *et al.* (15). As sensitizer the  $\text{Ru}^{2+}$  complex [N,N'-di(1-hexadecyl)-2,2'-bipyridine-4,4'-dicarboxamide]-bis(2,2'-bipyridine)ruthenium(II) was used (10). 1,1'-Diheptyl-4,4'-bipyridinium dibromide (heptylviologen,  $\text{C}_7\text{VBr}_2$ ) was purchased from Aldrich, EDTA from

Mallinckrodt. The ionophores valinomycin and gramicidin were obtained from Sigma and Calbiochem, respectively. Carbonyl cyanide m-chlorophenylhydrazone (CCCP) was a generous gift of Prof. W. Hubbell.

Vesicle Preparation. The vesicle dispersions containing PtdCho and the  $\text{Ru}^{2+}$ -complex at a molar ratio of 200:10, were prepared by the injection method (16) according to Ford et al. (10). Vesicle suspensions were prepared freshly before gel filtration and illumination. The vesicle concentration was estimated to be approximately  $1.3 \times 10^{-7}$  M assuming a mean diameter of vesicles of  $700\text{\AA}$  (17). For the generation of transmembrane potentials vesicles were prepared in a 0.3 M EDTA solution containing either a high potassium concentration, plus 50 mM sodium glycine, pH 8.5, or a high sodium concentration, plus 50 mM sodium glycine, pH 8.5.

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  $\text{K}^+$ -gradients with ratios  $(\text{K}^+)_{\text{in}}/(\text{K}^+)_{\text{out}}$  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  $(\text{K}_2\text{SO}_4)_{\text{out}}$ , and  $\text{Na}_2\text{SO}_4$  to make the ionic strength and the osmotic pressure of the continuous aqueous phase equal to that of the internal vesicle solution.  $\text{MgSO}_4$  ( $2 \times 10^{-3}$  M) 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  $\text{C}_7\text{V}^{2+}$  reduction. For example, to obtain a ratio of  $(\text{K}^+)_{\text{in}}/(\text{K}^+)_{\text{out}} = 10:1$ , vesicles containing 0.9 M  $\text{K}^+$ , 0.3 M EDTA, plus 50 mM sodium glycine buffer were passed through the

column which was equilibrated with 0.09 M  $K^+$ , 0.81 M  $Na^+$ , 0.002 M  $Mg^{2+}$ , 0.452 M  $SO_4^{2-}$ , plus 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 according to (18).

Illumination. After the addition of  $C_7V^{2+}$ , final concentration  $10^{-3}$  M, the vesicle suspension was transferred to a gas-tight cuvette and deaerated with scrubbed argon before illumination. The cuvette was irradiated with blue light (440-550 nm) using a 1000-W xenon arc lamp, according to Ford et al. (10). The temperature was held at  $26.0 \pm 0.2$  C, and the incident photon flux was  $(1.67 \pm 0.10) \times 10^{-5}$  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 for methylviologen radical,  $12,400 M^{-1}cm^{-1}$  (20).  $\phi_m$  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  $H^+$  at 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  $H^+$ , while 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 such as  $H^+$ ,  $K^+$ , and  $Na^+$ .

When the CCCP concentration in the vesicle suspension was increased, the quantum yield rose from  $4 \times 10^{-4}$  to a constant level of  $1.2 \times 10^{-3}$ . Maximal stimulation was already observed at a CCCP concentration of  $1.3 \times 10^{-7}$  M; that is 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 transport of cations in the same direction.

Although less pronounced at relatively low concentrations, the enhancing effect of gramicidin on the quantum yield was similar to that observed with CCCP. In the case of gramicidin about 10 molecules per vesicle were necessary to obtain maximal stimulation. When besides gramicidin CCCP was added in excess to the vesicle suspension, the quantum yield hardly increased further. This seems to indicate 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 ( $1.3 \times 10^{-8}$  M) was sufficient to stimulate transmembrane electron transport to the same extent as was 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 affect the quantum yield further. 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 the direction of an applied transmembrane electric field. Long-lasting transmembrane potentials were developed by the addition of valinomycin to vesicles having a gradient of  $K^+$  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 according to the Nernst

equation. For example, the experimentally-determined potential difference generated in vesicles having a  $(K^+)_{in}/(K^+)_{out}$  ratio of 90 is 115 mV interior more negative, while the calculated value is 117 mV. At a valinomycin concentration of 10 molecules per vesicle ( $1.3 \times 10^{-6}$  M) the quantum yield was increased approximately 2-fold by increasing the transmembrane potential to -115 mV. Similar results were found at a much lower valinomycin concentration of one molecule per 10 vesicles. Conversely, the quantum yield could be lowered by reversing the direction of the electric field. With both the high and low valinomycin concentrations/<sup>a</sup> limiting quantum yield was reached at  $4 \times 10^{-4}$ , which is similar to values obtained in the absence of valinomycin. A quantum yield of  $4 \times 10^{-4}$  represents a minimal rate of transmembrane electron transfer which cannot 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  $4.4 \times 10^{-3}$ . The combined effect of valinomycin (Fig. 1) and a transmembrane electric field (Fig. 2) on photo-induced electron transfer, therefore, resulted in a 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 photo-induced electron transfer across lipid bilayers. The charge imbalance is shown to be counteracted effectively by enhancing the cation permeability of the membrane by ionophores (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 to 8  $\mu\text{sec}$  (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 be reasonably expected from a carrier (21). This implies that gramicidin should be at least equally 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 besides acting as a  $K^+$ -carrier. One possibility might be 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 had already been indicated by Mazur *et al.* (26).

Transmembrane Potentials and Electron Transfer. Our results clearly demonstrate that photo-induced 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 is presented suggesting that the most likely mechanism for transmembrane electron transfer is electron exchange between the  $Ru^{2\pm}$  and  $Ru^{3\pm}$  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, since 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 amounted to  $4.4 \times 10^{-4}$ . In a comparable homogeneous system with  $\text{Ru}(\text{bipy})_3^{2+}$  as sensitizer the overall quantum yield for the reduction of viologen was found to be  $5 \times 10^{-2}$ . It appeared, however, that the luminescence quantum yield in the vesicle system was about 2-fold lower than in the homogeneous system. Furthermore, in the vesicle system only 20% of the photo-excited Ru-complex could be quenched by  $10^{-3}$  M viologen, while in the comparable homogeneous system 50% of the photo-excited  $\text{Ru}(\text{bipy})_3^{2+}$  was quenched. Thus, the highest quantum yield that can be expected under these circumstances for our vesicle system is about  $1 \times 10^{-2}$ . Fig. 2 (highest  $\phi_m = 4.4 \times 10^{-3}$ ) demonstrates 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 using excess of 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 means of flash photolysis. These studies might lead to more insight into the photochemical reactions that take place in a vesicle system.

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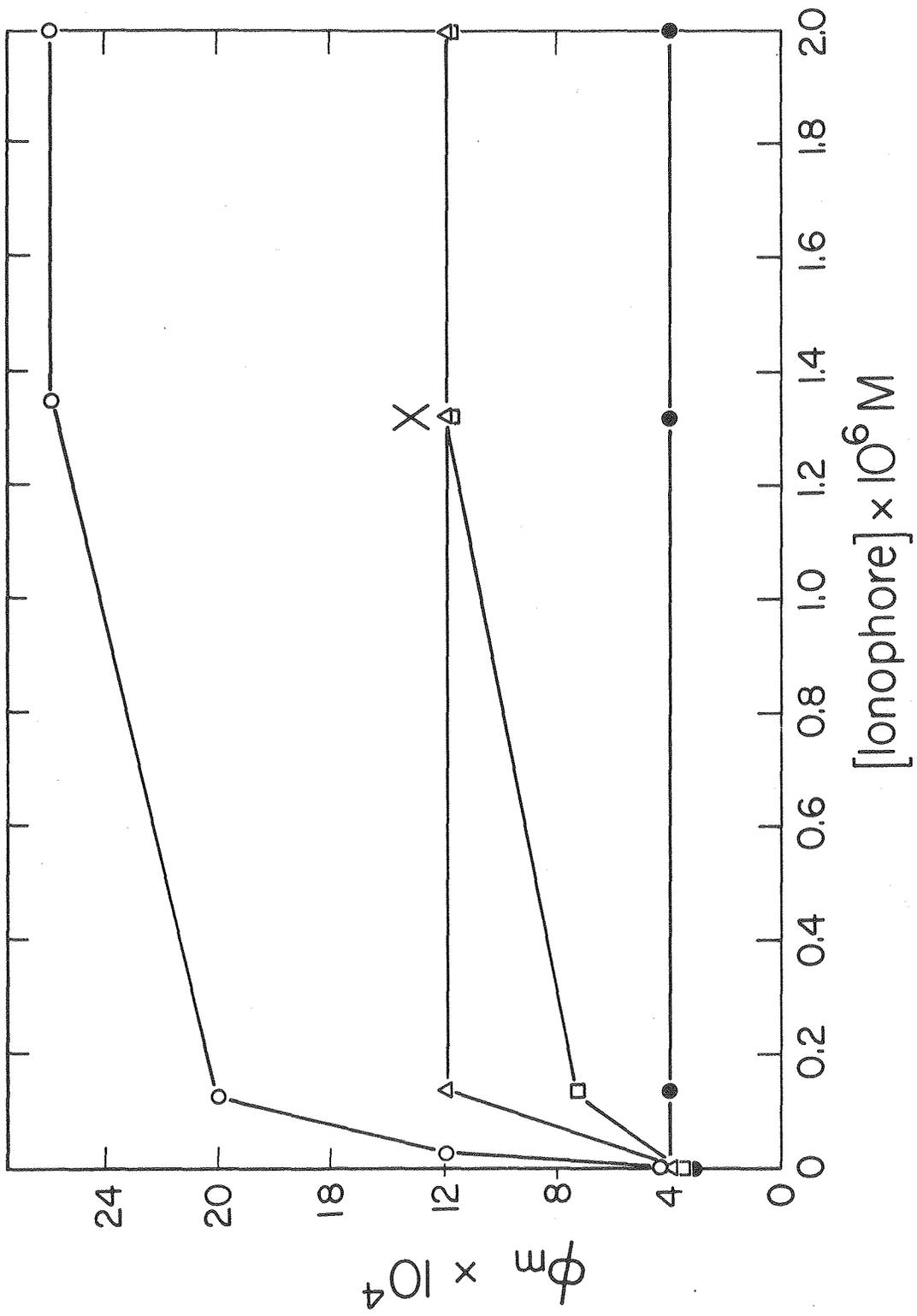
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## LEGENDS

Figure 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.  $\phi_m$ , initial time slope quantum yield; (O-O), valinomycin plus  $K^+$ ; (●-●), valinomycin plus  $Na^+$ ; ( $\Delta$ - $\Delta$ ), CCCP; ( $\square$ - $\square$ ), gramicidin; (x) CCCP plus gramicidin.

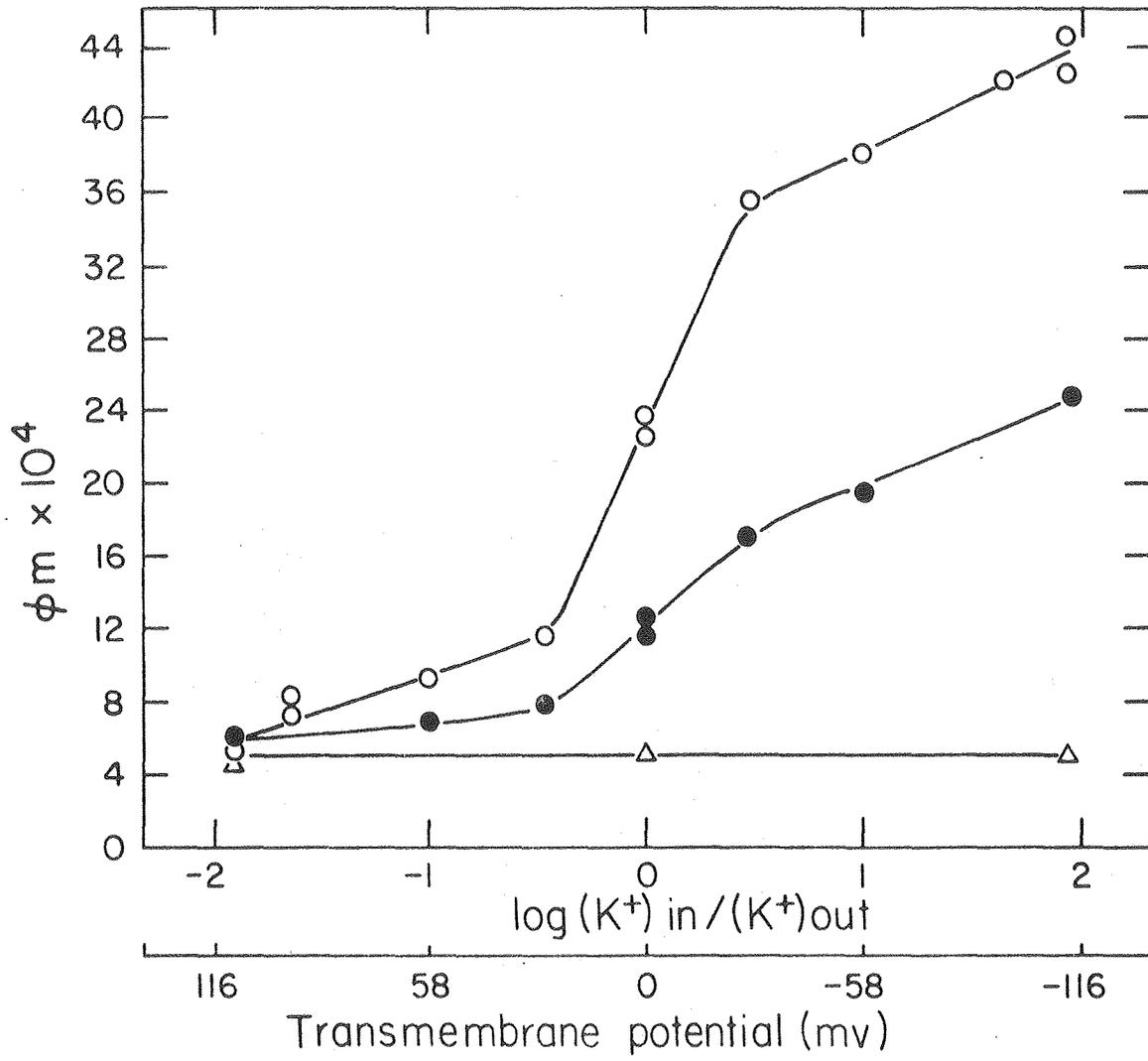
Figure 2: Effect of transmembrane potentials on the quantum yield of heptylviologen reduction in the presence of different concentrations of valinomycin. Experiments were performed as described in Materials and Methods.  $\phi_m$ , initial time slope quantum yield. (O-O), Valinomycin,  $1.3 \times 10^{-6}$  M; (●-●), valinomycin,  $1.3 \times 10^{-7}$  M; ( $\Delta$ - $\Delta$ ), no valinomycin. It should be noted that the transmembrane potential scale does not apply for the data obtained without valinomycin.



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FIG. 1



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FIG. 2