J. Membrane Biol. 50, 241–255 (1979)

Anionic Detergents as Divalent Cation Ionophores across Black Lipid Membranes

Jonathan J. Abramson* and Adil E. Shamoo**

Department of Radiation Biology and Biophysics,
University of Rochester School of Medicine and Dentistry,
Rochester, New York 14642

Received 30 January 1979; revised 11 June 1979

Summary. Three ionic detergents commonly used in membrane-bound protein isolation and reconstitution experiments, SDS, cholate, and DOC, are shown to act as divalent cation ionophores when incorporated into black lipid membranes made from either oxidized cholesterol or a mixture of phosphatidylcholine and cholesterol (PC/cholesterol = 5:1 mg). At a concentration greater than or equal to 1 μM, SDS shows large selectivity differences between cations and anions and among the different cations tested (Ba^{2+}, Ca^{2+}, Sr^{2+}, Mg^{2+}, and Mn^{2+}). Deoxycholate and cholate at concentrations greater than 4×10^{-4} M and 10^{-3} M, respectively, also act as divalent cation ionophores. The selectivity sequence measured for these two detergents is evidence for a strong ionic interaction between the divalent cation and the anionic charged groups on the detergent. In the case of cholate, the conductance depends on the third or fourth power of the cholate concentration and shows a linear dependence on $CaCl_2$ concentration. The conductance for deoxycholate depends on the sixth or seventh power of the DOC concentration and is also linearly dependent on the $CaCl_2$ concentration. In an oxidized cholesterol black lipid membrane in the presence of 5 mM $CaCl_2$, small concentrations of $LaCl_3$ (< 1 μM) inhibit the ionophoric activity of each of the detergents tested. Evidence is presented to show that this inhibitory effect is a nonspecific effect on oxidized cholesterol BLM's, and is not due to a direct effect of La^{3+} on detergent-mediated transport.

The use of anionic detergents is important in the purification and reconstitution of many membrane-bound proteins involved in ion transport across biological membranes. Anionic detergents have been used in the purification and reconstitution of $(Na^+ + K^+)$-ATPase (Kyte, 1971; Goldin & Tong, 1974), in the reconstitution of the purple membrane from *Halobacterium halobium* cells (Racker & Stoekenius, 1974), and in the purification and reconstitution of $(Ca^{2+} + Mg^{2+})$-ATPase and its tryptic fragments into both planar and vesicular membrane systems

* *Present address:* Department of Physics, Portland State University, P.O. Box 751, Portland, Oregon 97207.

** *To whom reprint requests should be made:* Department of Biological Chemistry, University of Maryland School of Medicine, 660 W. Redwood Street, Baltimore, Maryland 21201.

0022-2631/79/0050-0241 $03.00

(MacLennan, 1970; Racker, 1972; Shamoo & MacLennan, 1974; Shamoo *et al.*, 1976; Stewart, MacLennan & Shamoo, 1976; Abramson & Shamoo, 1978; Shamoo, 1978).

To distinguish between the effects of the solubilized transport protein and the detergent used in the solubilization or reconstitution procedure, studies must be performed to measure the effects of these detergents. Although anionic detergents are widely used in the isolation of proteins and their reconstitution, only a small number of studies have appeared reporting the transport properties of anionic detergents on membranes (Seufert, 1965; Seufert, 1973; Ksenzhek, Omel'chenko & Koganov, 1974; Ksenzhek *et al.*, 1975; Antonov, Korepanova & Vladimirov, 1976). All of these studies deal with the effect of anionic detergents in the presence of monovalent cations. The effects of detergents on the conductivity and selectivity of membranes to divalent cations have not previously been dealt with. Our laboratory has a special interest in the effect of detergents on divalent cation conductance in black lipid membranes, since these detergents were used in the purification of proteins that possess ionophoric activity (Shamoo & MacLennan, 1974; Shamoo *et al.*, 1976; Stewart *et al.*, 1976; Abramson & Shamoo, 1978; Jeng, Ryan & Shamoo, 1978; Shamoo, 1978).

In this paper we study the effects of sodium dodecyl sulfate (SDS); the dihydroxy-bile salt, deoxycholate (DOC); and the trihydroxy-bile salt, cholate, on the conductivity and selectivity properties of black lipid membranes made from either oxidized cholesterol or a mixture of phosphatidylcholine and cholesterol in the presence of various divalent cations.

Materials and Methods

Oxidized cholesterol is prepared by the method of Tien, Carbone, and Davidowicz (1966). Egg lecithin (phosphatidylcholine) is purchased from Supelco. Cholesterol, cholic acid, deoxycholic acid, and histidine are purchased from Sigma. HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid) is purchased from Calbiochem. Sodium dodecyl sulfate (Sequanal Grade) is purchased from Pierce. Cholesterol, deoxycholic acid, and cholic acid are twice recrystallized (MacLennan, 1970). The PC/cholesterol membrane-forming solution is made by adding 5 mg egg lecithin and 1 mg twice recrystallized cholesterol to 500 μl decane.

Conductance Measurements

Black lipid membranes are made by using either PC/cholesterol (5:1 mg) or oxidized cholesterol. Membranes are formed across a 1-mm diameter hole in a Teflon cup in the presence of salt solutions on both sides of the membrane. Membranes are formed with

a tapered Teflon brush. Calomel electrodes are used both to apply a given voltage and to measure the current passing through the membrane. Triangular voltage pulses of amplitude ± 50 mV, frequency 1 cycle/min, are applied across the membrane by using a function generator as the voltage source. The current through the BLM is amplified by a current amplifier in series with the membrane (Keithley model 427 current amplifier). Solutions are buffered with either 5 mM HEPES, Tris at pH 7.3, or with 5 mM histidine, HCl at pH 7.3.

To measure the relative cation *vs.* anion selectivity ($P_{Ca^{2+}}/P_{Cl^-}$), a $CaCl_2$ gradient is applied across the membrane. As the conductance increases, when the detergent is added in equal amounts to both sides of the membrane, the voltage intercept at zero current remains unchanged. This voltage intercept at zero current is related to the relative permeability ($P_{Ca^{2+}}/P_{Cl^-}$) using a modified Goldman equation (Shamoo & Goldstein, 1977). In a similar manner, the relative divalent cation permeability can be determined. Equal concentrations of two different divalent cation chloride salts are placed on opposite sides of a BLM. The detergent is added and the voltage intercept at zero current is recorded as the conductance increases. By knowing both this voltage intercept and the value of $P_{Ca^{2+}}/P_{Cl^-}$, the relative divalent cation permeability can be calculated (Shamoo & Goldstein, 1977). In some experiments the relative cations *vs.* anion permeability is measured by using a gradient of a divalent salt other than Ca^{2+} (e.g., a $MgCl_2$ gradient is used to measure $P_{Mg^{2+}}/P_{Cl^-}$). All solutions are made with distilled deionized water.

Results

In Table 1, we show the mean voltage intercept and the standard error along with the calculated relative permeability for an oxidized cholesterol black lipid membrane (BLM) in the presence of sodium dodecyl sulfate (SDS), cholate, and deoxycholate (DOC). Solutions were buffered at pH 7.3 with either histidine or HEPES buffer. Under most situations HEPES is the preferred buffer. Its association with divalent cations is negligible (Good *et al.*, 1966). As can be seen for those experiments in which independent measurements were made with histidine buffer and HEPES buffer, the voltage intercepts in the presence of histidine are higher than those in which only HEPES buffer is present. These results indicate that histidine binds Ca^{2+} greater than Cl^- and that histidine binds more of the other divalent cations tested (Ba^{2+}, Sr^{2+}, Mn^{2+}, and Mg^{2+}) than it binds Ca^{2+}. This is in agreement with the previous observation that Mn^{2+} binds to histidine but not to HEPES (Good *et al.*, 1966; Sillén & Martell, 1971). The binding of divalent cations to histidine makes it an undesirable buffer in which to measure divalent cation permeability.

In measuring the permeability of an oxidized cholesterol BLM with the detergent SDS, it is found that in the presence of HEPES buffer there are large increases in capacitance observed. The capacitance of

Table 1. Mean voltage intercept (in mV) ±SE and calculated relative permeabilities of an oxidized cholesterol BLM in the presence of different anionic detergents[a]

		SDS		Cholate		DOC	
		Voltage intercept (mV)	Relative perm.	Voltage intercept (mV)	Relative perm.	Voltage intercept (mV)	Relative perm.
2:1 $CaCl_2$	Hist. buffer	5.42±0.60 (n=3)	3.51	3.06±0.51 (n=4)	1.83	4.56±0.48 (n=4)	2.68
Perm=$P_{Ca^{2+}}/P_{Cl^-}$	HEPES buffer			2.00±0.27 (n=4)	1.46	4.47±0.34 (n=8)	2.61
$CaCl_2$ *vs.* $BaCl_2$	Hist. buffer	−1.38±0.13 (n=2)	0.88	3.13±0.30 (n=6)	1.38	3.58±0.32 (n=6)	1.41
Perm=$P_{Ca^{2+}}/P_{Ba^{2+}}$	HEPES buffer			1.75±0.35 (n=7)	1.21	1.50±0.33 (n=6)	1.15
$CaCl_2$ *vs.* $SrCl_2$	Hist. buffer	−0.38±0.14 (n=4)	0.97	3.13±0.19 (n=6)	1.38	5.30±0.37 (n=5)	1.68
Perm=$P_{Ca^{2+}}/P_{Sr^{2+}}$	HEPES buffer			1.68±0.18 (n=7)	1.20	1.60±0.13 (n=5)	1.16
$CaCl_2$ *vs.* $MnCl_2$	Hist. buffer	3.63±0.44 (n=6)	1.40	−0.29±0.09 (n=7)	0.97	−1.00±0.33 (n=6)	0.91
Perm=$P_{Ca^{2+}}/P_{Mn^{2+}}$	HEPES buffer			−1.91±0.15 (n=8)	0.82	−2.19±0.13 (n=8)	0.82
$CaCl_2$ *vs.* $MgCl_2$	Hist. buffer	3.00 (n=2)	1.32	3.88±0.40 (n=6)	1.50	6.20±0.57 (n=5)	1.84
Perm=$P_{Ca^{2+}}/P_{Mg^{2+}}$	HEPES buffer			3.14±0.20 (n=7)	1.42	4.50±0.53 (n=6)	1.55
		$P_{Ba^{2+}} > P_{Sr^{2+}} > P_{Ca^{2+}} > P_{Mg^{2+}} > P_{Mn^{2+}}$ (hist buffer) Sequence (I)		$P_{Mn^{2+}} > P_{Ca^{2+}} > P_{Sr^{2+}} \geqq P_{Ba^{2+}} > P_{Mg^{2+}}$ (HEPES buffer) Sequence (III)		$P_{Mn^{2+}} > P_{Ca^{2+}} > P_{Ba^{2+}} \geqq P_{Sr^{2+}} > P_{Mg^{2+}}$ (HEPES buffer) Sequence (III)	

[a] Numbers in parentheses indicate the number of membranes and hence the number of measurements of the voltage intercept at zero current. The relative permeabilities are calculated using modified Goldman equation (Shamoo & Goldstein, 1977). Solutions are buffered with either 5 mM HEPES, Tris, pH 7.3, or with 5 mM histidine, HCl, pH 7.3 Equal concentrations of detergent are present on both sides of the BLM. In the first row, voltage intercepts measured in the presence of 10 mM $CaCl_2$ on one side of the BLM, and 5 mM $CaCl_2$ on the other side of the BLM. The relative permeability ($P_{Ca^{2+}}/P_{Cl^-}$) is higher for higher voltage intercepts. In all later entries, 5 mM $CaCl_2$ is on one side of the BLM and 5 mM of a different divalent cation chloride salt is on the opposite side of the membrane (e.g., $SrCl_2$). Higher voltage intercepts correspond to a higher relative calcium permeability (e.g., $P_{Ca^{2+}}/P_{Sr^{2+}}$ increases with increased voltage intercept). The sequence numbers referred to for each detergent tested are derived by Sherry on theoretical grounds (Sherry, 1969). Low sequence numbers correspond to weak electrostatic interaction between the divalent cation and fixed anionic sites on the detergent. High sequence numbers correspond to strong electrostatic interaction.

the already thinned BLM increases by as much as a factor of ten over a period of approximately 5 min. This effect is not observed when histidine buffer is used instead of HEPES buffer or when the BLM is made from PC/cholesterol instead of oxidized cholesterol. This large increase in capacitance cannot be explained in terms of further thinning of the BLM. We believe it is due to a surface effect at the interface between the BLM and the Teflon cup. Sodium dodecyl sulfate in the presence of HEPES buffer must cause the membrane to move away from the cup and cause the membrane to bow. This would cause an increase in the surface area of the BLM and explain the observed increase in capacitance. To avoid this phenomenon, all measurements with SDS using an oxidized cholesterol BLM were performed with a histidine buffer. From our measurements with cholate and DOC we know that the derived values for $P_{Ca^{2+}}/P_{Cl^-}$ and for $P_{Ca^{2+}}/P_{D^{++}}$ (where $D^{++}=Ba^{2+}$, Sr^{2+}, Mn^{2+} or Mg^{2+}) are all slightly overestimated when histidine is used as the buffer instead of HEPES. Therefore, we believe that, in the case of SDS, the relative permeabilities reported are all overestimated.

In Table 2 we show the mean voltage intercept and the calculated relative permeabilities with a PC/cholesterol BLM for the three anionic detergents tested. All solutions are buffered with 5 mM HEPES, Tris, pH 7.3. Because of technical reasons the divalent cation permeability was measured with respect to chloride in each of these experiments. Phosphatidylcholine/cholesterol (5:1 mg) in decane forms stable membranes; however, initially it is difficult to form stable membranes. To prevent diffusion of different salts across the hole in the Teflon cup, the membrane is initially formed with 5 mM of the divalent cation salt on both sides of the membrane. After the formation of stable membranes, a small volume of concentrated salt is added to one side of the bilayer. The solution is stirred, and the voltage intercept is measured. After breakage, the membrane is easily reformed. The voltage intercept at zero current after the membrane breaks is also carefully monitored in order to insure that the salt gradient has not significantly dissipated. Calculations of the relative divalent cation permeability using the gradient method yields the same results as the calculated permeability when different divalent salts are on opposite sides of the BLM.

There is a significant difference in the calculated permeabilities when comparing oxidized cholesterol and PC/cholesterol membranes. In particular, there is a large shift in the relative permeability of Mn^{2+}. For both cholate and DOC, in an oxidized cholesterol BLM the membrane shows a high permeability to Mn^{2+}, while in a PC/cholesterol BLM

Table 2. Mean voltage intercept (in mV) ±SE and calculated relative permeabilities of a PC/cholesterol (5:1 mg) BLM in the presence of different anionic detergents[a]

	SDS		Cholate		DOC	
	Voltage intercept (mV)	Relative perm.	Voltage intercept (mV)	Relative perm.	Voltage intercept (mV)	Relative perm.
2:1 $CaCl_2$ Perm $= P_{Ca^{2+}}/P_{Cl^-}$	4.94 ± 0.24 ($n=8$)	3.00	3.19 ± 0.33 ($n=4$)	1.89	2.42 ± 0.29 ($n=6$)	1.59
2:1 $BaCl_2$ Perm $= P_{Ca^{2+}}/P_{Ba^{2+}}$	6.19 ± 0.31 ($n=2$)	0.64	2.79 ± 0.16 ($n=6$)	1.10	2.96 ± 0.33 ($n=7$)	0.89
2:1 $SrCl_2$ Perm $= P_{Ca^{2+}}/P_{Sr^{2+}}$	4.71 ± 0.32 ($n=6$)	1.07	1.05 ± 0.54 ($n=10$)	1.56	1.28 ± 0.46 ($n=13$)	1.26
2:1 $MnCl_2$ Perm $= P_{Ca^{2+}}/P_{Mn^{2+}}$	5.88 ± 0.23 ($n=6$)	0.72	-0.22 ± 0.59 ($n=8$)	1.96	0.22 ± 0.25 ($n=12$)	1.53
2:1 $MgCl_2$ Perm $= P_{Ca^{2+}}/P_{Mg^{2+}}$	3.18 ± 0.37 ($n=5$)	1.59	1.93 ± 0.54 ($n=5$)	1.31	1.92 ± 0.39 ($n=6$)	1.11
	$P_{Ba^{2+}} \geqq P_{Mn^{2+}} > P_{Ca^{2+}} \geqq P_{Sr^{2+}} > P_{Mg^{2+}}$ Sequence (II)		$P_{Ca^{2+}} \geqq P_{Ba^{2+}} > P_{Mg^{2+}} > P_{Sr^{2+}} > P_{Mn^{2+}}$ Sequence (IV)		$P_{Ba^{2+}} > P_{Ca^{2+}} \geqq P_{Mg^{2+}} \geqq P_{Sr^{2+}} > P_{Mn^{2+}}$ Sequence (II)	

[a] Numbers in parentheses indicate the number of membranes and hence the number of measurements of the voltage intercept at zero current. The relative permeabilities are calculated using a modified Goldman equation (Shamoo & Goldstein, 1977). All solutions are buffered with 5 mM HEPES, Tris, pH 7.3. Equal concentrations of detergent are present on both sides of the BLM. In all entries the voltage intercept at zero current is measured in the presence of a 2:1 salt gradient (e.g., 10 mM $SrCl_2$ on one side of the BLM, 5 mM $SrCl_2$ on the other side of the BLM). The relative permeability shown is as described in the leftmost column. Higher voltage intercepts correspond to a higher permeability for the divalent cation as compared to chloride. Sequence numbers are described in the caption to Table 1.

the membrane shows a very low permeability to Mn^{2+}. The surface charge distribution of the membrane as defined by the lipid appears to strongly influence the selectivity of the conducting unit.

The concentration of SDS used in these experiments is approximately 10–20 μM, although there is an increase in conductance of the BLM at concentrations as low as 1–2 μM SDS. Deoxycholate and cholate at concentrations greater than 4×10^{-4} M and 10^{-3} M, respectively, also act as divalent cation ionophores in the presence of a 5 mM salt concentration. As we can see from the relative permeabilities, SDS distinguishes most strongly between cations and anions. It also shows the largest difference in permeability between the different divalent cations tested.

To better describe the mechanism by which these detergents transport divalent cations across a membrane, the steady-state conductance is measured as a function of detergent concentration and the salt concentration for the detergents, DOC and cholate. Sodium dodecyl sulfate did not yield steady-state conductance values and hence was not examined in this manner. In Fig. 1 *A* and *B* we plot the log of the steady-state specific conductance *vs.* the log of the detergent concentration in the presence of 5 mM $CaCl_2$ on both sides of an oxidized cholesterol BLM. The raw data is fit to the form:

$$G = G_o + A[\text{Det}]^N$$

where G is the specific conductance, G_o is the specific conductance of the undoped bilayer, and [DET] is the detergent concentration in moles per liter. The fit is done using the MLAB on-line modeling laboratory which performs a least squares fit using the Marquand-Levenberg iteration curve fitting algorithm. The values for N returned plus or minus the standard deviation are

$$\begin{aligned} \text{cholate:} \quad & N = 3.49 \pm 0.37 \\ \text{deoxycholate:} \quad & N = 6.45 \pm 0.55. \end{aligned}$$

Cholate forms either a trimer or a tetramer, while deoxycholate forms either a hexamer or a heptamer.

To determine if the oligomer formed by cholate requires the association of detergents from different sides of the bilayer, membranes were formed with detergent added on only one side on the membrane. The conductance increased immediately upon the addition of the detergent to one side of the bilayer to a value that is equal to the value that we obtain when the same concentration is applied to both sides of the

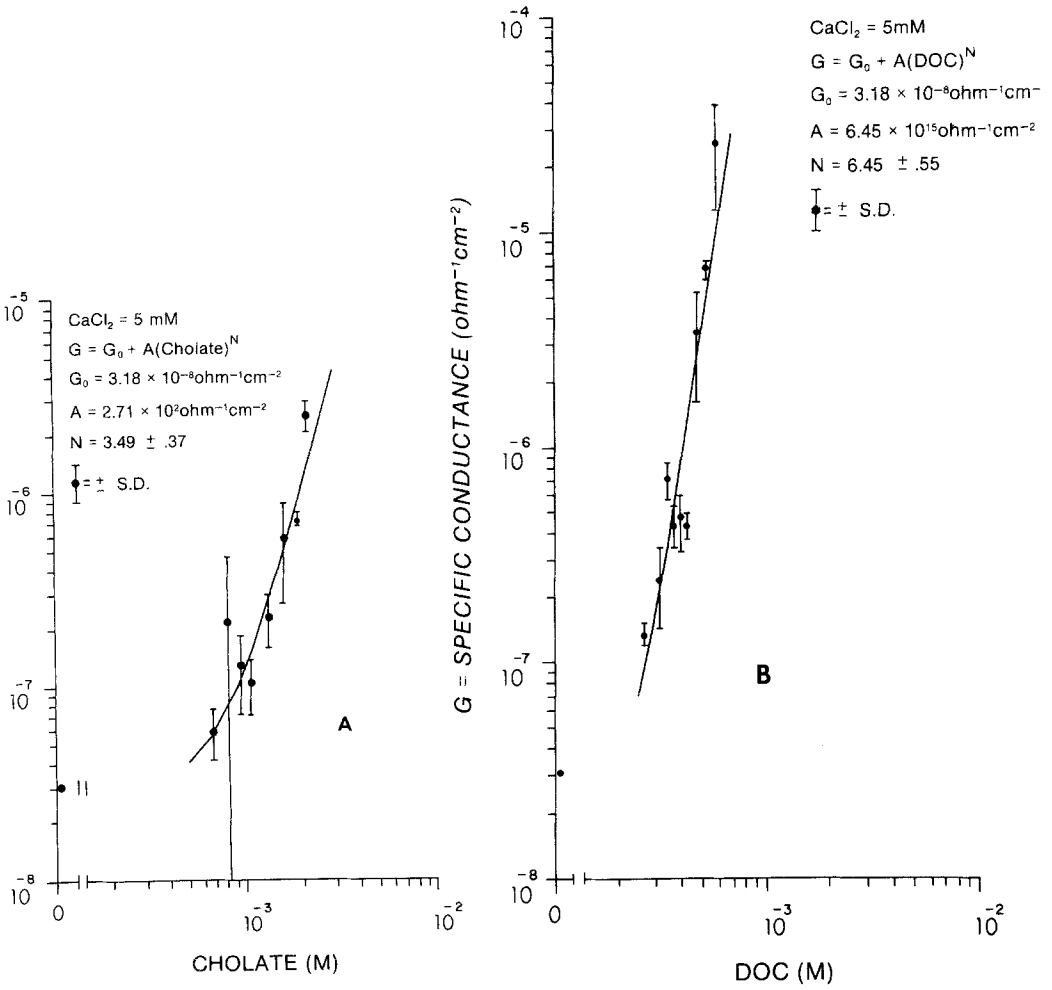

Fig. 1. *A:* Log of the specific conductance *vs.* log of the cholate concentration. The slope of the line determined by the method of least squares is 3.49 ± 0.37. *B:* Log of the specific conductance *vs.* log of the deoxycholate (DOC) concentration. The slope of the line drawn is 6.45 ± 0.55. Both *A* and *B* are in the presence of 5 mM $CaCl_2$, buffered with 5 mM HEPES, Tris, pH 7.3

BLM. The level of conductance is related to the amount of detergent that equilibrates in the lipid phase. This equilibration appears to occur relatively quickly. In all subsequent membranes there is an increase in conductance to a significantly lower value. At a slightly slower rate the detergent seems to diffuse across the bilayer, probably through the annulus of the BLM. The decrease in the level of conductance is related to the decreased concentration of detergent on the side of the BLM

to which the detergent was initially added. The final steady-state conductance level is the same as one would obtain if half of the amount of detergent initially added was added to both sides of the membrane.

In Fig. 2*A* and *B* we show the specific conductance as a function of the $CaCl_2$ concentration in the presence of cholate and DOC, respectively. The measurements were performed with an oxidized cholesterol BLM. In both cases the slope of the best fit line as determined by the method of least squares is consistent with $N=1$. There is no evidence of saturation at high $CaCl_2$ concentration as might be expected if the detergents acted as mobile carriers, binding one Ca ion per conducting complex. The most likely explanation of the observed data is that both DOC and cholate act as channels. Cholate forms a conducting complex with either three or four monomers which aggregate in the membrane. In the case of DOC, either 6 or 7 monomers combine to form a conducting channel.

The effect of $LaCl_3$ on the ionophoric properties of an oxidized cholesterol and PC/cholesterol BLM was investigated for both cholate and DOC. In Fig. 3 we show the effect of addition of $LaCl_3$ to the specific conductance of an oxidized cholesterol BLM doped with cholate. Addition of as little as 2 μM $LaCl_3$ in the presence of 5 mM $CaCl_2$ dramatically decreases the specific conductance of the membrane. When identical experiments were performed with a PC/cholesterol BLM, instead of seeing a similar effect there is an increase in ionophoric activity observed. This stimulatory effect is found to be partially due to a large conductance of $LaCl_3$ in the presence of either cholate or DOC. It seems unlikely that $LaCl_3$ would act to inhibit detergent mediated transport in an oxidized cholesterol BLM and act to stimulate ionophoric activity in a PC/cholesterol BLM.

To determine if either the stimulation or inhibition of ionophoric activity is a nonspecific effect, the effect of $LaCl_3$ on the ionophoric activity of the 20,000-dalton fragment of $(Ca^{2+}+Mg^{2+})$-ATPase (Shamoo, 1978) was examined in both a PC/cholesterol and an oxidized cholesterol BLM. It is found that in both bilayer systems low concentrations of $LaCl_3$ inhibit the ionophoric activity of the 20,000-dalton fragment from $(Ca^{2+}+Mg^{2+})$-ATPase. This observation led us to believe that the stimulatory effect seen in a PC/cholesterol BLM due to $LaCl_3$ is a real effect on the detergent complex while the inhibitory effect seen in an oxidized cholesterol BLM is a nonspecific effect on the lipid.

To see if $LaCl_3$ might stabilize an oxidized cholesterol BLM and cause the inhibitory effect that we have reported, we examined the stabil-

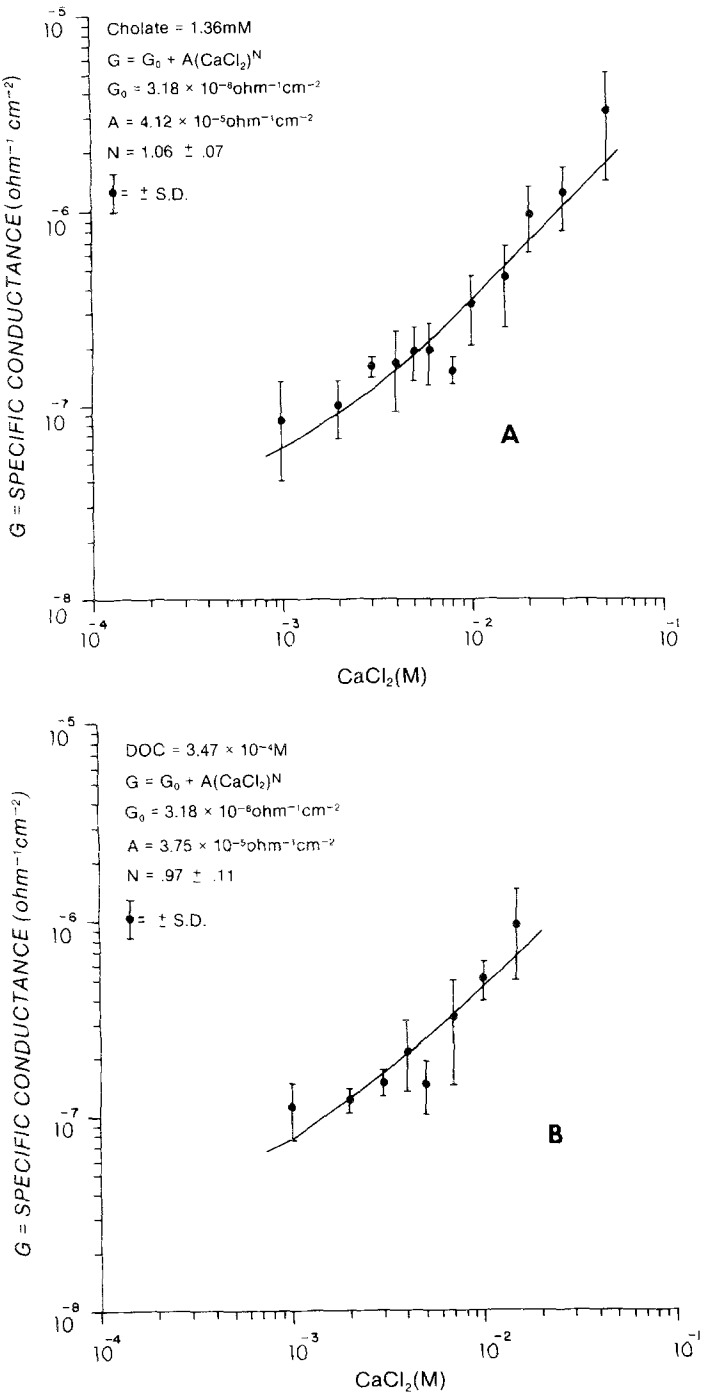

Fig. 2. *A:* Log of the specific conductance *vs.* log of the $CaCl_2$ concentration in the presence of 1.36 mM cholate. The slope of the line determined by the method of least squares is 1.06 ± 0.07. *B:* Log of the specific conductance *vs.* log of the $CaCl_2$ concentration in the presence of 0.35 mM deoxycholate (DOC). The slope of the line drawn is 0.97 ± 0.11. Both *A* and *B* are buffered with 5 mM HEPES, Tris, pH 7.3

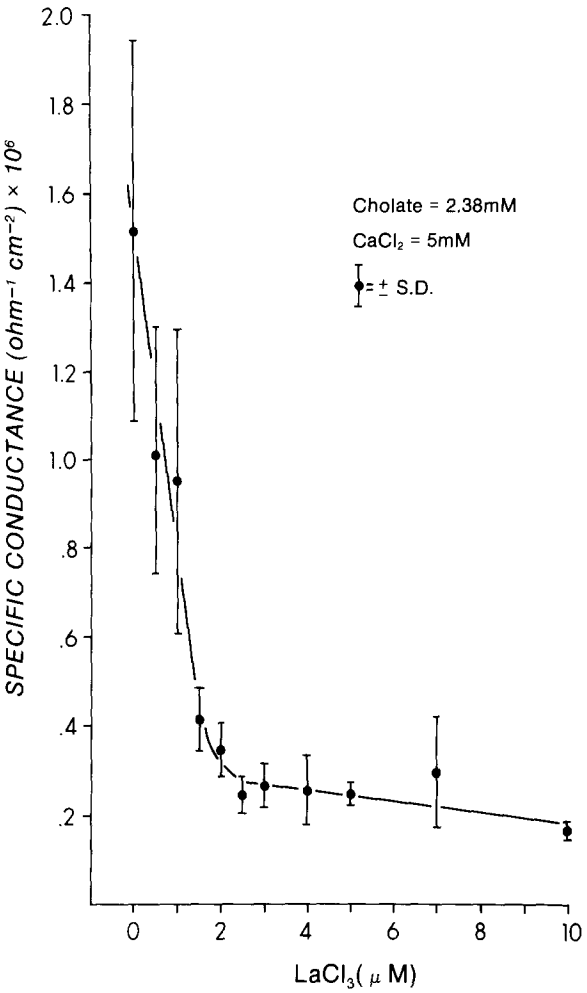

Fig. 3. Specific conductance *vs.* $LaCl_3$ concentration. 5 mM $CaCl_2$ and 2.38 mM cholate are on both sides of an oxidized cholesterol BLM. The solutions are buffered with 5 mM HEPES, Tris, pH 7.3

Table 3. The stability of an oxidized cholesterol black lipid membrane as a function of applied voltage and $LaCl_3$ concentration in the bathing solution on both sides of the BLM[a]

$LaCl_3$ concentration (μM)	Applied voltage (mV)					
	50	75	100	125	150	200
0.	–	↑				
5.	–	–	–	↑		
35.	–	–	–	–	–	↑

[a] For all entries, the bathing solution on both sides of the membrane contains 5 mM $CaCl_2$ and is buffered with 5 mM HEPES, Tris, pH 7.3.

– indicates no increase in conductance; ↑ indicates that membrane breakage occurs within 2 min.

ity of an undoped oxidized cholesterol BLM as a function of $LaCl_3$ dosage and increased the applied voltage until the membrane broke. As we see in Table 3, the voltage necessary to cause breakage of the bilayer increases significantly upon addition of a small amount of $LaCl_3$. The inhibitory effect that we see with $LaCl_3$ with an oxidized cholesterol BLM is consistent with a stabilizing of the BLM. This may well be accompanied by a squeezing out of the material used for doping the membrane.

Discussion

We have shown in this study that sodium dodecyl sulfate (SDS), deoxycholate (DOC), and cholate all act as divalent cation ionophores. In the presence of 5 mM $CaCl_2$, SDS is active at concentrations as low as 1 μM. It also shows large selectivity differences between cations and anions and between the different divalent cations tested. Deoxycholate and cholate are active at a somewhat higher concentration ($\sim$1 mM).

The relative permeabilities derived in Tables 1 and 2 act as a fingerprint to identify the detergents studied. Comparison of the relative permeabilities of the protein of interest with the permeability of the contaminating detergent used in the isolation of the protein should enable one to determine if the transport properties of the protein are in fact not due to the detergent. Measurement of the concentration of the detergent bound to the protein using radioactively labeled detergent enables one to determine if the concentration of contaminating detergent is sufficient to cause the ionophoric effects observed (Shamoo *et al.*, 1976). Being below the threshold concentration of detergent reported in this article is, however, not absolute proof of the lack of a detergent effect. Conceivably, the protein could act to increase the incorporation of the detergent into the BLM. If the effect observed is partially due to contaminating detergent, further removal of detergent from the protein should change the measured permeabilities in a predictable manner. Removal of detergent below some value should lead to no further changes in the permeabilities measured. At this point, the detergent is no longer effecting the permeability properties of the protein in question. In work carried out in our laboratory, similar studies have shown that the detergents used in the isolation of the tryptic fragments of $(Ca^{2+} + Mg^{2+})$-ATPase from sarcoplasmic reticulum are not influencing the ionophoric activity and selectivity of either the isolated enzyme or it's tryptic fragments (Shamoo & MacLennan, 1974; Shamoo *et al.*, 1976; Stewart *et al.*, 1976; Abramson & Shamoo, 1978; Shamoo, 1978).

Measurements of the relative cation permeability of membranes doped with detergents enables us to better understand the nature of the interaction between the divalent cation and the anionic charge groups on the detergent. Sherry (1969) has modelled the interaction of divalent cations with two univalent negatively charged sites separated by a fixed distance. He assumes that the system can be totally defined in terms of the electrostatic interaction of the divalent cation with fixed anionic charge and in terms of ion hydration energy. Seven selectivity sequences are derived from this analysis. Sequence (I) corresponds to the weakest electrostatic interaction between the divalent cation and the anionic sites, and sequence VII corresponds to the strong-field case, in which the smallest divalent cations are preferred. Both cholate and deoxycholate have selectivity sequences that are indicative of a stronger electrostatic interaction than is present in the case of SDS. We also note that the nature of interaction is influenced by the membrane. Changing from an oxidized cholesterol BLM to a PC/cholesterol BLM not only changes the magnitude of the relative permeabilities but also alters the selectivity sequence.

By plotting the specific conductance *vs.* the concentration of the detergent in the solution, we are able to determine that there is an oligomeric aggregation for either cholate or DOC in order to form a conducting unit. Cholate forms either a trimer or a tetramer, and deocycholate forms either a hexamer or a heptamer. Conducting oligomeric units can form from either side of the membrane. There appears to be no requirement for association of complexes on opposite sides of the membrane. For both detergents the specific conductance is linear with $CaCl_2$ concentration over a large range of salt concentration. There is no saturation evident as one would expect if the detergents acted as carriers. These detergents probably act as channels in the transport of Ca^{2+} across the membrane. The evidence presented is not, however, conclusive proof that the detergents tested act as channels.

In a recent study by Bangham and Lea (1978) the specific conductance of membranes made with a solution of phosphatidylcholine/phosphatidylethanolamine (2.5:8 mg) in the presence of 0.1 M NaCl in the bathing solution was plotted as a function of the concentration of DOC and cholate. In both cases conductance was close to linear with the DOC or cholate concentration. Their data seems to indicate that in the presence of monovalent cations the conducting unit is a monomer, while in this study, in the presence of divalent cations, we show that higher order oligomeric aggregates are required for the detergents to act as ionophores.

The inhibitory effect of micromolar concentrations of $LaCl_3$ on the

ionophoric activity of cholate and deoxycholate in an oxidized cholesterol BLM appears to be due to $LaCl_3$ stabilizing the BLM. This data helps stress the importance of testing the effect of different ionophores and inhibitors on bilayers made from different lipids. The inhibitory effect of $LaCl_3$ is a nonspecific effect. It appears not to directly act on the conducting oligomeric unit, but instead it acts to tighten up the oxidized cholesterol BLM and probably squeezes out the detergent.

This paper is based on work performed under contract with U.S. Department of Energy (DOE) at the University of Rochester Biomedical and Environmental Research Project and has been assigned Report Number UR-3490-1513. This paper was also supported in part by NIH Grant 1 R01 18892; program project grant ES-10248 from NIEHS; and the Muscular Dystrophy Association.

This work was done during the tenure of Jonathan J. Abramson as a research fellow of the Muscular Dystrophy Association. Adil E. Shamoo is an established investigator of the American Heart Association.

References

Abramson, J.J., Shamoo, A.E. 1978. Purification and characterization of the 45,000-dalton fragment from tryptic digestion of $(Ca^{2+}+Mg^{2+})$-adenosine triphosphatase of sarcoplasmic reticulum. *J. Membrane Biol.* **44**:233

Antonov, V.F., Korepanova, E.A., Vladimirov, Y.A. 1976. Bilayer membrane charged by detergents as a model to study the role of the surface change in ionic permeability. *Stud. Biophys.* **58**:87

Bangham, J.A., Lea, E.J.A. 1978. The interaction of detergents with bilayer lipid membranes. *Biochim. Biophys. Acta* **511**:388

Goldin, S.M., Tong, S.W. 1974. Reconstitution of active transport catalyzed by the purified sodium and potassium ion stimulated adenosine triphosphatase from canine renal medulla. *J. Biol. Chem.* **249**:5907

Good, N.E., Winget, G.D., Winter, W., Connolly, T.N., Izawa, S., Singh, R.M.M. 1966. Hydrogen ion buffers for biological research. *Biochemistry* **5**:467

Jeng, A.Y., Ryan, T.E., Shamoo, E.A. 1978. Isolation of a low molecular weight Ca^{2+}-carrier from calf heart inner mitochondrial membrane. *Proc. Nat. Acad. Sci. USA* **75**:2125

Ksenzhek, O.S., Gevod, V.S., Omel'chenko, A.M., Koganov, M.M. 1975. Study of the discrete conductance of bilayer membranes in the presence of sodium dodecylsulfate. *Elektrokhimiya* **11**:1566

Ksenzhek, O.S., Omel'chenko, A.M., Koganov, M.M. 1974. Discrete conductance induced in bilayer lipid membranes by sodium dodecyl sulfate. *Dokl. Biophy.* **218**:61

Kyte, J. 1971. Purification of the sodium and potassium-dependent adenosine triphosphatase from canine renal medulla. *J. Biol. Chem.* **246**:4157

MacLennan, D.H. 1970. Purification and properties of an adenosine triphosphatase from sarcoplasmic reticulum. *J. Biol. Chem.* **245**:4508

Racker, E. 1972. Reconstitution of a calcium pump with phospholipids and a purified Ca^{++}-adenosine triphosphatase from sarcoplasmic reticulum. *J. Biol. Chem.* **247**:8198

Racker, E., Stoeckenius, W. 1974. Reconstitution of purple membrane vesicles catalyzing light-driven proton uptake and adenosine triphosphatase formation. *J. Biol. Chem.* **249**:662

Seufert, W.D. 1965. Induced permeability changes in reconstituted cell membrane structure. *Nature (London)* **207**:174

Seufert, W.D. 1973. The interaction of anionic detergents with lipid bilayer membranes. *Biophysik* **10**:281

Shamoo, A.E. 1978. Ionophorous properties of the 20,000-dalton fragment of $(Ca^{2+}+Mg^{2+})$-ATPase in phosphatidylcholine:cholesterol membranes. *J. Membrane Biol.* **43**:227

Shamoo, A.E., Goldstein, D.A. 1977. Isolation of ionophores from ion transport systems and their role in energy transduction. *Biochim. Biophys. Acta* **472**:13

Shamoo, A.E., MacLennan, D.H. 1974. A Ca^{++}-dependent and -selective ionophore as part of the $Ca^{++}+Mg^{++}$-dependent adenosine triphosphatase of sarcoplasmic reticulum. *Proc. Nat. Acad. Sci. USA* **71**:3522

Shamoo, A.E., Ryan, T.E., Stewart, P.S., MacLennan, D.H. 1976. Localization of ionophoric activity in a 20,000 dalton fragment of the adenosine triphosphatase of sarcoplasmic reticulum. *J. Biol. Chem.* **25**:4147

Sherry, H.S. 1969. The ion-exchange properties of zeolites. *In:* Ion Exchange, II. p. 89. Decker, New York

Sillén, L.G., Martell, A.E. 1971. Stability Constants. Suppl. 1, Special Publication 25. The Chemical Society, London

Stewart, P.S., MacLennan, D.H., Shamoo, A.E. 1976. Isolation and characterization of tryptic fragments of the adenosine triphosphatase of sarcoplasmic reticulum. *J. Biol. Chem.* **251**:712

Tien, H.T., Carbone, S., Davidowicz, E.A. 1966. Formation of black lipid membranes by oxidation products of cholesterol. *Nature (London)* **212**:718