initially (120 min) 20.5, 26.7, 52.8, and the extrapolated values were 19.8, 26.6, 53.6.

The isopropylcyclohexene isomers were identified by comparison of their GC retention times with those of authentic samples, using a 2-m silver nitrate/tetraethylene glycol packed column made according to the procedure of Cope et al.[35] The assignments had previously been confirmed by using the HP-1 capillary column and dibrominating the olefins by the method of Crabtree et al.[36] and by comparing GC retention times with those of the dibromides of the authentic olefins.

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Mechanisms of Viologen-Mediated Charge Separation across Bilayer Membranes Deduced from Mediator Permeabilities

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Abstract: The mobilities of several bipyridinium ions across the hydrocarbon bilayer membranes of dihexadecyl phosphate (DHP) small unilamellar vesicles have been determined by measuring the uptake of ^{14}C-radiolabeled analogs. The *N*-alkyl-*N'*-methyl-4,4'-bipyridinium (viologen, C_nMV^{2+}) dications and the *N*-methyl-4,4'-bipyridinium cation were membrane-impermeable. However, the C_nMV^{+} radical cations were permeable, provided that the *n*-alkyl chain length did not exceed 12 carbon atoms. Diffusion rates obeyed a simple first-order rate law; rate constants for $n = 1$ and $n = 6$ were nearly identical, and the constant for $n = 12$ was about 3-fold lower. From these values and the measured diffusion potential, the permeability coefficient of the *N,N'*-dimethyl-4,4'-bipyridinium radical cation was estimated to be 2×10^{-8} cm s^{-1} at 23 °C. When $n = 16$, radiolabel exchange was biphasic, with the major fraction of DHP-bound $C_{16}MV^{+}$ undergoing no transmembrane diffusion. This behavior confirms earlier suggestions, based upon the dynamics of viologen-mediated transmembrane redox reactions, that the short-chain viologens act as mobile charge relays, whereas the long-chain congeners transfer charge primarily by electron tunneling.

Introduction

Oxidation-reduction reactions have been shown to occur across bilayer membranes in numerous asymmetrically organized redox systems.[1] These reactions are of considerable topical interest in relation to applications in photoconversion, "molecular" electronics, and biomimicry.[1,2] In every case reported, either the reactants themselves were membrane-bound or the membrane contained additional redox components capable of mediating the overall reaction. The requirement for membrane-localized redox components is consistent with the general nature of electron transfer processes, since the bilayer membrane presents an insulating barrier of 40-50-Å thickness, a distance which is too large to allow electron tunneling at appreciable rates between reactants confined to the aqueous compartments.[3] The molecular details of these reactions are not well characterized, however. Two alternative limiting pathways are[1a] (i) that membrane-bound reactants which are transversely juxtaposed in the opposite bilayer leaflets undergo electron exchange or (ii) that they diffuse across the bilayer, thereby acting as mobile redox carriers. Only in pathway i does transmembrane electron transfer actually occur.

The measurement of redox rates alone does not provide a basis for distinguishing between the limiting pathways.[1a] Comparison of the redox rates with independently measured rates of transmembrane diffusion of the reaction components could, in principle, resolve this issue. For example, if diffusion by all available pathways were much slower than the overall transmembrane oxidation-reduction rate, then the reaction would involve electron transfer; in contrast, if one or more redox components were to diffuse on the same timescale as the net transmembrane redox rate, then reaction by a diffusional pathway would be plausible.[1a] Quantitative information on transmembrane diffusion of the reaction components has generally been unavailable, however.

We have sought to identify transmembrane redox pathways in simple vesicular systems for which the requisite diagnostic rate information can be obtained. Specifically, we have utilized anionic dihexadecyl phosphate (DHP) vesicles, which strongly bind cationic *N*-alkyl-*N'*-methyl-4,4'-bipyridinium ions (C_nMV^{2+}, with $n = 1-16$). We have shown that addition of membrane-impermeable strong reductants to the external medium caused one-electron reduction of internal viologens to their corresponding radical cations (C_nMV^{+}) by processes mediated by the externally bound viologens.[1a,4,5] As with all charge separation processes

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across closed membranes, the transmembrane redox step was electrogenic and required charge-compensating ion movement to maintain electroneutrality in the aqueous subphases separated by the membrane. For viologens containing short alkyl chains ($n \leq 10$), this function was served by the external C_nMV^+ radical cations, which were taken up in an approximately 1:1 stoichiometric ratio with each internal C_nMV^{2+} ion reduced.[4-6] However, for the longer-chain analogs, not all of the externally bound C_nMV^+ ions could be translocated.[1a,5] Correspondingly, reduction of internal C_nMV^{2+} ions was incomplete unless lipophilic cations were added to the reaction medium. The fraction of C_nMV^+ ions that were mobile decreased progressively with increasing alkyl chain length, so with $n = 16$ only about 30% of the radical cation could be taken up. These data were interpreted to indicate the simultaneous operation of two distinct reaction pathways, one diffusional in the sense that electron transfer was accompanied by comigration of the C_nMV^+ radical cation, and the other necessarily involving transmembrane electron exchange since the C_nMV^+ ions contributing to this pathway were unable to diffuse across the bilayer.

This interpretation implies the existence of at least two distinct binding domains for the long-chain analogs which do not interconvert on the timescales of the redox reactions. Kinetic mapping studies have revealed that two distinguishable reaction environments were produced when the long-chain C_nMV^{2+} ions were bound to the DHP external interface, whereas no such heterogeneity could be detected for the short-chain analogs.[7,8] Further, optical spectroscopic studies indicated that the extent of aggregation of the corresponding externally bound radical cations increased progressively with the viologen alkyl chain length.[5,9] Additional physical studies gave results suggesting that the nature of membrane binding for short- and long-chain viologens is fundamentally different. Specifically, the data are consistent with a binding model in which the short-chain viologens are only electrostatically bound to anionic phosphate headgroups at the aqueous hydrocarbon interfaces, whereas the long-chain viologens preferentially intercalate within the surfactant amphiphiles to form part of the bilayer structure.[5,10,11] Assignment of individual microstructures to specific reaction pathways is not yet possible because their supramolecular organization is not sufficiently well-described. However, covalently linked viologen radical cation dimers are DHP membrane-impermeable,[5] and studies involving simultaneous spectroscopic and electrochemical measurements to locate MV^+ in DHP vesicles have suggested that the multimeric form of MV^+ is also membrane-impermeable.[12] Thus, the electron tunneling pathway might be associated with aggregated viologens. These viologens are also presumably intercalated, since then the effective transmembrane electron transfer distance would be 20–25 Å for juxtaposed reactants. This separation distance is consistent with measured transmembrane redox rate constants.[1a] In contrast, surface-adsorbed reactants would be separated by at least 40 Å and would require some membrane-penetrating diffusional motion to reduce transverse electron tunneling distances.[1a,3]

In the present study, we have used ^{14}C-radiolabeling techniques to measure diffusional rates of representative alkyl methyl viologens in DHP small unilamellar vesicles (SUVs). These studies, which are the first to measure directly viologen diffusional dynamics across bilayers, support our mechanistic models and provide the basis for systematic investigations of how supramolecular architectures and forces of self-assembly influence the diffusional properties of dopant species. The measurement of viologen permeabilities also allows identification of the charge-carrying species in transmembrane redox reactions proceeding by diffusional mechanisms. Because the radical cations disproportionate slightly,[9] i.e., $2C_nMV^+ \rightleftharpoons C_nMV^{2+} + C_nMV^0$, it is possible that C_nMV^0 is the actual electron shuttle, in effect carrying the electron and charge-compensating ion as a single entity. Recent kinetic studies on the transmembrane reduction of $C_{16}MV^{2+}$ by $S_2O_4^{2-}$ ion across phosphatidylcholine membranes have, in fact, been interpreted as indicating that $C_{16}MV^0$ is the membrane-localized redox carrier in that system.[13]

Experimental Section

Materials. Dihexadecyl phosphate (Aldrich) was recrystallized from methanol after being filtered to remove insoluble impurities. The chloride salts of *N*-alkyl-*N'*-methyl-4,4'-bipyridinium and *N*-alkyl-4,4'-bipyridine (C_nB^+) ions were synthesized by Dr. David H. Thompson (OGI) according to previously established procedures.[11] The corresponding ^{14}C-methyl-labeled alkylmethylviologens ($C_n^{14}MV^{2+}$) were synthesized from appropriate C_nB^+ chlorides and [^{14}C]methyl iodide (Sigma, specific activity 9.3 mCi/mmol) as follows: 0.01 mmol $^{14}CH_3I$ in 1 mL of dry CH_3CN was added to a 10-fold excess (0.1 mmol) of C_nBCl in 14 mL of CH_3CN, contained within a thick-walled glass ampule. The ampule contents were frozen by immersion in liquid nitrogen, and the ampule was sealed under vacuum with a torch and placed in an oven at 82 °C for 16 h. The seal was then broken, and a 40-fold excess (4 mmol) of nonradioactive methyl iodide was added. The ampule was resealed as before and returned to the oven for an additional 8 h. The solvent was then evaporated with a stream of nitrogen, and the yellow-orange solid was washed repeatedly on a filter with dry ether followed by dry acetone until the filtrates were colorless. The solid was then dissolved in water and characterized spectrophotometrically using published spectral parameters.[7] Absorption bands assignable to C_nMV^{2+} and I^- ions appeared at 260 and 228 nm, respectively. Anaerobic reduction with excess sodium dithionite caused complete conversion to the radical cations with absorption maxima at 602 nm, identical to those of the corresponding nonradioactive viologens. The [^{14}C]-*N*-methyl-4,4'-bipyridine ($^{14}MB^+$) iodide was synthesized analogously in a single step. Both radioactive (0.01 mmol) and nonradioactive (0.06 mmol) CH_3I were added to a 22-fold excess (1.54 mmol) of 4,4'-bipyridine in 14 mL of benzene. After being sealed, the ampule was heated at 82 °C for 16 h, the solvent was removed as before, and excess bipyridine was removed by washing with dry ether. The remaining solid was then dissolved in H_2O and stored as a stock solution. The electronic absorption spectrum of this solution was identical to those of nonradioactive MB^+ solutions. Anaerobic addition of $Na_2S_2O_4$ did not alter the spectrum; in particular, no coloration attributable to viologen impurities was observed. Sodium dithionite solutions (Fluka, 87%) were freshly prepared prior to each set of experiments by dissolving weighed amounts of the free-flowing solid in deoxygenated buffer and determining their concentrations spectrophotometrically using $\epsilon_{316} = 6.9 \times 10^3\ M^{-1}\ cm^{-1}$.[14] Other chemicals were of the best available grade and used as received. Water was purified using a reverse osmosis/ion exchange system followed by distillation from a quartz still.

Vesicle Preparation. Vesicle suspensions were prepared by sonicating 4.3 mg/mL of DHP in 10–11 mL of 20 mM Tris chloride, pH 8.0, using a Heat Systems Ultrasonic W185F sonicator equipped with a flat tip titanium horn. Two 10-min pulses separated by a 5-min interval for cooling were applied at a power setting of 4.0 to obtain a clear solution. Particulate matter was removed by passing the solution through a 0.2-μm cellulose nitrate filter. The suspension was then centrifuged for 90 min at 100 000*g* in a Beckman L8-70M ultracentrifuge, and the supernatant containing small unilamellar vesicles was decanted from the pellet for subsequent experimentation. Vesicles containing entrapped MV^{2+}, C_6MV^{2+}, or MB^+ were prepared by sonicating DHP in buffer containing 0.2–1 mM pyridinium cation, followed by removal of extravesicular cations by passage down a 0.7- × 8-cm cation exchange column (Bio-Rad AG50W-X8, 200–400 mesh) that had been equilibrated with buffer. Quantitative removal of external viologens was confirmed by the absence of detectable radical cation formation upon adding sodium dithionite to anaerobic suspensions.[4] Repetitive passage of vesicle suspensions through the column caused no further loss of the viologen dications or MB^+, as determined spectrophotometrically. Spectra were corrected for light scattering by referencing against suspensions of vesicles that contained no pyridinium cations but were otherwise identical to the sample suspensions. The extent of entrapment was 10–25% of the initially added

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cation and was highly reproducible from preparation to preparation under a given set of conditions. Following spectrophotometric analysis to determine the internal viologen or MB^+ concentration, C_nMV^{2+} or MB^+ was added from the stock solutions to give the desired concentrations in the external medium. This procedure allowed preparation of vesicles containing ^{14}C-labeled and unlabeled components that were asymmetrically distributed across the bilayer membrane. Binding of the longer-chain analogs $C_{12}MV^{2+}$ and $C_{16}MV^{2+}$ was so strong that they could not be removed from the external DHP vesicle surface by ion exchange chromatography.

Radioactive Exchange Experiments. Rates of viologen diffusion across the bilayer membrane were generally determined by measuring the time course of the uptake of externally added ^{14}C-radiolabeled viologen. The analysis entailed chromatographing fractions of the vesicle suspensions taken at varying times following initiation of the exchange and determining simultaneously the radioactivity and total concentration of entrapped viologen in the vesicle-containing eluate. Specifically, a 0.7- × 4.5-cm AG50W-X8 column was used; its void volume was determined to be 0.6 mL with 2×10^6 MW blue dextran. With the buffer level at the top of the resin, 0.5 mL of the vesicle suspension was added and passed through the column, followed by washing with 1.0 mL of the buffer. This procedure gave essentially complete recovery of vesicles that were depleted of external viologen in a 2-fold diluted solution. The concentration of entrapped viologen was determined spectrophotometrically; the sample was then placed in a Wheaton scintillation vial containing 4 mL of Ecolite scintillation cocktail (ICN) and homogenized by vortex-mixing, and its radioactivity was counted using a Beckman LS-3150T counter. Counting times were adjusted to give a count per minute accuracy exceeding 3%. The total radioactivity in the vesicle suspensions was obtained by diluting 0.5 mL of the reaction solution with 0.5 mL of the buffer and preparing the scintillation solution as described above. To study transmembrane diffusion of viologen radical cations, 1.5-mL fractions of the vesicle suspensions were placed in optical cuvettes fitted with rubber septa and deoxygenated with a stream of water-saturated, oxygen-scrubbed argon. Then, 15–100 μL of 1 mM dithionite solution was added anaerobically with a microsyringe while the vesicle suspension was vigorously stirred with a magnetic microbar. The sample was incubated for a predetermined time, during which its optical spectrum was periodically taken, and then the reaction was quenched by quickly air-saturating the sample with a syringe. The product solution was analyzed as described above. Reoxidation of the radical cations was complete within 1 s. Because O_2 is freely permeable to the DHP membrane, the oxidation of internally localized viologen radical cations is not electrogenic.[1a,4] Furthermore, dication exchange across the DHP bilayer is extremely slow (Results). Given these conditions, aeration effectively "freezes" the radical cation exchange reaction and allows application of the relatively slow ion exchange analytical procedure. Because the long-chain C_nMV^{2+} ions (n = 12 or 16) could not be removed from the vesicle exterior by cation exchange chromatography, it was not possible for these ions to prepare particles containing a single viologen with asymmetrically distributed radiolabel. In these cases, transverse diffusion was estimated by adding the long-chain viologens to suspensions of vesicles preloaded with $[^{14}C]$-MV^{2+} and measuring the subsequent rate of release of radioactivity using the procedures described above.

To determine if there was a specific influence of dithionite on the exchange kinetics, several measurements were made using the triplet-excited [5,10,15,20-tetrakis(4-sulfonatophenyl)porphinato]zinc(II) ion ($ZnTPPS^{4-}$) as the reductant.[15] The complete reducing system included 150 nM $ZnTPPS^{4-}$ photosensitizer and 160 μM dithiothreitol, the latter added as an electron donor. Illumination of the vesicle suspensions for a few seconds with visible light generated external viologen radical cation at concentrations that were comparable to the concentrations used in the exchange reactions initiated by $S_2O_4^{2-}$ reduction. The light source was a 1.5-kW xenon lamp whose output was focused and passed through aqueous $CuSO_4$ and Corning GG 400-nm cutoff filters before striking the sample.

All experiments were done at ambient temperature (23 ± 2 °C). Optical spectra were taken on a Hewlett-Packard 8452A diode array spectrophotometer interfaced to a ChemStation data acquisition/analysis system.

Results

Transmembrane Diffusion of C_nMV^{2+} and MB^+ Ions. No radioactivity was found within vesicles containing 50–100 μM nonradioactive MV^{2+}, C_6MV^{2+}, or MB^+ after incubation for 5–30 h in a medium containing equimolar quantities of the corresponding ^{14}C-radiolabeled compounds. The total concentration of entrapped material remained constant over this period. No radioactivity accumulated in empty vesicles when incubated in ^{14}C-radiolabel-containing media, and no leakage of entrapped $[^{14}C]$-MV^{2+} from loaded vesicles into a viologen-free medium could be detected within 30 h. Bubbling with argon or adding sodium dithionite to the external aqueous phase had no effect upon these results; dithionite did not reduce MV^{2+} when the two reagents were separated by the DHP membrane (e.g., Figure 2), consistent with earlier results.[4] These data establish both that DHP membranes are impenetrable to C_nMV^{2+} and MB^+ cations with respect to both simple diffusion and diffusive exchange and that dithionite does not alter these characteristics.

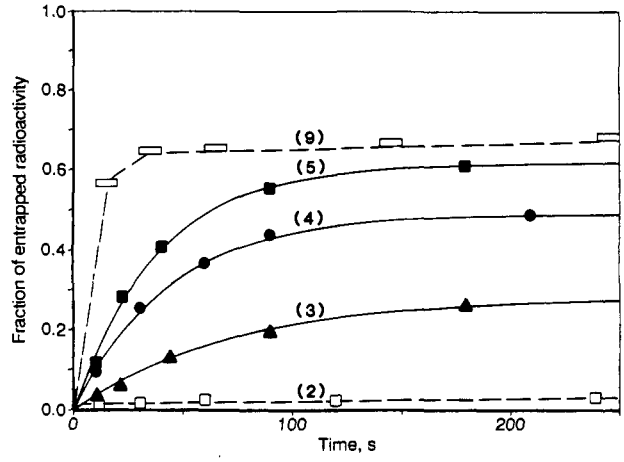

Figure 1. Time course of $[^{14}C]$methyl viologen accumulation by DHP vesicles. Numbers in parentheses indicate corresponding data entries in Table I, which gives experimental conditions and kinetic constants obtained as best-fit parameters to eq 1. Solid lines are calculated from eq 1; dashed lines have no theoretical significance. The broad bars in curve 9 reflect experimental uncertainty arising from the time required to quench the reaction with O_2 when dithionite was in excess.

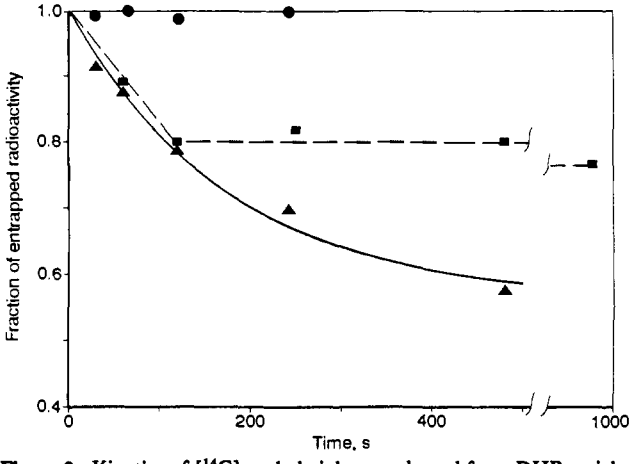

Figure 2. Kinetics of $[^{14}C]$methyl viologen released from DHP vesicles containing long-chain viologen radical cations. Initial conditions: triangles (▲), $[MV^{2+}]_i$ = 54 μM, $[C_{12}MV^+]_o$ = 19 μM, $[C_{12}MV^{2+}]_o$ = 33 μM; squares (■), $[MV^{2+}]_i$ = 57 μM, $[C_{16}MV^+]_o$ = 25 μM, $[C_{16}MV^{2+}]_o$ = 25 μM; circles (●), $[MV^{2+}]_i$ = 54 μM, $[S_2O_4^{2-}]_o$ = 13 μM, no external viologen. All runs were at 23 °C with [DHP] = 6 mM.

Transmembrane Diffusion of the MV^+ Radical Cation. For DHP suspensions in which $[^{14}C]$-MV^{2+} was initially located in the external medium, partial reduction with dithionite led to radioactivity uptake (Figure 1). The maximal extent of accumulation (F_{max}) was dependent upon the fraction of the total viologen in the internal aqueous phase, and the kinetics obeyed an exponential rate law

$$F_t = F_{max}[1 - \exp(-kt)] \quad (1)$$

where F_t represents the fraction of radioactivity located inside the vesicles at a given time. Kinetic data for reactions under various

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Table I. Kinetic Parameters for C^{14}-MV^+ Uptake in MV^{2+}-Containing DHP Vesicles[a]

entry no.	[DHP] (mM)	$[MV^{2+}]_i$[b] (μM)	$[MV^{2+}]_o$[b] (μM)	$[MV^+]$[c] (μM)	F_∞	F_{max}	10^3k[d] (s^{-1})	β	10^3k_i[e] (s^{-1})
1	6.2	0	100	40	0	0.03		0.40	
2	5.9	0	79	26	0	0.03		0.33	
3	5.8	40	94	23	0.30	0.28	16	0.24	19
4	5.8	43	39	24	0.52	0.49	23	0.61	18
5	5.8	43	23	19	0.65	0.62	26	0.83	19
6	6.0	71	77	28	0.48	0.49	14	0.36	19
7	5.9	79	79	24	0.50	0.48	12	0.30	19
8	5.9	80	81	27[f]	0.50	0.49	14	0.33	21
9	2.9	40	40	28	0.50	0.49	30	0.70	21
10	6.2	41	41	56	0.50	0.70	≥70	1.4	≥35[g]

[a] At 23 °C, concentration ratios F_{max}, F_∞, and β are defined by eqs 1, 5, and 8, respectively. [b] Concentrations before initiating exchange. [c] Determined spectrophotometrically following $S_2O_4^{2-}$ addition. [d] From eq 1. [e] From eq 8. [f] Photochemically generated. [g] Reaction occurs with net MV^+ uptake, useful for qualitative comparisons only since eq 5 is not valid under these conditions.

experimental conditions are given in Table I. Identical results were obtained when the $ZnTPPS^{4-}$–dithiothreitol photoreduction system was substituted for $S_2O_4^{2-}$ ion (Figure 1; Table I, entry 8); very similar results were also obtained when the radiolabel was initially contained within the DHP vesicles as [^{14}C]-MV^{2+}, except that reaction in this case involved a loss of radioactivity from the particles. So long as the $S_2O_4^{2-}$ equivalent concentration did not exceed the external MV^{2+} concentration, the total amount of entrapped viologen remained constant within ±2% over the time course of exchange. When $S_2O_4^{2-}$ was added in excess of the amount required to reduce external MV^{2+}, a net uptake of the viologen occurred which was coincident with the reduction of the internal MV^{2+} ions. This effect, which is attributable to the electrogenic nature of transmembrane redox reactions in these systems, is well understood.[1a,4-6] However, the relatively high radiolabel exchange rates (Figure 1, curve 9) and slow, kinetically complicated O_2 oxidation rates[5] observed under these conditions precluded accurate kinetic analyses by these methods. The uptake was also too rapid to measure when ~35 μM radiolabeled MV^+ radical cation was added to DHP vesicles preloaded with ~35 μM internal MV^+. The minimum sampling time for these studies was ~15 s. Empty vesicles accumulated small but readily detectable amounts of radioactivity in media containing [^{14}C]-MV^+ ions (Figure 1, curve 2).

Transmembrane Diffusion of C_nMV^+ Ions. The dynamical behavior of the uptake of $C_6{}^{14}MV^+$ was very similar to that described above for [^{14}C]-MV^+. Kinetic data are given in Table II. Transmembrane diffusion was also investigated for several systems containing different viologens on opposite sides of the membrane. These studies provided a means for estimating transverse diffusional rates of the viologen dications and radical cations whose chain lengths exceeded 10 carbon units. Because these C_nMV^{2+} ions could not be removed from the vesicle exterior surface by cation exchange chromatography, their diffusional dynamics could not be studied in symmetrical systems. The kinetics of radiolabel uptake by vesicles containing internal C_6MV^{2+} and external $MV^{2+/+}$ were nearly identical to the transmembrane radiolabel exchange kinetics for $MV^{2+/+}$ and $C_6MV^{2+/+}$ ions; however, the rate was several-fold slower when MV^{2+} was encapsulated and C_6MV^{2+} was in the aqueous phase (cf. lines 3 and 4, Table II). Release of [^{14}C]-MV^{2+} from DHP vesicles containing externally bound $C_{12}MV^{2+}$ or $C_{16}MV^{2+}$ ions also did not occur until the external C_nMV^{2+} ions were partially reduced. For $C_{12}MV^{2+}$, radioactivity release followed exponential kinetics (Figure 2) with a rate constant that was several-fold slower than that measured for the shorter-chain analogs (Table II). For $C_{16}MV^{2+}$, the reaction behavior was more complex. Specifically, relatively little exchange occurred, and it was complete within about 2 min (Figure 2). Unlike the other reductions, $C_{16}MV^+$ formation occurred on the same timescale as ^{14}C-radiolabel uptake, and about 30% of the radical formed was multimeric, i.e., aggregated.[5] For all other viologens, reduction by dithionite was practically instantaneous and gave greater than 90% monomeric viologen radical.[5] For all of the reactions studied, no detectable changes occurred in the total internal viologen concentration over the time range of the studies, again indicating that radiolabel diffusion was not accompanied by any significant net mass transport.

Discussion

Kinetic Model for Transmembrane Exchange. The absence of exchange of ^{14}C-radiolabeled viologen dications between inner aqueous and external C_nMV^{2+} pools establishes that the DHP bilayer membrane is impermeable to these ions. Therefore, the exchange that occurs upon partial one-electron reduction of the external ions must be attributed to transmembrane diffusion of the radical cations or neutral MV^0 diradicals formed by their disproportionation, i.e., $2C_nMV^+ \rightleftharpoons C_nMV^{2+} + C_nMV^0$. We will assume that the radical cations are the diffusing species and then justify this assumption by quantitative arguments based upon the results.

Self-exchange rate constants for electron transfer between the cations in homogeneous solutions, e.g.,

$$[^{14}C]\text{-}MV^{2+} + MV^+ \rightleftharpoons [^{14}C]\text{-}MV^+ + MV^{2+}$$

are about 10^8 M^{-1} s^{-1}.[16-18] Therefore, equilibration of the radiolabeled viologen radical cation with unlabeled dications in the aqueous phases is expected to occur with reaction half lives shorter than 1 ms under the experimental conditions (Table I). Because accumulation (or loss) of the radiolabel is slow relative to this reaction (Figures 1 and 2), the transmembrane diffusion step (Figure 3) must be rate-limiting. Under these conditions, the inward flux of radioactivity (R_i) is given by

$$R_i = J_i\alpha(1 - F_t) \quad (2)$$

where J_i is the total inward flux, α is the initial fraction of the externally localized viologen that is radiolabeled, and F_t is as previously defined. R_i is, therefore, the product of the flux times the probability that the entering viologen is radiolabeled. Similarly, the outward flux of radioactivity (R_o) is given by

$$R_o = J_o\alpha[MV]_o/[MV]_iF_t \quad (3)$$

In this equation, $[MV]_o$ and $[MV]_i$ refer to the total viologen (MV^+ and MV^{2+}) concentrations in the external and internal aqueous phases, respectively, and J_o is the total outward flux. The fraction of entrapped radioactivity can be expressed as

$$F_i = \int_0^t (R_i - R_o)dt/\{[^{14}C]\text{-}MV\}_0 \quad (4)$$

Except for a net inward diffusion of a small amount of MV^+ at the start of the reaction (discussed below), the total amounts of internal and external viologens were unchanged over the course of the radiolabel exchange reactions. This result indicates that $J_i = J_o = J$ and that the ratio $[MV]_o/[MV]_i$ is constant. Given these constraints, the solution to eqs 2–4 is

$$F_t = F_\infty\{1 - \exp(-Jt/F_\infty[MV]_o)\} \quad (5)$$

where $F_\infty = [MV]_i/([MV]_o + [MV]_i)$. Comparing eqs 1 and 5, we find that $F_{max} = F_\infty$ and the apparent first-order rate constant

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Table II. Kinetic Parameters for Transmembrane Diffusion across DHP Vesicles Containing Various Combinations of C_nMV^{2+} Ions[a]

internal C_nMV^{2+}	external $C_mMV^{2+/+}$	F_{max}[b]	$10^3 k_i$[c] (s^{-1})
MV	[^{14}C]-MV	0.48	19
C_6MV	$C_6{}^{14}$MV	0.43	21
C_6MV	[^{14}C]-MV	0.45	18
MV	$C_6{}^{14}$MV	0.43	3.3
[^{14}C]-MV	C_{12}MV	0.44	5.7

[a] [DHP] = 6 mM, [C_nMV] = [C_mMV], at 23 °C. [b] Calculated from eq 1, theoretical value for F_∞ = 0.5. [c] Calculated from eqs 1 and 8.

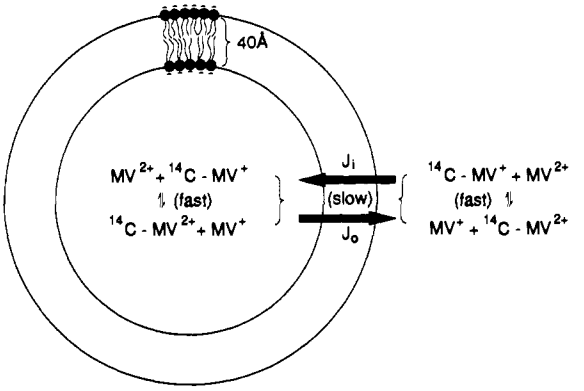

Figure 3. Kinetic model for radioactive exchange across the DHP bilayer membrane. The heavy arrows indicate total inward (J_i) and outward (J_o) fluxes of viologen radical cations; the dications are membrane-impermeable.

k is given by $k = J/F_\infty[MV]_o$. In this equation, the concentration dependence of the flux is unspecified. Assuming $J_i = k_i[MV^+]_o$ (and $J_o = k_o[MV^+]_i$), we write

$$k_i = kF_\infty[MV]_o/[MV^+]_o$$

In these equations, k_i is the first-order rate constant for inward diffusion of the viologen radical and $[MV^+]_o$ is its concentration in the external aqueous phase.

Because, in this model, the radical cations are the diffusing species, their final concentrations in the external phase following equilibration across the bilayer are very nearly equal to the total viologen radical concentration, e.g.,

$$[MV^+]_o + [MV^+]_i = MV^+{}_T \approx [MV^+]_o \quad (6)$$

This is a consequence of the electrogenic nature of the diffusion process. Inward diffusion of the radical ions down their concentration gradients establishes a diffusion potential ($\Delta\psi$), with the vesicle interior positively charged with respect to the exterior. This developing potential opposes further net inward flux of C_nMV^+, ultimately achieving a balance when

$$\Delta\psi = -(2.3RT/F) \log ([C_nMV^+]_i/[C_nMV^+]_o) \quad (7)$$

This transmembrane equilibrium distribution was determined in our systems by measuring the net viologen radical uptake by DHP vesicles containing no viologen dication. As discussed in the Experimental Section, subsequent air oxidation effectively trapped translocated viologen radicals as the dications within the vesicles. The extent of viologen uptake was very small (Figure 1, curve 2), amounting to a few percent of the total viologen radical present in the suspensions. On the basis of measured diffusion rates, the equilibration process establishing the diffusion potentials occurred within the first few seconds after exchange was initiated by dithionite reduction of the external C_nMV^{2+} ion. Careful examinations of the MV^+ uptake by empty vesicles indicated biphasic kinetics with a rapid step on this timescale followed by a slower net accumulation occurring over several hours. This slow step is just detectable in curve 2 of Figure 1; it is attributable either to charge-compensating electrolyte leakage that partially dissipates the membrane potential, allowing further inward net diffusion of the MV^+ ion, or to electroneutral diffusion of MV^0 (discussed below). In any event, these results establish the approximate equality given by eq 6; therefore, the inward first-order rate constant for transmembrane diffusion of C_nMV^+ ions is approximated by

$$k_i \approx (F_\infty/\beta)k \quad (8)$$

where $\beta = MV^+{}_T/[MV]_o$, the fraction of externally located viologen that was reduced upon the initiation of exchange of radiolabel between the pools.

Comparison with Experiment. This simple model accurately predicts the observed experimental behavior for transmembrane radiolabel exchange involving the MV, C_6MV, and C_{12}MV cations (Tables I, II) but not the $C_{16}MV^{2+/+}$ ions (Figure 2). Specifically, for the shorter-chain analogs the reaction was first order, with the kinetically determined values of F_{max} nearly equaling the theoretically expected values of F_∞, and, for exchange of $MV^{2+/+}$ (the most extensively studied ion), the diffusion constant k_i was invariant within uncertainty under conditions where the parameters k, F_∞, and β were varied widely (Figure 1, Table I).

The transmembrane potential estimated from eq 7 can also be compared to values calculated from fundamental parameters. As discussed above, the magnitude of entrapment of C_nMV^+ was small and, therefore, relatively uncertain. Nonetheless, we calculate from the data in Table I (entries 1 and 2) that ~8% of the external MV^+ is translocated in establishing the diffusion potential. On the basis of approximate spherical symmetry with inner and outer radii of 80 and 120 Å,[11] respectively, the vesicle inner membrane surface area is ~31% of the total area. Since the viologen radical is interfacially adsorbed, translocation to the inner surface causes an approximate 2-fold increase in its effective concentration. Thus, for 8% translocation, $[MV^+]_i/[MV^+]_o \approx 0.19$ and, from eq 7, $\Delta\psi \approx -43$ mV at ambient temperatures. A calculation using a spherical plate capacitor model with dimensions of the vesicle and a dielectric constant ϵ of 2.2 gave $\Delta\psi$ ranging from −44 to −54 mV for the number of charges transferred in these experiments. The calculated potential is only weakly dependent upon vesicle size but is strongly dependent upon the chosen ϵ value. We have assumed a value reported for lipids[19] which is consistent with about 2-fold greater values measured for *interfacial regions* of anionic vesicles whose hydrocarbon chains are in the gel phase.[20] These conditions are appropriate for DHP vesicle suspensions.[11] The good correlation between experimentally determined and predicted values for $\Delta\psi$ supports our interpretation that the uptake of MV^+ by empty DHP vesicles represents a Donnan-type equilibration and, consequently, that the radicals are the only membrane-permeable ions in the microphase assemblies.

In this model, the constant k_i is not the intrinsic rate constant (k_p) for penetration of the bilayer but rather is that value multiplied by a factor representing a repulsive potential originating in the transmembrane diffusion potential, i.e., $k_i = k_p e^{\phi/RT}$. Similarly, $k_o = k_p' e^{-\phi'/RT}$, where outward diffusion is accelerated by the membrane potential. If the potential barrier is symmetrical, so that $k_p = k_p'$ and $\phi = \phi'$, then $\phi = F(\Delta\psi/2)$ and $k_i = k_p e^{F(\Delta\psi/2RT)}$, where F is the Faraday constant. From the data in Table I, it follows that $k_p \approx 4 \times 10^{-2}$ s^{-1}; assuming a bilayer width of ~40 Å, this amounts to a permeability coefficient of 2×10^{-8} cm s^{-1}.

Transmembrane Diffusion of MV^0. As mentioned previously, it is possible that MV^0 formed by MV^+ disproportionation is the actual diffusing species. Bilayer penetration by neutral lipophiles can be very rapid,[17] with first-order rate constants as large as $k = 1.2 \times 10^4$ s^{-1} having been reported for the protonophore carbonyl cyanide *m*-(chlorophenyl)hydrazone.[21] However, several arguments indicate that C_nMV^0 diffusion is unimportant in our experiments. First, net fluxes of material are expected to be different for electroneutral (MV^0) and electrogenic (MV^+) transport. Electroneutral transport in compartmented systems will proceed

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until the chemical potentials of the diffusing species are equal in all compartments. Assuming that the activity coefficients at both DHP vesicle interfaces are identical, equilibrium is achieved when $[MV^0]_i = [MV^0]_o$. Likewise, if the MV^+ disproportionation constants $K_d = [MV^0][MV^{2+}]/[MV^+]^2$ are equal at both interfaces, then at equilibrium, $[MV^{2+}]_o/[MV^{2+}]_i = ([MV^+]_o/[MV^+]_i)^2$. Using this equation, we calculate that under certain experimental conditions, e.g., Table I, entry 5, equilibration would require inward translocation of as much as 27% of the total initial external viologen. However, negligible translocation was measured. Second, $K_d = 6 \times 10^{-6}$ for MV^+ bound at the external interface of the DHP vesicle.[9] The calculated equilibrium concentration of the neutral diradical is extremely low, i.e., $[MV^0] = 10^{-11}$ M under the experimental conditions. To account for the net rate of radiolabel exchange, the required rate constant for MV^0 transmembrane diffusion would be $k_i' = k_i[MV^+]_o/[MV^0]_o \approx 4 \times 10^3\ s^{-1}$. While this value is the same magnitude as the diffusion rate constants measured for the most mobile lipophiles, it is far greater than the rate constants for molecules with structural characteristics similar to those of viologens.[1b] For example, rate constants for quinones and hydroquinones are typically $k \approx 10\ s^{-1}$.[22] Additionally, diffusional rates are generally sensitive to the membrane bilayer phase, increasing dramatically upon passing through the phase transitions corresponding to side-chain melting.[1b,23] The rate constants cited above for other molecules were measured for vesicles in their fluid, or liquid crystalline, phase, whereas DHP vesicles are in their solid, or gel, phase at ambient temperatures. (The DHP major phase transition appears at 45 °C in Tris buffers, the media used in these experiments.[11]) Raman spectroscopic investigations of the alkyl chain C–H-coupled vibrational motions in DHP vesicles have been interpreted as indicating an unusually high degree of ordering.[11] This observation, which correlates with the relatively high phase transition temperature, suggests that the energetic barrier to transmembrane molecular diffusion is especially large for DHP membranes. Specifically, the disruption of alkyl chain packing accompanying the conformational dislocations required to reorient the viologen in the opposite bilayer leaflet implies a relatively large unfavorable contribution to the energy barrier. These two factors, i.e., phase differences and the high intrinsic stability of the DHP gel phase, should lower transmembrane diffusion in DHP by several orders of magnitude relative to phospholipid bilayer membranes. Thus, it is improbable that the diffusion of MV^0 can account for the measured rates of radiolabel exchange, and, consequently, MV^+ must be the diffusing species. This conclusion is supported by recent experiments demonstrating that transmembrane oxidation of photogenerated MV^+ by $Co(bpy)_3^{3+}$ ion occurs with a rate constant nearly identical to k_p under conditions where the MV^0 concentration is infinitesimal (S. Lymar, unpublished work).

Structural Factors Affecting Diffusion Rates. The most significant factors controlling ion permeabilities are the magnitude and distribution of electrostatic charge. Thus, the viologen dications and the MB^+ ion, in which the charge was localized upon quaternary nitrogen atoms, were impermeable, but the radical cations, containing a single charge delocalized over the heterocycle skeletal framework, diffused across the bilayers. These differences can be rationalized in terms of stronger forces of solvation and attraction to the highly anionically charged membrane interface[7,15] by the localized charges, which increase the potential barrier for bilayer penetration by desolvated cations.

Among the radical cations, permeabilities of MV^+ and C_6MV^+ were nearly identical (Table II). Apparently, the lower diffusion coefficient expected for the larger C_6MV^+ ion is compensated by its increased hydrophobicity,[24,25] lowering its intrinsic permeability barrier. The asymmetry observed when the uptake of radiolabeled C_6MV^+ by vesicles preloaded with MV^{2+} was compared to the uptake of radiolabeled MV^+ by C_6MV^{2+}-loaded vesicles (Table II) has its origins, at least in part, in the position of the redox equilibrium

$$C_6MV^+ + MV^{2+} \rightleftharpoons C_6MV^{2+} + MV^+$$

For DHP vesicle-bound viologens, the $C_6MV^{2+/+}$ reduction potential is about 30 mV greater than that for the $MV^{2+/+}$ ion.[9] The equilibrium position, therefore, lies to the left. As a consequence, C_6MV^+ ions diffusing across the bilayer will not distribute randomly with the internal MV^{2+} pool but will be in statistically higher concentration. Hence, the probability of their outward diffusion is greater, and the apparent net uptake rate will be lowered. This factor was not significant for the system measuring inward diffusion of MV^+ ion, because the translocated ion was effectively trapped by electron transfer to the C_6MV^{2+} pool. The lower rate for $C_{12}{}^{14}MV^+$ uptake in MV^{2+}-loaded vesicles cannot be ascribed to this effect, however, because the $C_{12}MV^{2+/+}$ reduction potential is about 30 mV *lower* than that for the $MV^{2+/+}$ ion.[9] Consequently, it must indicate a lower intrinsic permeability for the longer-chain analog, despite its great lipophilicity. Based upon discontinuous shifts occurring at $n = 12$ in various physical properties for the DHP-bound C_nMV^{2+} series, we have proposed that the longer-chain viologens form part of the bilayer structure, whereas the short-chain analogs are only interfacially bound.[5,9-11] The surprising result is that the intercalated ions are less mobile in the transverse direction. This effect is even more pronounced for the major fraction of bound $C_{16}MV^+$ ions, which do not undergo transmembrane diffusion on the timescales of these experiments (Figure 2).

Implications for Transmembrane Charge Separation. As described in the Introduction, viologen-mediated transmembrane reduction of MV^{2+} and short-chain C_nMV^{2+} ions is accompanied by translocation of a radical cation, whereas for $C_{16}MV^{2+}$, the major pathway is blocked unless membrane-permeating lipophilic ions are added to the medium.[1b,4-6] This differential behavior suggests that two distinct reaction pathways are expressed in these systems, one involving reduced viologens as diffusing redox carriers and the other involving transmembrane electron transfer accompanied by charge-compensating transverse diffusion of other, redox-inactive lipophilic ions. The diffusional properties of DHP-bound C_nMV^{2+} ions revealed in these studies are fully consistent with this interpretation. The radiolabeled exchange of short-chain C_nMV^+ ions between inner and outer aqueous phases clearly demonstrates that these ions are mobile across the DHP bilayer membrane. Furthermore, the exchange rates measured under conditions in which a *net* reduction of internal MV^{2+} by external dithionite occurred (Figure 1, curve 9) or in which the internal compartmented viologen was also reduced were too rapid to measure using these sampling techniques. The rates were sufficiently fast in comparison to net transmembrane redox rates[4,5] to support the concept that these reactions involve C_nMV^+ ions as redox carriers.

For the $C_{16}MV^+$ ion, the biphasic nature of radiolabel exchange (Figure 2) is consistent with other physical studies that indicate heterogeneous binding.[1a,5,7-11,26] Only about one-third of the bound $C_{16}MV^+$ was mobile with respect to diffusion through the bilayer, a value which corresponds to the fraction of $C_{16}MV^{2+}$ ions undergoing transmembrane reduction in the absence of added lipophilic ions.[5] The major fraction of $C_{16}MV^+$ was immobile with respect to diffusion through the DHP bilayer, implying the predominance of an electron transfer pathway in this system.

The remarkable observation presented in this study is that seemingly innocuous changes in dopant structure, i.e., lengthening a pendent carbon chain, can have profound effects upon their dynamical behavior in supramolecular assemblies, i.e., to the extent that entirely different reaction pathways are expressed.

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