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Optical measurement of aqueous potassium concentration by a hydrophobic indicator in lipid vesicles

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An assay was developed for K^+ in aqueous solution at neutral pH. The method was based on the change in optical absorbance of the hydrophobic indicator 7-(*n*-decyl)-2-methyl-4-(3′,5′-dichlorophen-4′-one)indonaphthl-1-ol (MEDPIN) in phospholipid vesicles. Formation of a ternary complex between a valinomycin-K^+ pair and the anionic form of MEDPIN in the bilayer resulted in an absorption band at 584 nm. K^+ concentration was determined by monitoring the MEDPIN absorbance at 584 nm and MEDPIN quenching of lissamine rhodamine B sulfonylphosphatidylethanolamine (L-RhB-PE) fluorescence by an energy-transfer mechanism. Both the fluorescence intensity and lifetime of L-RhB-PE decreased by more than 25% upon addition of 50 mM K^+. Kinetic studies using stopped-flow photometry showed a single-exponential reaction of MEDPIN and valinomycin in vesicles with aqueous K^+ (maximum rate 1.7 s^{-1}) that was dependent upon [valinomycin] and [K^+]. The lipid surface charge was shown to influence the ratio of anionic to neutral MEDPIN at constant pH, and to alter the sensitivity of MEDPIN absorbance to aqueous [K^+]. A 1:20 neutral/negative lipid mole ratio was optimal for K^+ detection at pH 7.4. Spectroscopic and kinetic data suggest that the optical response of MEDPIN to K^+ involves the formation of a ternary complex between K^+, valinomycin and MEDPIN.

1. Introduction

The serum K^+ concentration is an important indicator of the physiological and clinical state of a patient. Currently available methods for K^+ measurement are time-consuming and require blood samples to be drawn. A practical optical method that provides bedside analysis would be beneficial to both patient and physician. We previously described a simple method for the colorimetric detection of the alkali metals potassium and sodium using an indicator dye [1]. The procedure was based on ion-pair solvent extraction using an ionophore and the nonpolar dye 7-(*n*-decyl)-2-methyl-4-(3′,5′-dichlorophen-4′-one)indonaphthl-1-ol (MEDPIN). Cation selectivity was achieved by use of an ion-selective ionophore. The reaction of MEDPIN with the ionophore-ion complex resulted in a large shift in the MEDPIN absorption spectrum that was dependent on aqueous cation concentration.

The previous studies, using MEDPIN in an organic solvent, required a two-phase system that had a slow reaction time due to diffusion of the cation over large distances. The two-phase system was also not amenable to kinetic studies of the rate and mechanism of the cation interaction with ionophore and MEDPIN, nor could it be used to develop a fluorescence quenching assay for K^+ using an energy-transfer mechanism. Phospholipid vesicles (liposomes) can serve as a dispersed solvent for hydrophobic molecules such as MEDPIN. Vesicles can be formed with various sizes and properties [2], used as the medium for stopped-flow

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kinetic studies to define reaction mechanisms [3], and used for energy-transfer measurements that couple the fluorescence of a nonselective indicator fluorophore with the change in indicator absorption [4].

We describe here the detection of K^+ using the colorimetric indicator MEDPIN in phospholipid vesicles. The reaction was made specific with the ionophore valinomycin. The anionic form of the MEDPIN molecule forms a stable ternary complex with the valinomycin/K^+ pair in the phospholipid bilayer. The MEDPIN optical response to changes in K^+ concentration was optimized by variation of the liposome surface charge. Kinetic studies were performed by making use of the spectroscopic changes in liposome-bound MEDPIN in response to changes in aqueous K^+ and pH. Energy transfer between a fluorescently labeled phospholipid and the anionic form of MEDPIN was used to develop a fluorescence assay for detection of K^+ concentration.

2. Materials and methods

2.1. Materials

MEDPIN was a generous gift from Dr. Steve Charlton (Miles Laboratories, Inc., Elkhart, IN). Valinomycin was obtained from Sigma (St. Louis, MO). Chloroform solutions of egg phosphatidylcholine (EPC), egg phosphatidylglycerol (EPG) and lissamine rhodamine B sulfonylphosphatidylethanolamine (L-RhB-PE) were purchased from Avanti Polar Lipids (Birmingham, AL). Dimethyldioctadecylammonium bromide (DDAB) was obtained from Kodak (Rochester, NY). All other chemicals were reagent grade.

2.2. Vesicle preparation

Sonicated unilamellar vesicles (SUV) were prepared by bath sonication using a Laboratory Supplies sonicator (Hicksville, NY). Vesicles were prepared with lipid compositions of EPC, EPC : EPG (1 : 1 mole ratio), EPC : DDAB (1 : 1 mole ratio) and EPC : EPG (20 : 1 mole ratio). Stock MEDPIN or L-RhB-PE solutions in chloroform were added to lipid solution in chloroform and rotatory evaporated under N_2. The dried MEDPIN-lipid mixture was suspended in 25 mM Tris-Hepes buffer at pH 7.45 and sonicated under N_2 for 1 h at 23° C. Sonicated vesicles were centrifuged in a Beckman L3-50 ultracentrifuge (Palo Alto, CA) for 1 h at 35000 rpm in an SW 50.1 swinging-bucket rotor. The upper 60% of the supernatant in each tube was retained. Valinomycin was added from an ethanolic stock solution (25 mg/ml) immediately before experiments. Vesicles were sized using a Coulter N4 submicron particle analyzer (Hialeah, FL). Vesicles containing L-RhB-PE, MEDPIN, or L-RhB-PE + MEDPIN had a diameter of 63 ± 22 nm (mean $\pm$ S.D.).

2.3. Absorption measurements

Absorption spectra were recorded with a Hewlett Packard model 8452A diode array spectrophotometer (Palo Alto, CA). Spectra were taken at room temperature in quartz cells of 1 cm path length.

2.4. Kinetic studies

Kinetic measurements were performed on a Hi-Tech Scientific SF-51 stopped-flow apparatus with temperature control (Salisbury, U.K.). The instrument dead time was less than 2 ms. A 0.075 ml aliquot of lipid vesicle suspension was mixed with buffered solutions at specified K^+ or pH to give K^+ or pH gradients. A tungsten-halogen lamp and a single-grating monochromator set at 584 ± 1 nm were used as the excitation source. The time course of light transmittance (1 cm path length) was measured with a photomultiplier and interfaced to a Digital Equipment Corp. MINC/23 computer (Maynard, MA) for data storage and analysis.

2.5. Energy-transfer studies

Steady-state fluorescence spectra and lifetimes were measured on an SLM Instruments 48000 fluorometer (Urbana, IL). To eliminate inner filter effects in energy-transfer studies, measurements were made using concentrations of MEDPIN and

L-RhB-PE below 2 μM. Measurements of the fluorescence lifetime of L-RhB-PE were carried out by phase-modulation fluorometry at an excitation wavelength of 572 nm. Lifetimes were measured at 20 and 50 MHz modulation frequencies using glycogen in water as a zero-lifetime reference solution. Fluorescence was detected through a Schott Optical Glass OG 590 cut-on filter (Duryea, PA) for lifetime and energy-transfer measurements.

3. Results

Detection of K^+ is based on its solvent extraction by an ionophore from the aqueous to the lipid phase. MEDPIN and valinomycin are soluble in the lipid vesicles and insoluble in the aqueous phase. The phenolic hydroxy group of MEDPIN is deprotonated by a high aqueous pH, and by interaction with a valinomycin-K^+ complex in the organic phase [1].

Fig. 1 (upper panel) shows the structure of MEDPIN and the effect of solution pH on the absorption spectra of MEDPIN in neutral PC vesicles. The spectra show an increase in the 584 nm absorption band with increasing pH, with two distinct absorption maxima at 464 and 584 nm. The spectra have an isosbestic point at 497 nm, indicating the presence of only two forms of the MEDPIN molecule. The band at 464 nm was attributed to the neutral form of MEDPIN and that at 584 nm to the anionic species. Previous data obtained using octanol showed two absorption maxima at 472 and 594 nm with an isosbestic point at 497 nm [1]. The difference in position of the MEDPIN absorption peaks in octanol vs. lipid vesicles is attributed to the different solvent polarity and dielectric constants. The term 'shifted' will be used to denote an increase in absorbance at the 584 nm band of MEDPIN.

Fig. 1 (lower panel) demonstrates that the MEDPIN absorption spectrum is sensitive to the aqueous K^+ concentration. In the absence of K^+, valinomycin has no effect on the MEDPIN spectrum (not shown). MEDPIN (40 μM) and valinomycin (120 μM) in negative vesicles (1 : 1 EPC : EPG, 4 mM) show no absorbance band at

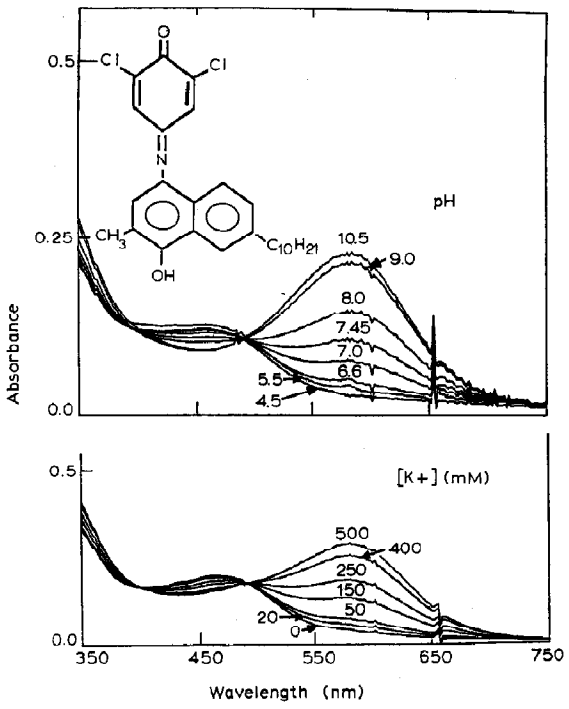

Fig. 1. (Upper) pH dependence of the absorption spectra of MEDPIN in vesicles. Spectra were obtained using 2 mM EPC vesicles containing 15 μM MEDPIN. Buffers consisted of 10 mM potassium hydrogen phthalate/NaOH (pH 4–6), 25 mM Tris-Hepes (pH 6–9), and 10 mM lysine-NaOH (pH > 9). (Lower) K^+ dependence of the absorption spectra of MEDPIN in vesicles containing valinomycin. EPG : EPC (1 : 1) vesicles were used (4 mM) containing 40 μM MEDPIN and 120 μM valinomycin in 25 mM Tris-Hepes buffer at pH 7.45. K^+ was added in 25-μl aliquots from a 2 M KCl stock solution. These and all subsequent experiments were performed at 23° C.

584 nm in a buffered solution at pH 7.45 in the absence of K^+. Small aliquots of a 2 M KCl solution were added to give final K^+ concentrations of 0–500 mM. With increasing K^+ concentration, there was a shift in the absorbance peak of MEDPIN from 464 to 584 nm. The intensity of the absorbance at 584 nm was dependent upon the aqueous K^+ concentration. There was an isosbestic point at 497 nm. No shift was observed when KCl was replaced by 100 mM NaCl, 100 mM LiCl, or in the absence of valinomycin at 100 mM KCl (not shown). 100 mM CsCl showed a shift that was 36% of that seen for K^+ which confirms the valinomycin cation selectivity se-

quence, $K^+ > Cs^+ \gg Na^+$ and Li^+, obtained using other techniques [5,6]. Results in dispersed solvents are thus similar to those obtained in the two-phase system [1]. The spectrum of MEDPIN (100 μM) sonicated without lipid showed a single absorption band at 270 nm that was not affected by 500 mM K^+ or pH (pH 6.6–9.0).

3.1. *Effects of surface charge*

Lipid vesicles with positive and negative surface charge were used to determine the sensitivity of the MEDPIN absorption shift to the surface charge of the vesicles. All vesicles were prepared with a MEDPIN/lipid mole ratio of 1:100. Neutral vesicles were made with EPC, negative vesicles with EPC and EPG (1:1 mole ratio), and positive vesicles with EPC and DDAB (1:1 mole ratio). Fig. 2 (upper) shows the dependence of the MEDPIN absorbance spectrum, expressed as the ratio of absorption at 584 nm to that at 468 nm, in vesicles with different surface charge. The surface charge of the membrane markedly influences the apparent pK_a value of MEDPIN. This result is in agreement with previous reports showing that the vesicle surface charge strongly influences the apparent pK_a value of membrane-bound indicator dyes containing a phenolic hydroxy group [7,8]. The negatively charged liposomes have the lowest absorbance at 584 nm and undergo the least conversion to the anionic form with variations of pH in the physiological range (pH 7.4).

The ratio of the absorbance of the anionic form to the neutral species of MEDPIN as a function of K^+ concentration at pH 7.45 is plotted in fig. 2 (lower). The positively charged liposomes have shifted the MEDPIN to the anionic form, and there is no further shift with K^+ addition. Neutral liposomes show a saturable absorbance ratio with increasing K^+ concentration. Sensitivity to $[K^+]$ between 0 and 50 mM is highest with these liposomes. Negatively charged vesicles show a linear response between 0 and 500 mM K^+ and display the least anionic MEDPIN at neutral pH in the absence of K^+.

Both neutral and negatively charged vesicles have desirable properties as carriers of MEDPIN and valinomycin. Neutral vesicles show a color change that is more sensitive to K^+ concentrations between 0 and 50 mM than observed in the negatively charged vesicles. However, MEDPIN was shifted by more than 50% toward its anionic form at serum pH, resulting in sensitivity of the absorption spectrum to small shifts in pH. In negatively charged vesicles, MEDPIN was in the uncharged form at serum pH and the absorption spectrum was not affected by small pH changes, however, the system was not as sensitive to low K^+ concentrations. To compromise, vesicles were prepared with a 1:20 mole ratio of EPG to EPC for further kinetic and energy-transfer experiments.

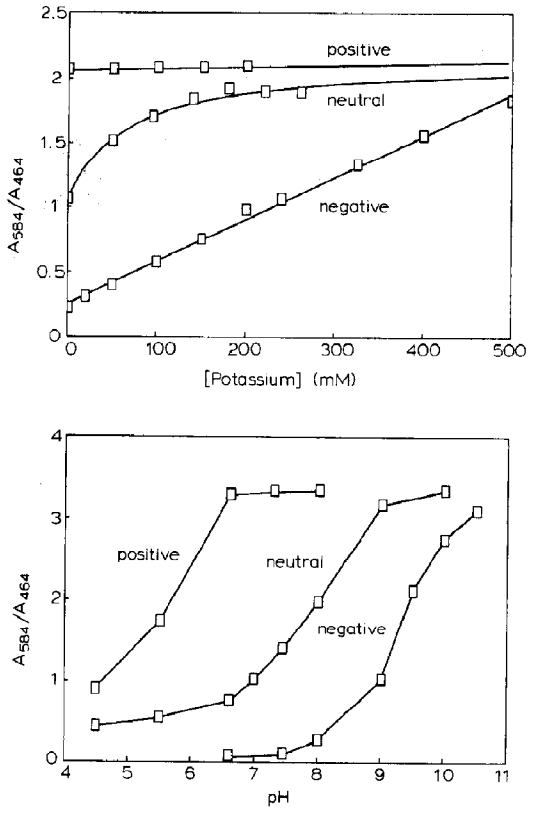

Fig. 2. (Upper) Dependence of the apparent pK_a of MEDPIN on the surface charge of the carrier vesicles. Positively charged, neutral and negatively charged vesicles were prepared from EPC:DDAB (1:1), EPC and EPC:EPG (1:1), respectively. Solutions contained 4 mM total lipid and 40 μM MEDPIN in the buffers given in the legend to fig. 1. (Lower) Dependence of the absorption of the anionic to neutral form of MEDPIN on K^+ concentration. Solutions were the same as in the upper panel with addition of 120 μM valinomycin. pH was 7.45.

3.2. Kinetic studies

Kinetic studies were performed to define the rates and reaction mechanism of the MEDPIN absorption change. The time courses of K^+- and H^+-induced MEDPIN absorption shifts, as MEDPIN is converted from its neutral to anionic form, are shown in fig. 3. Rapid mixing of the MEDPIN-labeled vesicles with K^+ resulted in a decrease in transmittance at 584 nm. The decrease had a single-exponential time course. The half-time of the decrease was a function of the K^+ concentration. There was no change in transmittance within the stopped-flow instrument dead time, indicating the absence of a MEDPIN reaction prior to the rate-limiting transmittance decrease (see section 4). Exposure of MEDPIN-labeled vesicles to a 1.55 pH unit inward proton gradient resulted in a rapid shift of MEDPIN to its anionic form. As shown in fig. 3, there is an 'instantaneous' transmittance decrease because the MEDPIN reaction was completed within the 2 ms dead time of the instrument.

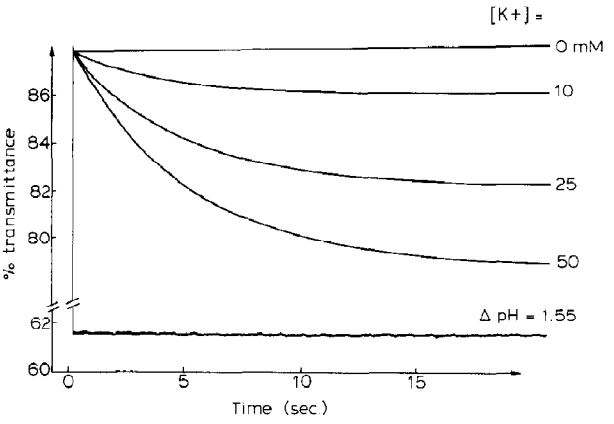

Fig. 3. Time course of MEDPIN transmittance in response to rapid addition of K^+ and change in external pH. EPG:EPC (1:20) vesicles were used containing 2 mM total lipid, 20 μM MEDPIN and 60 μM valinomycin in pH 7.45 buffer. This solution was mixed in a 1:1 ratio with a pH 7.45 buffer containing varying concentrations of KCl. Single-exponential functions are fitted through the data; time constants are summarized in table 1. The % transmittance at 584 nm was recorded. Solution pH was changed by mixture of vesicles with a pH 10.5 buffer to give a final pH of 9.

The dependence of the single-exponential time constant on valinomycin and K^+ concentration is summarized in table 1. The initial rate (reciprocal exponential time constant) of the transmittance change increases and becomes saturated with increasing concentrations of valinomycin in the presence of a limiting amount of MEDPIN and K^+ (100 mM). No change in transmittance was observed without valinomycin or in the absence of K^+. The rate of transmittance decrease also saturates with increasing K^+ concentration when MEDPIN and valinomycin concentration were kept constant. Mechanistic interpretations for these findings are discussed below.

Table 1

Kinetics of the MEDPIN signal in response to rapid K^+ addition

MEDPIN/valinomycin/K^+ reaction rate in lipid vesicles. EPG:EPC (1:20) was used to make vesicles containing 2 mM total lipid and 20 μM MEDPIN in pH 7.45 buffer. Valinomycin was added from an ethanolic solution to the concentrations shown. K^+ in pH 7.45 buffer was used for K^+ gradients. Transmittance measurements were made at 584 nm. Reaction rates (s^{-1}) determined from reciprocal single-exponential time constants are given as means ± S.D. and the standard deviation was taken from seven experimental points.

	[Valinomycin] (μM)						
	20	40	60	80	120	160	220
[K^+]							
100 mM	0.16 ± 0.02	0.24 ± 0.01	0.50 ± 0.04	0.89 ± 0.04	1.35 ± 0.07	1.84 ± 0.05	1.66 ± 0.08
	[K^+] (mM)						
	10	20	40	60	80	100	
[Valinomycin]							
40 μM	0.19 ± 0.02	0.21 ± 0.01	0.238 ± 0.005	0.263 ± 0.001	0.267 ± 0.004	0.291 ± 0.004	
120 μM	0.64 ± 0.01	0.73 ± 0.02	0.93 ± 0.02	1.10 ± 0.01	1.234 ± 0.001	1.283 ± 0.001	

3.3. Development of a fluorescence assay for K^+

Energy transfer was used to couple the absorption shift of MEDPIN with the fluorescence emission of L-RhB-PE. An increase in K^+ concentration would result in an increase in MEDPIN absorption at 584 nm, causing quenching of L-RhB-PE fluorescence. This approach relies on the dipole interaction of a donor fluorophore with an acceptor absorber when the emission band of the donor overlaps the absorption band of the acceptor, and when both molecules are in close physical proximity [4,9,10]. When these conditions are met, the energy from a photon absorbed by the fluorescent donor can be transferred to the nonfluorescent acceptor. The reduction of the donor emission intensity or lifetime is measured.

The emission spectrum of L-RhB-PE incorporated into unilamellar vesicles is shown in fig. 4, together with the absorption spectrum of MEDPIN and the MEDPIN-valinomycin-K^+ complex. L-RhB-PE was chosen as the lipid-soluble fluorescent indicator because of the excellent overlap of its emission spectrum with the absorption spectrum of the anionic form of MEDPIN, and its high quantum yield. An estimate of the L-RhB-PE

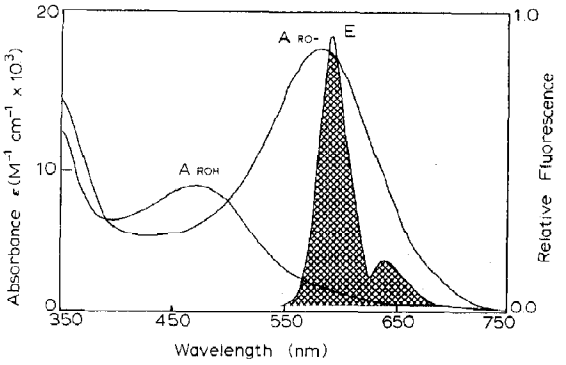

Fig. 4. Absorption spectra of neutral (A_{ROH}) and anionic (A_{RO^-}) MEDPIN and the emission spectra of L-RhB-PE in EPG : EPC (1 : 20) vesicles. L-RhB-PE was excited at 472 nm.

fluorescence decrease due to quenching by MEDPIN can be made using known parameters. The average donor-acceptor distance (R_0) at an energy-transfer (donor-quenching) efficiency of 50% can be calculated according to the equation [11],

$$R_0 = \left(JQ_0K^2n^{-4}\right)^{1/6}(9.79\times10^3)\ (\text{in Å})$$

where J denotes the donor-acceptor overlap in-

Table 2

Fluorescence quenching of L-RhB-PE by MEPDIN in lipid vesicles

EPG : EPC (1 : 20) was used to make vesicles containing 2 mM total lipid in pH 7.45 buffer. The molar ratio of L-RhB-PE to total lipid is 1 : 100. Vesicles with MEDPIN and valinomycin have 1 : 50 and 1 : 25 (MEDPIN : valinomycin) molar ratios, respectively. L-RhB-PE was excited at 472 nm.

Vesicle contents	Relative intensity					Lifetime (ns)		% decrease	
	pH 7.45			pH 9.0					
L-RhB-PE	1			0.98				−2	
L-RhB-PE + MEDPIN	0.46			0.27				−41	
	Relative intensity								
[K^+] (mM):	0	10	15	25	50	0	50	I(0–50)	(0–50)
L-RhB-PE	1	0.98	0.98	0.98	0.97	2.89	2.84	3.0	1.7
L-RhB-PE + valinomycin	0.95	0.95	0.94	0.94	0.93	2.87	2.86	2.1	0.4
L-RhB-PE + MEDPIN	0.44	0.43	0.42	0.42	0.41	1.46	1.37	6.8	6.2
L-RhB-PE + MEDPIN + valinomycin	0.43	0.39	0.37	0.34	0.31	1.46	1.09	27.9	25.3

tegral, calculated to be 1.85×10^{-13} cm^3/M for the MEDPIN/L-RhB-PE pair, K^2 a dipole-dipole orientation factor taken to be 2/3 for a random orientation, Q_0 the quantum yield of the donor in the absence of acceptor (0.94 for a rhodamine B derivative) and n the medium index of refraction (1.4 for lipid). From these values R_0 was calculated to be 55 Å using the absorption spectrum of the shifted MEDPIN and the emission spectrum of L-RhB-PE.

The intensity of L-RhB-PE in vesicles with and without MEDPIN at pH 7.45 and 9.0 is given in table 2 (upper). The molar ratio of L-RhB-PE to total phospholipid was 1 : 100 in all solutions. Vesicles with MEDPIN had a mole ratio of MEDPIN to total phospholipid of 1 : 50. L-RhB-PE-labeled vesicles showed an intensity decrease of 2% in going from pH 7.45 to 9.0. The presence of MEDPIN in the vesicles at pH 7.45 resulted in a 54% decrease in intensity because of the spectral overlap of rhodamine with the partially shifted MEDPIN. When the pH was increased from pH 7.45 to 9.0, vesicles containing L-RhB-PE and MEDPIN showed an additional 41% decrease in intensity due to the increased concentration of ionic MEDPIN. The fluorescence of the vesicles containing both MEDPIN and L-RhB-PE was 73% less than those containing L-RhB-PE alone (table 2).

The coupling of MEDPIN's spectral shift to the intensity of L-RhB-PE fluorescence was also shown with K^+ addition. Results from four possible combinations of L-RhB-PE and MEDPIN in liposomes are summarized in table 2 (lower). Fluorescence intensity decreases only in the presence of the anionic form of MEDPIN. Table 2 shows a decrease in L-RhB-PE fluorescence with increasing K^+ concentration due to formation of the MEDPIN-valinomycin-K^+ complex. Stepwise addition of 5 mM K^+ up to 50 mM gave a total decrease of 28% in signal intensity when L-RhB-PE, MEDPIN and valinomycin were present in the vesicles. K^+ addition to vesicles with L-RhB-PE and L-RhB-PE + valinomycin showed almost no change in fluorescence intensity. Addition of 50 mM K^+ to vesicles containing L-RhB-PE and MEDPIN with no valinomycin showed a 6.8% decrease in intensity.

Lifetime studies were performed to confirm that the decrease in L-RhB-PE fluorescence with increasing K^+ was due to energy transfer. An energy-transfer mechanism would predict parallel decrease in L-RhB-PE intensity and lifetime with increasing K^+ concentration. Inner filter effects were minimized in these experiments by using 1 μM L-RhB-PE and 2 μM MEDPIN, and an emission path length of 0.2 cm; the solution absorption was 0.025 at 574 nm. Table 2 (lower) shows the lifetimes and the percentage decrease in lifetime as K^+ is increased from 0 to 50 mM. The addition of MEDPIN to L-RhB-PE-labeled vesicles resulted in a 56% decrease in intensity and a 52% decrease in lifetime. The lifetime decrease upon addition of K^+ to vesicles containing L-RhB-PE, MEDPIN and valinomycin sample closely paralleled the decrease in L-RhB-PE fluorescence. These findings indicate an energy-transfer mechanism for the L-RhB-PE-MEDPIN interaction in vesicles.

4. Discussion

Lipid vesicles containing MEDPIN and valinomycin can be used to detect optically changes in aqueous K^+ concentration. The response of the MEDPIN complex to K^+ is sensitive, specific and rapid, indicating that lipid vesicles are a good dispersed solvent.

The apparent pK_a of MEDPIN in the membrane is sensitive to the vesicle surface charge. This results from a difference in the H^+ activity within the lipid bilayer and the solution due to the intramembrane potential profile. A positive lipid surface charge results in decreased interfacial/membrane H^+ activity, increased formation of anionic MEDPIN because of mass balance, and a consequent decrease in the apparent pK_a of MEDPIN. A negative lipid surface charge results in increased intramembrane H^+ activity, and by analogy, an increase in the apparent pK_a of MEDPIN. The alteration of vesicle composition and charge provides a useful approach to modify the cation sensitivity and optical response of MEDPIN for specific applications.

The kinetic results given in fig. 3 and table 2 show that the MEDPIN protonation/deprotona-

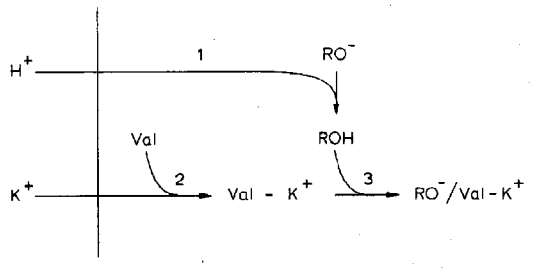

Fig. 5. Proposed mechanism for MEDPIN interaction with valinomycin and K^+ in phospholipid vesicles.

tion reaction occurs in less than 2 ms in response to a rapid change in aqueous pH. The MEDPIN-K^+-valinomycin reaction is slower and follows a single-exponential time course. No evidence was obtained for a faster component of the time course when K^+ was added to the MEDPIN-valinomycin liposomes. At a constant high concentration of K^+, the reaction rate increased and saturated with increasing valinomycin to a maximum rate of approx. 1.7 s^{-1}. At constant valinomycin concentration, the reaction rate increased and became saturated with increasing K^+. Taken together, these findings are consistent with the simple reaction mechanism shown in fig. 5. The MEDPIN protonation process (reaction 1) is extremely fast. The MEDPIN interaction with K^+ and valinomycin results from K^+-valinomycin binding (reaction 2), followed by a charge-transfer process (reaction 3) in which the ionizable MEDPIN proton is exchanged with the K^+-valinomycin complex. The saturation of the time constant with increasing K^+ or valinomycin indicates that reaction 3 is the rate-limiting process in the MEDPIN optical response.

Development of a fluorescence assay for K^+ was accomplished by coupling L-RhB-PE fluorescence with the MEDPIN absorption shift using a resonance energy-transfer technique. Aqueous K^+ concentrations between 0 and 50 mM were measured from the fluorescence intensity of L-RhB-PE. The K^+-shifted MEDPIN spectrum caused a parallel decrease in the L-RhB-PE intensity and lifetime of over 25%. These results provide a fluorescence intensity and lifetime approach for quantitation of K^+ concentrations in aqueous solutions.

In summary, the absorption spectrum of the lipophilic indicator MEDPIN is strongly influenced by the aqueous cation concentration when the cation is presented to organic phase MEDPIN by a cation-specific ionophore. The reaction can be accomplished in a two-phase system or using phospholipid vesicles as a dispersed solvent system. The reaction is rapid and sensitive, and can be made into a fluorescence assay by a coupled indicator technique using fluorescence energy transfer. These results have potential application in the clinical detection of cations in serum samples and in in vivo fiber-optic applications.

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