mobility change, and that additional alternative evidence should be sought.

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Thallous Ion Is Accumulated by Potassium Transport Systems in *Escherichia coli*†

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ABSTRACT: The accumulation of $^{204}Tl^+$ by *Escherichia coli* occurs primarily via either of two K^+ transport systems called Kdp and TrkA. Tl^+ influx is inhibited and Tl^+ efflux is stimulated by the addition of K^+ to the assay medium. Mutants defective in both the Kdp and TrkA systems accumulate little Tl^+. Uptake of triphenylmethylphosphonium, a lipid-soluble cation whose distribution is widely used to estimate the membrane electrical potential in bacteria, occurs to about the same extent in mutants that accumulate little Tl^+ as in strains that accumulate Tl^+ to high levels. These findings indicate that Tl^+ may be useful as a probe of bacterial K^+ transport systems but is not a reliable indicator of the membrane electrical potential in *E. coli*.

Interpretation of membrane-related phenomena in chemiosmotic terms requires a knowledge both of the concentration ratio of the ions or molecules involved and of the transmembrane electrical potential, $\Delta\psi$.[1] This potential can be estimated directly with microelectrodes in large cells, but in small cells or organelles indirect methods must be used

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[1] Abbreviations used: $\Delta\psi$, electrical potential difference across the cytoplasmic membrane (interior negative); Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Tris, Tris(hydroxymethyl)aminomethane; EDTA, (ethylenedinitrilo)tetraacetic acid; Mops, 3-(*N*-morpholino)propanesulfonic acid; $TPMP^+$, triphenylmethylphosphonium; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone.

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Table I: Bacterial Strains

strain	relevant genotype[a]	K^+ transport systems present	ref
FRAG-1	wild type	all	Epstein & Davies (1970)
TK1001	*kdpABC5 trkD1*	TrkA, TrkF	Rhoads et al. (1976)
TK2240	*trkD1 trkA*	Kdp, TrkF	*b*

[a] Only mutations affecting K^+ transport are listed. [b] Strain TK2240 is *kdp*$^+$ *nagA malA*$^+$, otherwise isogenic with strain TK405M (Rhoads & Epstein, 1978).

Table II: K^+ Transport System of *E. coli*[a]

system	K_m (mM)	V_{max} (μmol g^{-1} min^{-1})	regulation
Kdp	0.002	150[b]	repressible by K^+
TrkA	1.5	550	constitutive
TrkD	0.5	40	constitutive
TrkF	low rate, linearly proportional to K^+ concentration		constitutive

[a] Data are from Rhoads et al. (1976). [b] This value of the V_{max} is for fully derepressed cells.

(Rosen & Kashket, 1978). Useful indirect methods to measure $\Delta\psi$ include the equilibrium distribution of K^+ in the presence of valinomycin (Mitchell & Moyle, 1969), the equilibrium distribution of various permeant ions (Grinius et al., 1970), and changes in the absorbance or fluorescence intensities of dyes (Waggoner, 1976). These methods indicate the presence of large values of $\Delta\psi$ in energized mitochondria, chloroplasts, and gram-positive bacteria. A drawback of these indirect methods is that they cannot be used in gram-negative bacteria such as *Escherichia coli* unless the cells are first treated to increase the permeability of the outer membrane (Leive, 1968; West & Mitchell, 1972). Because such treatments may alter $\Delta\psi$, results obtained with the indirect methods in gram-negative bacteria may not be reliable indicators of the potential existing in untreated cells.

Bakker (1978) has proposed the use of Tl^+ distribution ratios to measure $\Delta\psi$ in *Streptococcus faecalis*, a gram-positive organism, and in *Escherichia coli*. The use of Tl^+ would have the distinct advantage of being taken up by untreated *E. coli*, so that perturbation of $\Delta\psi$ by permeabilizing treatments would be avoided. Earlier work had, however, suggested that Tl^+ uptake in *E. coli* was mediated by K^+ transport systems (Norris et al., 1976). We have examined Tl^+ accumulation in *E. coli* to determine its utility for measuring $\Delta\psi$. We here report independent experiments performed in two laboratories using somewhat different experimental protocols, showing that Tl^+ uptake occurs primarily via two K^+ transport systems. The equilibrium distribution of Tl^+ does not generally correspond to that observed for a permeant cation.

Experimental Procedure

Bacterial Strains. The strains of *E. coli* K-12 and their relevant properties are listed in Table I. The properties of the four K^+ transport systems of *E. coli* are summarized in Table II. The Kdp system is the only one not expressed constitutively; it is repressible and only expressed when cells are grown in medium of sufficiently low K^+ concentration (Rhoads et al., 1976).

Growth of Cells. Cells were grown at 37 °C in the minimal salts medium of Tanaka et al. (1967), containing 54 mM glycerol as the carbon source for the experiments of Figures 2 and 3. Cells were grown in K115 medium (Epstein & Kim, 1971), containing 10 mM glucose for the experiments of Figures 1 and 4–6. The Kdp system in strain TK2240 was derepressed by transferring cells to the same medium used for growth but with Na^+ replacing K^+ and allowing further growth for at least 2 h to achieve full derepression.

Uptake Measurements. For the experiments of Figures 1 and 4–6, cells were collected and washed by filtration, suspended at 9×10^9 cells/mL in 0.3 M Na–Hepes buffer (pH 7.5), and kept on ice until used. For uptake studies the suspension was diluted with 2 volumes of 0.3 M Na–Hepes (pH 7.5) containing 10 mM glucose and allowed to equilibrate at 30 °C for at least 5 min. Uptake was initiated by the addition of $^{204}Tl^+$ to 5 μM and about 0.05 μCi/mL unless otherwise stated. Samples were collected at suitable intervals on 0.45-μm pore size membrane filters (Millipore; type HA), washed with ice-cold 0.4 M glucose, 0.1 M Na–Hepes (pH 7.5), and 0.01 M $MgCl_2$, dried, and counted in a liquid scintillation counter. Intracellular concentrations were calculated by using a value of 2.5 μL/mg dry weight with dry weight estimated from turbidity measurements and a calibration curve (Rhoads et al., 1976). For the experiments of Figures 2 and 3, cells were treated with Tris and EDTA as described by Leive (1968). Cultures were harvested, washed twice with 0.1 M Tris-HCl (pH 8), suspended in the same buffer at 40 mg wet weight/mL, and incubated at 37 °C for 6 min. Then Tris–EDTA (pH 8) was added to 2 mM, and 2 min later $MgCl_2$ was added to 4 mM and the cells were harvested. The cells were washed twice in 20 mM Mops and 135 mM choline chloride (pH 7.2), suspended in this buffer at about 15 mg of protein per mL, and kept at 23 °C until used. For uptake assays the cells were diluted to 100–200 μg of protein per mL in an assay buffer containing 20 mM Mops (pH 7.2), 135 mM NaCl, and glucose (5 mg/mL) and incubated at 23 °C for 5 min. Uptake was initiated by adding $^{204}Tl^+$ to 10 μM and 0.5 μCi/mL. Samples of 0.2 mL were removed at intervals, collected on 0.45-μm pore size nitrocellulose filters (Matheson-Higgins Co., Inc., Woburn, MA), washed with 5 mL of 10 mM Mops and 200 mM choline chloride (pH 7.2), dried, and counted. $TPMP^+$ uptake was measured similarly, except that 2 μM tetraphenylboron was added to the assay buffer. [^{3}H]$TPMP^+$ was used at 10 μM and 1 μCi/mL, and filtration was done with 0.45-μm pore size cellulose triacetate filters (Gelman Metricel; Type GA-6). Protein was measured by a modification (Peterson, 1977) of the method of Lowry et al. (1951). Concentrations in the cells were calculated by using a value for internal volume of 2.26 μL/mg of cell protein.

Results

The rate of uptake of a substance which can cross the cell membrane barrier without interaction with a carrier is expected to be linearly dependent on the concentration of that substance. As shown in Figure 1A, this is true over the range of Tl^+ concentrations up to 0.1 mM. Likewise, over the lower range of concentrations, the steady-state level of Tl^+ accumulation is linearly proportional to external Tl^+ concentration (Figure 1B). At higher concentrations, however, the initial rate of uptake is no longer proportional to Tl^+ concentration (Figure 1C), suggesting uptake is by interaction with some component which limits uptake rate at higher Tl^+ concentrations.

Uptake of Tl^+ in mutants with different K^+ transport systems is shown in Figure 2. Tl^+ uptake is very extensive in strains FRAG-1 and TK1001, two strains in which the TrkA

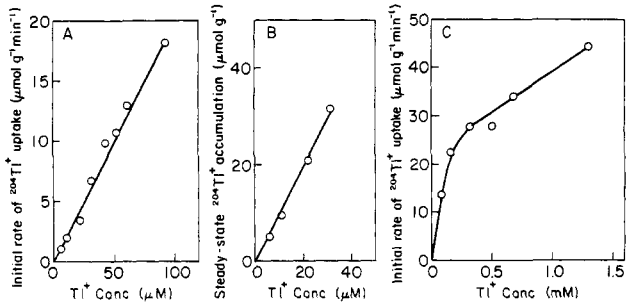

FIGURE 1: Relationship of initial rate and steady-state accumulation of $^{204}Tl^+$ as a function of Tl^+ concentration in strain TK1001. (A and C) Initial rate of uptake. (B) Steady-state accumulation after 25 min.

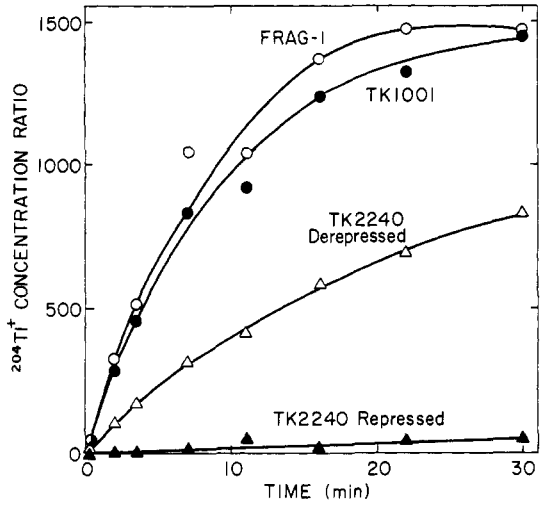

FIGURE 2: $^{204}Tl^+$ accumulation by Tris–EDTA-treated cells. Strains are (○) FRAG-1, (●) TK1001, (△) derepressed TK2240, and (▲) repressed TK2240.

system is present. Strain TK2240 takes up virtually no Tl^+ when grown in high-K^+ media to repress the Kdp system. When strain TK2240 is grown to K^+ limitation to derepress the Kdp system, Tl^+ uptake occurs but at a slower rate in these experiments than that in the two strains in which the TrkA system is present. Tl^+ uptake is energy dependent. Accumulation is abolished by an uncoupler, FCCP, at a concentration of 10 μM (data not shown). Other strains in which only the TrkD and TrkF systems are present accumulated little Tl^+, similar to the accumulation in repressed cells of strain TK2240 (data not shown). These results show that high levels of Tl^+ accumulation require the presence of either the TrkA or Kdp systems, suggesting that Tl^+ enters via these K^+ transport systems.

The accumulation of $TPMP^+$, a lipophilic cation considered to distribute itself in response to $\Delta\psi$ (Grinius et al., 1970), by the three strains studied here is shown in Figure 3. These results can be compared directly with those for Tl^+ in Figure 2 because both were done after the Tris–EDTA treatment needed to make the cells permeable to $TPMP^+$. Strain TK2240 in which the Kdp system is repressed takes up virtually no Tl^+ (Figure 2) but accumulates $TPMP^+$ at least as well as strains FRAG-1 and TK1001 (Figure 3) and likely forms a $\Delta\psi$ of similar magnitude. Thus, the inability to accumulate Tl^+ in the absence of TrkA or Kdp systems is not attributable to the absence of a $\Delta\psi$. Comparison of Figure 2 with Figure 3 shows that Tl^+ accumulation by the two strains with the TrkA system is about 1 order of magnitude higher than the respective $TPMP^+$ accumulation, whereas Tl^+ accumulation by repressed strain TK2240 is about 1 order of

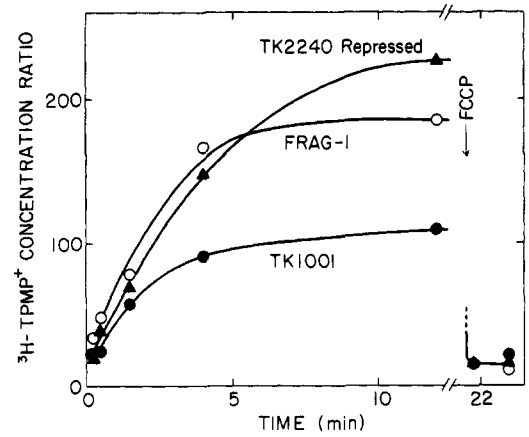

FIGURE 3: [^{3}H]$TPMP^+$ accumulation by Tris–EDTA-treated cells. At the arrow, FCCP was added to a final concentration of 10 μM. Strains are (○) FRAG-1, (●) TK1001, and (▲) repressed TK2240.

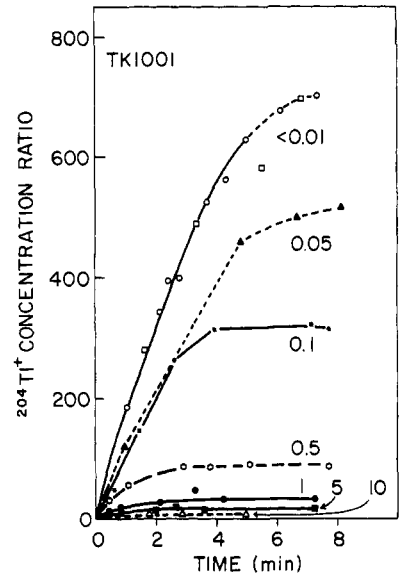

FIGURE 4: Inhibition of $^{204}Tl^+$ uptake by K^+ in strain TK1001. K^+ concentrations in millimolarity were (○) 0, (□) 0.005, (▲) 0.05, (*) 0.1, (○) 0.5, (●) 1, (■) 5, and (△) 10.

magnitude lower. Thus, Tl^+ and $TPMP^+$ accumulation ratios are not directly related in *E. coli*.

The accumulation of Tl^+ and $TPMP^+$ was not measured simultaneously in a double-layer experiment because tetraphenylborate was used as the lipophilic anion necessary to observe $TPMP^+$ uptake [our observations and those of others, i.e., Szmelcman & Adler (1976)]. Tetraphenylborate interacts with cations such as Tl^+, K^+, and choline to form insoluble salts (McClure & Rechnitz, 1966).

Uptake of Tl^+ is markedly inhibited by K^+, an effect supporting the idea that Tl^+ uptake occurs by K^+ transport systems. Inhibition in strain TK1001 is shown in Figure 4; both the initial rate of uptake and steady-state accumulation are progressively inhibited by K^+. At 10 mM K^+, Tl^+ uptake is completely inhibited. The amount of K^+ required to inhibit uptake is determined by the K^+ transport system present, as shown in Figure 5. Tl^+ uptake by the Kdp system in derepressed strain TK2240 is 50% inhibited by 3 μM K^+, a value close to the K_m of this system of 2 μM. Uptake by the TrkA system in strain TK1001 is 50% inhibited by 0.5 mM K^+, which is close to the K_m of this system of 1.5 mM. These results are consistent with competitive inhibition of Tl^+ uptake by K^+. The initial rate of Tl^+ uptake by strain TK2240 in these experiments was higher than that by strain TK1001, a

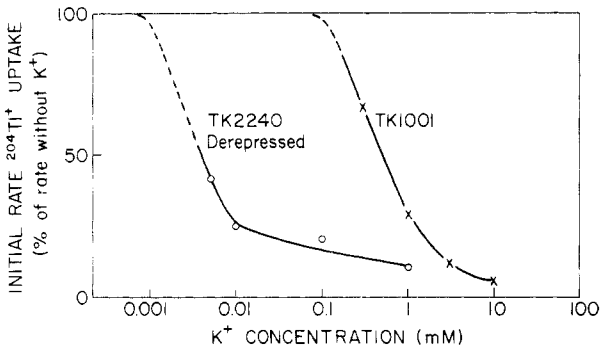

FIGURE 5: Inhibition of the initial rate of $^{204}Tl^+$ uptake by K^+. The rate is plotted as a percent of the rate in the absence of K^+. These rates were (○) derepressed strain TK2240, 0.05 μmol g^{-1} min^{-1}, and (×) strain TK1001, 0.21 μmol g^{-1} min^{-1}.

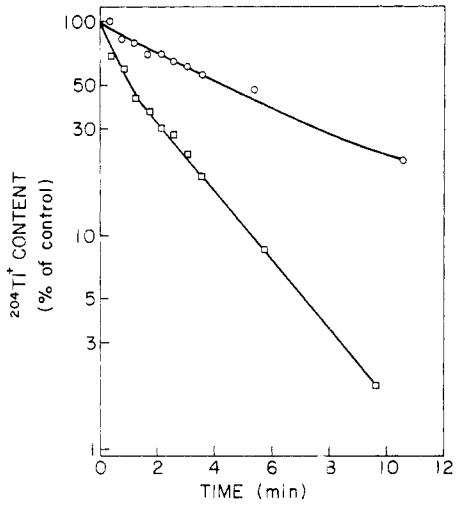

FIGURE 6: Effect of K^+ on $^{204}Tl^+$ efflux in strain TK1001. Cells were loaded for 20 min in 5 μM $^{204}Tl^+$ and then diluted 1:50 in 0.3 M Na–Hepes buffer (pH 7.5) with or without K^+. Samples were taken at the indicated times. (○) Control; (□) 20 mM K^+.

difference from the results of Figure 2 probably accounted for by the different buffers used in the two sets of experiments. The kinetics of inhibition by K^+ illustrated in Figure 5 confirm that Tl^+ uptake occurs via K^+ transport systems.

K^+ can also accelerate Tl^+ efflux. When cells of strain TK1001 which had accumulated Tl^+ to the steady state were diluted 50-fold in buffer containing no Tl^+, the rate of efflux in buffer containing 20 mM K^+ was about 3 times higher than when the buffer contained no K^+ (Figure 6). This stimulation is presumably due to exchange of Tl^+ for K^+ by the TrkA system (Rhoads & Epstein, 1978).

Discussion

The distribution of an ion across a membrane will be a reliable measure of $\Delta\psi$ only if its rate of movement by energy-coupled transport systems is much slower than its rate of passive movement across that membrane. It has been suggested that Tl^+ behaves as a permeant cation in erythrocytes (Skulskii et al., 1973) and in mitochondria (Melnick et al., 1976; Skulskii et al., 1978b). Because Tl^+ and K^+ have similar crystal and hydrated radii, the high permeability of the erythrocyte membrane to Tl^+, compared to K^+, has been attributed to specific and favorable interactions between Tl^+ and membrane ligands (Skulskii et al., 1978a). In erythrocytes, the distribution of Tl^+ is affected by the Na^+,K^+-ATPase, while in mitochondria Tl^+ inhibits K^+ movement across the membrane (Barrera & Gómez-Puyou, 1975). Thus, Tl^+ movement by energy-coupled mechanisms is significant in these systems.

Transport of Tl^+ by energy-coupled mechanisms is also seen in *E. coli*, as suggested by Norris et al. (1976). We find that (1) mutants lacking both the Kdp and TrkA systems fail to accumulate Tl^+ but form a large membrane potential as estimated by $TPMP^+$ accumulation, (2) Tl^+ uptake is inhibited by K^+ at concentrations corresponding to the K_m for K^+ uptake by the particular K^+ transport systems present, and (3) addition of K^+ stimulates the efflux of accumulated Tl^+. Recent studies of *S. faecalis* have shown that the presence of K^+ reduces the distribution ratio of Tl^+ (Bakker, personal communication).

The rate of Tl^+ movement by passive routes must be quite low, because only strains with either the Kdp or TrkA systems have high rates of Tl^+ uptake. Our data do not completely exclude high rates of passive movement of Tl^+, but, if they occur, they are masked by energy-coupled systems that excrete Tl^+. Excretion of Tl^+ could occur via the K^+/H^+ antiporter of *E. coli* (Brey et al., 1978). However, the conclusion is inescapable that movement by specific, energy-linked transport systems is at least comparable in magnitude to whatever passive movement occurs. Tl^+ movement is dictated by the kinetics and energetics of K^+ transport and by the presence of K^+ and other substrates of these systems. Direct comparison with $TPMP^+$ accumulation confirms this view: Tl^+ accumulation is at the mercy of K^+ transport systems (Figure 2), but $TPMP^+$ uptake is not (Figure 3). That $TPMP^+$ distribution ratios are unaffected by the particular K^+ transport system present is expected for a permeant ion but does not exclude the possibility that $TPMP^+$ is a substrate for other energy-linked transport systems and therefore may not be a reliable indicator of $\Delta\psi$.

Our findings establish that $^{204}Tl^+$, with a half-life of 3.9 years, is a substrate of the Kdp system. This isotope should be useful in studies of this system. The only previously known substrate of this system was K^+ (Rhoads et al., 1977), whose radioactive isotope, $^{42}K^+$, has an inconveniently short half-life of 12.4 h.

Acknowledgments

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Dependence of the Conformation of the Polar Head Groups of Phosphatidylcholine on Its Packing in Bilayers. Nuclear Magnetic Resonance Studies on the Effect of the Binding of Lanthanide Ions†

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ABSTRACT: Proton magnetic resonance spectra of vesicles of various sizes composed of egg phosphatidylcholine (PC) with varying concentrations of cholesterol differed in the apparent line width of the signal of the methylene protons of PC ($\Delta\nu_{1/2}$). They also varied in the extent of lanthanide-induced shifts of the ^{31}P and ^{1}H NMR signals of the corresponding nuclei of the polar head groups located on the outer surface of the vesicles ($\Delta\delta$). The differences in the lanthanide-induced shifts of the ^{31}P signals are fully accounted for by the ratio between the externally added lanthanide and the number of PC head groups available for interaction with the lanthanide ions. This was not the case for the changes in the ^{1}H NMR spectra. Here $\Delta\delta$ decreased with increasing $\Delta\nu_{1/2}$, suggesting that the packing of the PC paraffinic chains in the bilayer affects the conformation of the polar head groups; tightening of the packing probably results in a more extended conformation of the head groups. This conclusion is also supported by the larger effect lanthanides have on the ^{1}H chemical shift of the choline head groups on the outer surface of small unilamellar vesicles as compared to groups on the inner, tighter packed layer.

Small unilamellar phospholipid vesicles have been extensively used as models for biological membranes. Various techniques have been employed to study the mobility, viscosity, and local motions within the hydrophobic core of the bilayers and the dependence of these parameters on the composition of the liposomes (Bangham et al., 1974; Levine, 1972). Efforts have also been devoted to the investigation of surface properties of the model membranes, mainly the binding of cations to the membrane components. Thus, the binding of lanthanides, Ca^{2+}, and Mg^{2+} to phosphatidylcholine (PC)[1] vesicles has been investigated in terms of stoichiometry, apparent binding constants, and environmental conditions (Hauser et al., 1975, 1977). The affinity of cations for other phospholipids also has been measured, and a pronounced effect of negatively charged phospholipids has been demonstrated (Hauser et al., 1976a). Moreover, the effects of cation binding on both the conformation of the PC polar group (Hauser et al., 1976b) and the packing of the PC in the bilayer have been thoroughly studied (Hauser et al., 1975).

One question of importance, which has received relatively little attention, is how the surface properties of the membrane depend on the packing of the phospholipids in the bilayer. In a previous paper it has been shown that the polar head group exhibits a restricted flexibility, characterized by rapid transitions between two enantiomeric conformations (Seelig et al., 1977). Increasing the temperature resulted in a change in the average orientation of the polar head groups to a less extended conformation, with the *N*-methyl groups closer to the phosphorus atom (Gally et al., 1975). It is conceivable that any other process which leads to the loosening of the packing within bilayers might also result in a similar reduction of the distance between the *N*-methyl and the phosphate groups. The goal of the present work is to test this possibility.

Information on the conformation of the polar head groups of PC can be gained from the lanthanide-induced shifts of the NMR signals of various nuclei of these groups (Bystrov et al., 1971; Kostelnik & Castellano, 1972; Huang et al., 1974; Michaelson et al., 1974; Sears et al., 1976; Hauser, 1976; Hauser et al., 1976b). These measurements indeed reflect the conformation of those PC head groups to which the lanthanide is bound, which might differ from the conformation in the absence of bound ions (Yeagle et al., 1977; Brown & Seelig, 1977; Hauser et al., 1978).

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[1] Abbreviations used: PC, egg phosphatidylcholine; $[PC]_{tot}$, the total PC concentration in the dispersion; I_{in} and I_{out}, the intensities of the peaks of nuclei located on the inner and outer surfaces of the vesicles, respectively; $[PC]_{eff}$, the concentration of PC on the outer surface of the vesicles (equal to the multiplication of $[PC]_{tot}$ times the ratio of intensities $I_{out}/(I_{out} + I_{in})$); $\Delta\delta_H$ and $\Delta\delta_P$, the lanthanide-induced shifts of the signals of the polar groups of the phospholipid in the ^{1}H and ^{31}P NMR spectra, respectively; $\nu_{1/2}$, the full width at half-height of the ^{1}H NMR signal of the bulk methylene of PC.

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