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Supplementary Material Available: Complete tables of atomic coordinates and displacement parameters (5 pages); lists of observed and calculated structure factors (25 pages). Ordering information is given on any current masthead page.

Methyl Viologen Mediated Oxidation-Reduction Across Dihexadecylphosphate Vesicles Involves Transmembrane Diffusion

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Numerous reports have appeared describing oxidation-reduction across bilayer membranes.[1,2] Mechanisms proposed for specific systems include the following:[1] (i) electron tunneling across the hydrocarbon barrier between interfacially bound redox partners,[3-6] (ii) molecular diffusion of bound redox components across the barrier,[7-9] and (iii) formation of barrier-penetrating aggregates, or electron-conducting "channels", across the bilayer.[10,11] Nonetheless, the actual reaction mechanisms remain obscure due to the general unavailability of transverse diffusion rates, possible loss of compartmentation of reactants, particularly in photochemical systems,[12,13] and the ambiguities inherent in deducing reaction mechanisms from rate data, which form the primary evidence in most systems studied.[1,2] The reactions of dihexadecylphosphate (DHP) vesicle-bound methyl viologen (MV^{2+}) described in this report are unique in allowing deduction of molecular details of a transmembrane redox event from the product composition and microphase distribution. Specifically, we have found that MV^{2+} bound at the outer vesicle interface mediates reduction of inner-localized MV^{2+} by dithionite ion in bulk solution in a manner that requires comigration of MV^+ with the electron transferred across the membrane barrier.

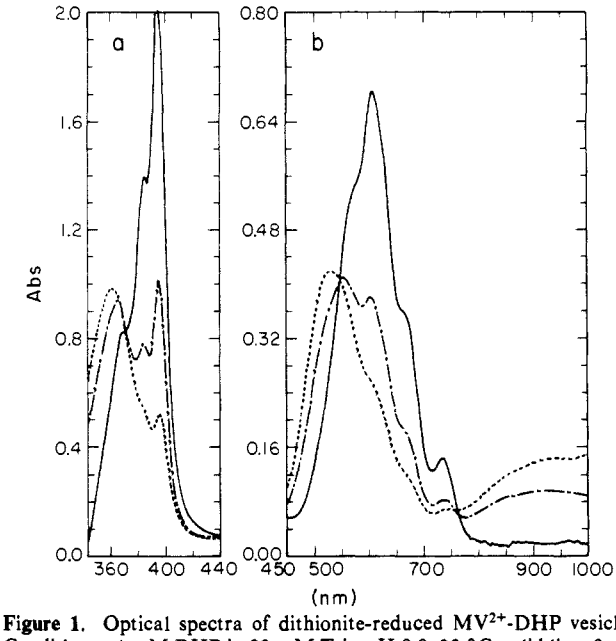

Figure 1. Optical spectra of dithionite-reduced MV^{2+}-DHP vesicles. Conditions: 4 mM DHP in 20 mM Tris, pH 8.0, 23 °C; solid line, 9 μM MV^{2+} outside only; dotted line, 40 μM MV^{2+} on both surfaces; dot-dashed line, 20 μM MV^{2+} inside, 30 μM MV^{2+} outside. All spectra were scaled to a total concentration of 50 μM MV^+ for comparison purposes.

Vesicles containing widely varying ratios of externally and internally bound MV^{2+} were prepared[14] by sonication of DHP in the presence of MV^{2+} followed by removal of external MV^{2+} by chromatography on cation exchange or dextran gels.[13] After spectrophotometrically determining the amount of occluded MV^{2+}, viologen was readded to the external medium to give the desired inside/outside ratio. Passive diffusion of MV^{2+} across the bilayer is very slow;[13] these ratios are maintained until vesicle integrity is lost by aggregation/fusion processes occurring over a period of several days. Dithionite ion does not penetrate the membrane and is incapable of directly reducing MV^{2+} bound at the opposite vesicle interface. Thus, no MV^+ was formed when $S_2O_4^{2-}$ was added to vesicle suspensions containing only internal MV^{2+}. Viologen reduction did occur in vesicles containing MV^{2+} bound at only the outer or at both interfaces.[6] When the amount of external MV^{2+} exceeded the internal MV^{2+}, all of the viologen was $S_2O_4^{2-}$-reducible; when the amount of internal MV^{2+} exceeded the external MV^{2+}, only a fraction of the total MV^{2+} equal to twice the external MV^{2+} was reducible. Upon oxygenation, MV^{2+} distributions were redetermined by using the chromatographic/spectrophotometric methods described above. Because O_2 is freely membrane-permeable, the MV^{2+} distribution should closely approximate the original MV^+ distribution following the reductive reaction. It was found that approximately one MV^{2+} had translocated from outside to inside the vesicle per internal MV^{2+} reduced.[15] Identical results were obtained when Cr^{II}(EDTA) was used in place of $S_2O_4^{2-}$ as the reductant.

The forces driving inward migration of MV^+ apparently arise from membrane polarization; i.e., in the absence of ion movement, transmembrane electron transfer is electrogenic. Electroneutrality can be restored by diffusion of ions in response to the developing potential; in this instance, lipophilic MV^+ comigrates with the electron. Addition of 50 μM tetraphenylphosphonium ion as an alternate lipophilic cation decreased the percentage of outer MV^+ migration by about 30-40%. When $Fe(CN)_6^{3-}$, which is not membrane-permeable, was used in place of O_2 as the oxidant, the

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(15) When $S_2O_4^{2-}$ was in large excess and the initial external/internal MV^{2+} ratio exceeded unity, the amount of translocated MV^+ was greater than the initial internal MV^{2+} concentration. Under these conditions, translocation of the additional MV^+ was $S_2O_4^{2-}$ concentration-dependent, suggesting that it arose by additional transmembrane redox cycling during aerobic oxidation of the system.

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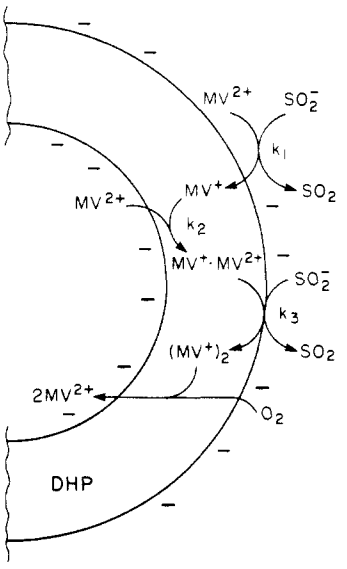

Figure 2. Reaction pathway for transmembrane redox. The k_2 step, represented as viologen dimerization, is complex.

inside–outside MV^{2+} product distribution after redox cycling was very nearly identical with the initial distribution before reduction. This result is expected since transmembrane oxidation of internal MV^+ would be electrogenic in the opposite sense to internal MV^{2+} reduction in the absence of transverse ion migration. Thus, the same forces driving inward diffusion of MV^+ during reduction drive its outward diffusion when a membrane-impermeable oxidant is used.

Reduction of MV^{2+} bound only at the external interface of DHP vesicles gave predominantly monomeric radical ion product[16] (Figure 1, solid line). The amount of monomer remained greater than 85% of the total reduced viologen at $[MV^{2+}]/[DHP]$ ratios ranging from 0.0025–0.015 and was still 40% at the very high ratio of 0.15. In contrast, when equimolar MV^{2+} was present at the opposing vesicle interfaces or when internal MV^{2+} was in excess (r = 0.01–0.04), the product optical spectrum corresponded primarily to the multimeric form of the radical[16] (Figure 1, dotted line). When external MV^{2+} was in excess, the amount of multimer formed was approximately equal to the initial concentration of MV^{2+} on the inner surface, the remainder being monomeric MV^+ radical cation (e.g., Figure 1, dot-dashed line). These observations indicate that the multimeric form of the radical is formed in a stoichiometric ratio of one viologen each from the inner and outer vesicle interfaces and that reduction of inner bound MV^{2+} is associated with aggregation.[17]

Reduction of DHP vesicles containing internally and externally bound MV^{2+} exhibited biphasic kinetics. Relative amplitudes for the two steps measured at various wavelengths indicated that monomeric and multimeric MV^+ were the principal products of the fast and slow reaction steps, respectively. These observations establish that aggregation is coincident with transmembrane redox under steady-state conditions. With $S_2O_4^{2-}$ in excess, the fast step was first order and gave a rate constant, k_1, similar to the constant for dithionite reduction of MV^{2+} in solution.[18] The rate for the slow step was about 10^2-fold less than the fast reaction step and with equimolar MV^{2+} initially at both interfaces followed simple second-order kinetics with k_2 = 1.3 (±0.4) × 10^4 M^{-1} s^{-1} in 20 mM Tris, pH 8.0, 23 °C, [DHP] = 1–2 mM, and $[MV^{2+}]/[DHP]$ = 0.025–0.081; k_2 was independent of the monitoring wavelength and $S_2O_4^{2-}$ concentrations measured over the range $[S_2O_4^{2-}]$ = 0.41–2.2 mM.

A reaction scheme consistent with these facts is illustrated in Figure 2. Here, rapid reduction of externally bound MV^{2+} (k_1) precedes reduction of internally localized MV^{2+} (k_2), which occurs either by rate-limiting formation of a mixed-valent MV^{2+}–MV^+ dimer or slow electron exchange between external MV^+ and internal MV^{2+}, followed by rapid dimerization. The MV^{2+}–MV^+ dimer is subsequently rapidly reduced (k_3) to $(MV^+)_2$ by externally localized $S_2O_4^{2-}$. Upon oxygenation, the MV^{2+} ions derived from the dimer are found inside the vesicle, as expected from the system electrostatics. Since k_1, $k_3 \gg k_2$, the rate law is given by $d[MV^+]_T/dt = k_1[MV^{2+}]_o[SO_2^-] + k_2[MV^+]_o[MV^{2+}]_i$, where subscripts T, o, and i refer to total MV^+ in the system and MV^{2+} bound at outer and inner vesicle interfaces, respectively. With equimolar inner and outer $[MV^{2+}]$, $[MV^+]_o \simeq [MV^{2+}]_i$ for the slow step, so that $d[MV^+]_T/dt \simeq k_1[MV^{2+}]_o[SO_2^-] + k_2[MV^{2+}]_i^2$.

Our present efforts are directed at probing mechanistic details of the transmembrane redox step and exploring the generality of the mechanism. Consistent with our observations, a report has recently appeared[9] suggesting comparable dynamic behavior for *N,N'*-dihexadecyl-4,4'-bipyridinium^{2+}-mediated transmembrane electron transfer between $S_2O_4^{2-}$ and $Fe(CN)_6^{3-}$ ions separated by phosphatidylcholine liposomal membranes.

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Registry No. DHP, 2197-63-9; MV^{2+}, 4685-14-7; S_2, 14844-07-6.

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Moderately Strong Intramolecular Magnetic Exchange Interaction between the Copper(II) Ions Separated by 11.25 Å in $[L_2Cu_2(OH_2)_2(\mu\text{-terephthalato})](ClO_4)_2$ (L = 1,4,7-Trimethyl-1,4,7-triazacyclononane)

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Owing to its fundamental importance, the study of long-range magnetic interactions has been an active field of research in recent years.[2] The terephthalato dianion has been proved to be an appropriate bridging unit to design magnetic systems with a separation of 11–12 Å between the two magnetic centers.[3-5] In all of these studies the intramolecular magnetic interactions, to the disappointment of the research workers, were negligibly small.

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