APPARENT INHIBITION OF PHOTOREDOX REACTIONS OF MAGNESIUM OCTAETHYLPORPHYRIN AT THE LIPID BILAYER-WATER INTERFACE BY NEUTRAL QUINONES

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ABSTRACT Neutral quinones rapidly equilibrate across the lipid bilayer, hereby rendering the photoeffects seen in pigmented bilayers sensitive to the redox properties at both interfaces. The lack of photoeffect by quinones themselves and their apparent quenching reactions with aqueous acceptors is thus explained. An aqueous donor is needed on one side to break the symmetry and to allow vectorial electron transfer to be recorded. It is concluded that the neutral quinone accumulates on the polar side of the interface with respect to the hydrophobic pigment. The system may allow the study of kinetics of proton transfer accompanying the redox reactions of the quinones.

INTRODUCTION

A previous paper has determined the redox span of photochemical electron transfer across a lipid bilayer-water interface (Ilani and Mauzerall, 1981). It was quite natural that all electron acceptors used were ions. In particular, the various quinones which acted at a relatively low concentration were sulfonated. The analogous neutral quinones, although not readily water soluble, could nevertheless be added to one side of the membrane in small volumes of an alcoholic solution. This, however, did not only fail to produce a characteristic photo-response, but it also abolished the responses to any of the other aqueous acceptors. The mechanism of this "quenching" effect and the ability of the "hydrophobic" quinones to act as electron acceptor for the excited porphyrin in a membraneous setting are the subjects of this presentation.

MATERIALS AND METHODS

Details of the experimental setup are as given in the previous paper (Ilani and Mauzerall, 1981).

Three neutral quinones were used in this study. 1,4-napthoquinone, 2,5-dichloro-*p*-benzoquinone (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N.Y.), and coenzyme Q_1 (a gift from Dr. Kaback of Roch Institute for Molecular Biology, Nutley, N. J.). Ethanol solutions of the quinones were prepared and aliquots were added to one side of the membrane.

RESULTS

Fig. 1 shows the main features of photoresponses of porphyrin containing membranes when exposed to neutral quinones: (*a*) addition of quinones to one side of the membrane produces

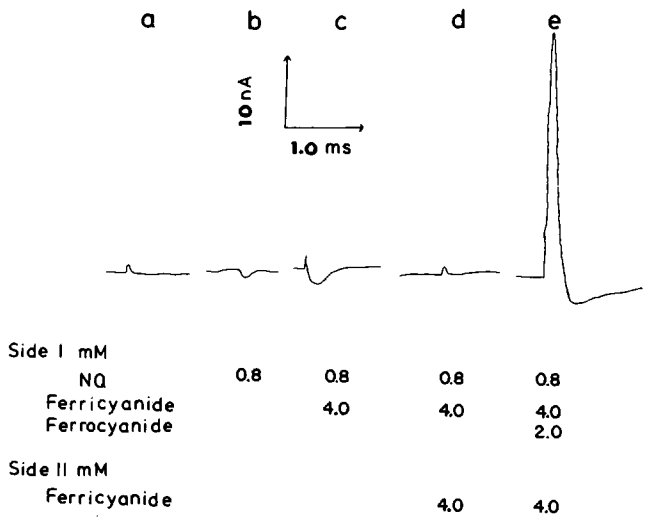

FIGURE 1 Records of photocurrent after pulse irradiation of MgOEP-containing membranes. The solutions on both sides of the membrane contained 100 mM NaCl and 1 mM phosphate buffer at pH 6.5. Naphthoquinone(NQ), ferricyanide, and ferrocyanide were added to either side of the membrane as indicated. Base line before laser shot represents zero current level. Upward deflection signifies electron flow from side I to side II. Various artifacts due to the spark gap of the laser are seen in records *a*, *c*, and *d*. Note that response *e* contains a clear "overshoot," indicative of a back reaction at membrane interface II. This back reaction cannot be accounted for by the small contamination of ferricyanide solution with ferrocyanide. Thus, naphthoquinone functions as a redox carrier across the membrane, (see Fig. 2) reducing the ferricyanide on side II and oxidizing the ferrocyanide on side I. The extent of back reaction seen in *e* corresponds to the presence of about 0.03–0.05 mM ferrocyanide at side II.

only negligible photocurrent, as if these quinones could not act as electron acceptors. (*b*) Subsequent addition of an established active acceptor to either side of the membrane produces only a very small or negligible photocurrent. (*c*) However, addition of an electron donor like ferrocyanide to one side of the membrane produces a full-fledged photoresponse.

Thus, the neutral quinones differed from the other water soluble acceptors in that their action could be demonstrated only if excess electron donor was added to one side of the membrane. Under these conditions the peak photocurrent dependence upon quinone concentration was similar to the other acceptors, i.e., I_{max} and K could be determined from a hyperbolic fit of the data. I_{max} was determined relative to ferricyanide or diquat, as explained in the previous paper (Ilani and Mauzerall, 1981).

From Table I it can be seen that: (*a*) the neutral quinones can elicit I_{max} very similar to other established aqueous acceptors. (*b*) K is roughly the same for the magnesium octaethyl porphyrin (MgOEP), and the chlorophyll *a*-containing membranes. (*c*) K for naphthoquinone is about four times higher than K for naphthoquinone-2-sulfonate.

In interpreting the results of the experiments with neutral quinones, it was considered proper to demonstrate that the neutral quinones can readily cross the thin lipid membrane. If a quinone of a suitable redox potential is chosen, its reduction by ferrocyanide present on one side of the membrane should lead to reduction of ferricyanide on the other side of the membrane and therefore to the appearance of a biphasic current wave indicative of the back

TABLE I

I_{max} AND K FOR NEUTRAL QUINONES IN MgOEP AND CHLOROPHYLL-*a* MEMBRANES

Acceptor	Pigment (M)			
	MgOEP		Chlorophyll *a*	
	I_{max}	K	I_{max}	K
		mM^{-1}		mM^{-1}
1,4-Naphthoquinone	100, 90	6, 7	100	7
Coenzyme Q_1	90	12	100, 90	10, 6
Naphthoquinone-2-Sulfonate	100	1.5	100	2.5

reaction of the porphyrin cation (P^+) with the reduced species (Hong and Mauzerall, 1976). This was demonstrated clearly when 2,5 dichlorobenzoquinone was used as the neutral electron acceptor (Fig. 2). The latter quinone at neutral pH has approximately the same redox potential as the ferro-ferricyanide redox pair. The shuttle of the reduced and oxidized forms of the neutral hydroquinone and quinone across the membrane can readily account for the pronounced expression of back reaction observed in the flashed membrane having ferricyanide and dichlorobenzoquinone on one side and ferrocyanide on the other side (Fig. 2). The ability of quinones to mediate redox reactions across lipid membranes was demonstrated in liposome preparations by Hauska (1977).

DISCUSSION

The results of the experiments described in this paper support the following interpretation. The neutral quinones accumulate at the membrane interface to which they are added as do the sulfonated quinones studied in the earlier paper. This can be inferred from the very high K value. As indicated in the previous paper (Ilani and Mauzerall, 1981), a $K > \sim 1$ mM^{-1}

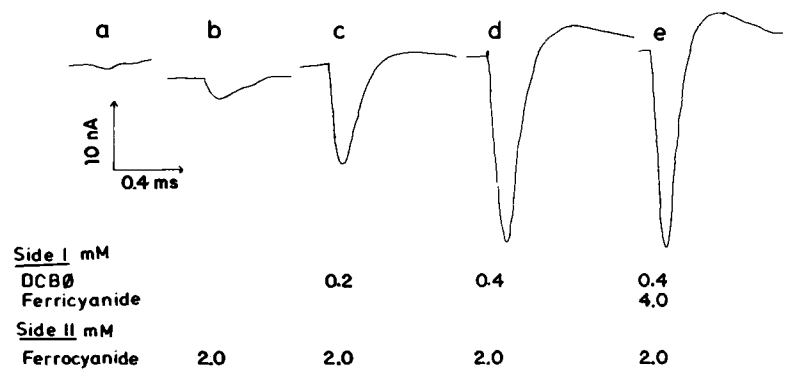

FIGURE 2 Photocurrent after pulse irradiation of chlorophyll *a*-containing membranes. Solutions as in Fig. 1. DCBQ (2-5-dichlorobenzoquinone), ferrocyanide and ferricyanide were added to either side of the membrane as indicated. A back reaction is evident in records *d* and *e* by the presence of a clear upward phase of the current. In record *e* the presence of a significant rapid back reaction is expressed also by the narrowing of the main current wave. Base line before laser shot represents zero current level. Downward deflection signifies electron flow flowing from side II to side I.

implies that the electron acceptor must be concentrated at the membrane interface relative to its density in the bulk solution. Unlike the sulfonated quinones, the neutral analogues can readily cross the membrane as demonstrated clearly for dichlorobenzoquinone. Therefore, the neutral quinones accumulate on the other interface as well. Thus, the addition of neutral quinones to either side of the membrane creates a symmetric distribution of electron acceptors at both interfaces that leads to equal probabilities of vectorial electron transfer in opposite directions. This accounts for the failure of these substances to produce a detectable photoresponse and their ability to abolish the photoresponse of other acceptors.

Adding ferrocyanide to one side of the membrane leads to annihilation of the photocurrent response from that interface because of rapid back reaction of ferrocyanide with the oxidized pigment; thus, the response from the other interface becomes visible.

These results imply that even though there is absorption of neutral quinones at the interface and that these quinones can cross the membrane, there is no tight attachment of semiquinone anions or protonated semiquinones to the membrane. If this were so, it would be expected that back reaction of pigment cation with tightly bound semiquinones would occur. Since I_{max} is very close to that with ferricyanide, it implies that the reduced quinone can readily escape by diffusion from the site of its reduction, and thus avoid back reaction with the porphyrin cation.

Moreover, the fact that I_{max} is close to 100% (i.e., equal to that with noninterface-crossing ions such as ferricyanide) implies that electron transport to quinone present inside the bulk membrane is negligible. Such an intramembraneous electron transfer, if it were to occur, would contribute a transmembrane current of opposite sign (Masters and Mauzerall, 1978) and therefore should have expressed itself by a decrease in I_{max}. These arguments suggest that either the neutral quinone concentration in the bulk membrane is very low relative to its interfacial concentration, or else that in the bulk membrane the energy level of the vacant orbital in the quinones is higher than the energy of the excited electron. The latter argument seems to be untenable for a compound like dichlorobenzoquinone since the latter redox potential at pH 7.0 is around +0.4 V, whereas the cutoff level for electron transfer by chlorophyll-a containing membranes is at the level of −0.6 V vs. Standard Hydrogen Electrode (Ilani and Mauzerall, 1981). Moreover, we conclude that most of the quinone is towards the aqueous side of the membrane relative to the pigment. If this were not so, more P^+ and Q^- (semiquinone anion) would recombine as the Q^- diffused out of the lipid because of the Coulomb field (Ballard and Mauzerall, 1980). Thus, there is a gradient of concentration of neutral quinone in the membrane with maxima at each interface. Oxygen behaves like a neutral quinone; the photoeffects seen in Fig. 2*b* can be explained in this way.

The findings described in this paper are therefore more compatible with the assumption that the concentration of neutral quinones in the membrane proper is indeed small compared with its concentration in the interfacial region. Similar accumulation at membrane-water interfaces has been suggested for hydrophobic ions like tetraphenylboron (Anderson and Fuchs, 1975). This suggests that a relatively small neutral molecule as a quinone whose net dipole moment is close to zero but which has strong local dipoles behaves like a surfactant in the sense that its minimal free energy will occur at the interface between two bulk water-lipid phases rather than in either one of the phases.

The ability to follow the kinetics of these charge transfer processes into the μs time range opens the possibility of measuring the kinetics of the movements of charged molecules across the interface: either the escape of semiquinone anion or its protonation by proton transfer in the reverse direction.

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