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Permeability studies of lipid vesicles from alkalophilic *Bacillus firmus* showing opposing effects of membrane isoprenoid and diacylglycerol fractions and suggesting a possible basis for obligate alkalophily

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Previous studies of the membrane lipids of extremely alkalophilic bacilli had indicated that both facultative and obligate alkalophiles contained a substantial fraction of isoprenoid lipid as well as high concentrations of cardiolipin. Facultative alkalophiles differed from obligate strains in having a phospholipid fatty acid composition that would be expected to result in a more ordered membrane structure. Current studies of ion permeability in vesicles prepared from lipids from obligately alkalophilic *Bacillus firmus* RAB and its facultatively alkalophilic strain, OF4, support the suggestion that membranes of the latter strain form a tighter barrier structure, with the difference especially pronounced at near neutral pH values. The water permeability of whole cells and the reflection coefficients for acetamide in vesicles were also consistent with a tighter membrane in the facultatively alkalophilic strain than in the obligately alkalophilic strain. The permeability properties of vesicles prepared from phospholipids from these organisms were studied as a function of the addition of either homologous membrane isoprenoid or diacylglycerol. For each permeability parameter that was assayed, in lipids from both strains, the isoprenoid fraction decreased the permeability, whereas the diacylglycerol fraction increased the permeability of the vesicles to solute.

Introduction

Alkalophilic bacilli that grow at pH 10.5 to 11.5 maintain a cytoplasmic pH of about 8.5 [1]. The extremely high external pH and the large difference between the pH values on the two sides of the cytoplasmic membrane constitute challenges to the structural and functional integrity of the membrane. In a recent study, we compared the membrane lipids of *Bacillus firmus* RAB and *Bacillus alcalophilus*, which are obligate alkalophiles that grow poorly or not at all below pH 9.0, with facultatively alkalophilic strains such as *Bacillus firmus* OF4, that exhibit substantial growth both at extremely alkaline and near neutral pH values [2]. Strain OF4 was of particular interest because, with respect to DNA homology, this strain is indistinguishable from *B. firmus* RAB [3]. We were interested both in those properties that may relate in some special way to the ability to grow at very high pH and in those properties that might form the basis for facultative vs. obligate alkalophily. It was found that membranes of all the *Bacillus* strains that grew at very high pH possessed a high content of anionic lipids, espe-

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cially cardiolipin, and also contained a substantial content of isoprenoids, including β-carotene, phytoene, and tetraanhydrobacterioruberin, in the neutral lipid fraction [2]. With respect to differences between obligately and facultatively alkalophilic strains, the most striking observation was that whereas the obligate strains possessed a remarkably high content of fatty acids that were unsaturated, branched chain, or both, the facultative strains possessed a lower total content of these fatty acid species, and virtually lacked the monounsaturated fatty acids that abounded in the obligately alkalophilic strains [2]. We suggested that the fatty acid composition of the phospholipids of obligate alkalophiles might result in a membrane that was so disordered that, at near neutral pH values, its integrity was insufficient to maintain adequate barrier function for viability and growth. Such a suggestion was consistent with earlier observations (see, for example, Ref. 4) that indicated a loss of membrane integrity of the obligate alkalophiles at neutral or near neutral pH.

In the current study, permeability measurements were undertaken in vesicles that were prepared from *B. firmus* RAB and strain OF4 membrane lipids. We sought to ascertain whether functional studies would support the above hypothesis with respect to a possible basis for obligate alkalophily. In addition, we studied the effect of inclusion of the isoprenoid fraction vs. inclusion of diacylglycerol, the other major neutral lipid in these organisms, on the permeability of the phospholipid vesicles.

Materials and Methods

Bacterial strains and growth conditions. B. firmus strains RAB and OF4 were isolated in our laboratory, as described previously [3,5]. For these studies, both strains were grown at pH 10.5 in carbonate-buffered medium supplemented with 50 mM D,L-malate, trace salts, and 0.1% yeast extract [6]. The organisms were grown at 30 °C, in 20-liter carboys with forced aeration. The cells were harvested, at the early stationary phase, using a Millipore ultrafiltration unit.

Preparation of right-side-out vesicles and total membrane lipids. Lipids were prepared from right-side-out membrane vesicles so that contamination by cell wall components would be avoided. The vesicles were prepared from washed whole cells by the lysozyme method of Kaback [7]. Vesicles prepared in this way had undetectable levels of contaminating muramic acid [2]. The protein content of the preparations was assayed by the method of Lowry et al. [8] using bovine serum albumin as a standard. Total lipids were extracted from the right-side-out vesicles by the procedure of Bligh and Dyer [9]. The extracted lipids were stored at −80 °C. under N_2.

Separation of neutral and polar lipid fractions. The total membrane lipids were fractionated on a silicic acid column by stepwise elution with chloroform, acetone, and then methanol. Individual lipid fractions that were eluted from the column were pooled and purified to a single lipid species by silica gel thin-layer chromatography. Purified lipids were obtained by extracting the scraped gel with chloroform and methanol (2:1, v/v) as described previously [2].

Separation of isoprenoids. Neutral lipids were developed on a preparative thin-layer chromatogram as described by us previously [2]. The fraction IV, which contained the isoprenoids, was scraped from the plate and extracted with ethyl ether/hexane (0.25:99.75, v/v).

Preparation of lipid vesicles containing trapped Ca^{2+}, Rb^+, or Cl^-. Thin lipid films were prepared from indicated mixtures of lipids in vials. For loading of liposomes with Ca^{2+}, lipids were dispersed in 10 mM imidazole (pH 8.0) containing 135 mM NaCl and 150 mM $CaCl_2$ (with $^{45}Ca^{2+}$ to a specific radioactivity of 23.5 Ci/g). To entrap Rb^+, we dispersed the lipids in 10 mM imidazole (pH 7.2 or 8.0) or in 10 mM Trizma buffer (pH 9.0) containing 150 mM RbCl (with $^{86}Rb^+$ to a specific radioactivity of 25.4 Ci/g). Entrapment of Cl^- was accomplished by suspending the thin lipid film in 10 mM Tris buffer containing 100 mM NaCl (pH 8.0). After the dispersions were shaken with a Vortex mixer, unilamellar vesicles were prepared by sonication for 40 min under nitrogen. Sonication of the suspensions containing $^{86}Rb^+$, $^{45}Ca^{2+}$, or Cl^- was conducted in a cup horn through which cold water was circulated. The vesicles were stored at 4 °C for several hours prior to gel filtration. Undispersed lipid and metal

released from the sonication probe were removed from the suspensions by centrifugation for 20 min at 12 000 × *g*. The suspensions were passed through columns (1.5 × 30 cm) of Sephadex G-50 to remove untrapped Rb^+, Ca^{2+}, and Cl^-. Gel filtration was conducted at room temperature as described previously [10,11]. The final total lipid concentration was determined as described elsewhere [2].

Assays of $^{86}Rb^+$ and $^{45}Ca^{2+}$ efflux. Ionophore-mediated efflux was measured as described previously [10]. Stock solutions of valinomycin in dimethylformamide and of A23187 in ethanol were prepared to final concentrations of 3.0 μM and 50 nM, respectively. A 10 μl aliquot of valinomycin or A23187 solution or solvent control was added to 1-ml suspensions of $^{86}Rb^+$- or $^{45}Ca^{2+}$-loaded liposomes, respectively. The vesicles were placed in Visking tubing (1 cm diameter) that was knotted so as to allow mixing of the contents upon suspension of the bags in 4 ml of 10 mM imidazole containing either 150 mM choline chloride (for the Rb^+ experiments) or 135 mM NaCl (for the Ca^{2+} experiments). Aliquots (100 μl) of the dialysates were taken at various times and were analyzed for radioactivity by scintillation spectrometry.

The time courses of ionophore-induced ion efflux were measured in triplicate from given preparations, and were examined in 2–4 batches of liposomes from independent lipid preparations. The rates of efflux for a given ion in a given preparation varied within 5%. Under the conditions used, the passive diffusion of the cations studied was a negligible percentage of the experimental values.

Assays of Cl^- efflux. The efflux of Cl^- from pre-loaded vesicles was assayed as described previously [11]. Vesicles (1 ml), in a Visking dialysis bag, were suspended in 10 ml of Tris buffer containing 100 mM $NaNO_3$ (pH 8.0). The chloride concentration of the dialysate was monitored using a chloride-sensitive electrode. Quadruplicate determinations were made on each of several independent samples, using an electrode that was calibrated in the range of 10 μM to 1 mM chloride.

Reflection coefficients and osmotic permeability Reflection coefficients for acetamide were determined for various cell and vesicle preparations using a Durrum-Gibson stopped-flow spectrophotometer as described previously [12]. The light transmitted at 180° was monitored at 450 nm and was linear with time following a 150 ms disturbance period. Changes in cell or vesicle volume arising from shrinking and swelling were calculated from transmittance changes according to relationships described by Bangham et al. [13], and the reflection coefficient, σ, was calculated according to methods also described by others [14,15]. The reflection coefficient represents the permeability of the solute acetamide relative to water, with higher values indicating relatively greater impermeability of the solute in a given preparation. The relative values for the different preparations are given more weight in these studies than the absolute values of the reflection coefficients, because the absolute values obtained by the methods employed may be somewhat undercorrected, albeit consistently, for unstirred water layers [16].

Measurements of osmotic permeability of cells were also conducted by monitoring transmittance changes, upon sudden imposed changes in osmolarity, using a Durrum-Gibson stopped flow spectrophotometer. The experimental procedure and calculations were precisely as described by Bittman et al. [17].

Materials. Authentic lipids were purchased from Serdary Research Laboratories, Sigma Chemical Co., or Altech Associates, Inc. Applied Science Division. Organic solvents were obtained from Aldrich Chemical Co. Valinomycin and A23187 were obtained from Eli Lilly, and $^{45}Ca^{2+}$ and $^{86}Rb^+$ were purchased from New England Nuclear.

Results

Ion efflux from vesicles prepared from B. firmus RAB lipids is more rapid than that from OF4, and homologous isoprenoids decrease the rates of efflux

Valinomycin-mediated efflux of rubidium, A23187-mediated efflux of calcium, and efflux of chloride ion were measured in vesicles prepared from total membrane lipids, membrane phospholipids, or membrane phospholipids to which either homologous isoprenoids or diacylglycerol was added to a percentage approximating its representation in the native membrane lipids. The

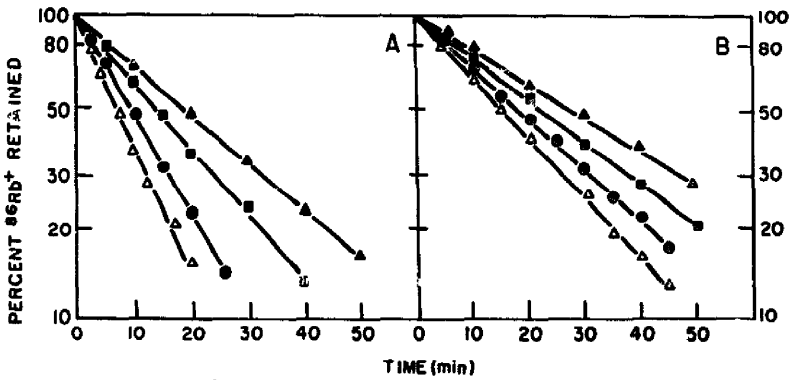

Fig. 1. Valinomycin-mediated efflux of $^{86}Rb^+$ from vesicles prepared from membrane lipids from obligately and facultatively alkalophilic *B. firmus*. Liposomes were prepared from lipids that were isolated from membranes of *B. firmus* RAB and OF4 that had been grown at pH 10.5, as described under Materials and Methods. Efflux from $^{86}Rb^+$-loaded vesicles in the presence of valinomycin is presented as first order plots for vesicles prepared from: total membrane lipids (●); membrane phospholipids (PL, ■); PL plus 6% isoprenoids (▲); and PL plus 1,2-diacylglycerol, 10% (△). Panel A shows the data for *B. firmus* RAB and panel B shows the data for strain OF4. The isoprenoids and diacylglycerol added were from the homologous strains. The assays were conducted at pH 8.0 at 25 °C, as described under Materials and Methods.

patterns of efflux are shown for both *B. firmus* RAB and strain OF4 in Figs. 1–3. Calculated rates of efflux are summarized in Table I. That table also shows values obtained for the efflux of each ion from vesicles prepared from commercially obtained phospholipids, in ratios approximating those found previously in the alkalophiles, either in the presence or absence of added isoprenoid from *B. firmus* RAB. For the artificial vesicles, in assays conducted at pH 8.0, efflux of each of the three ions was much slower than that observed with any of the vesicle preparations from either of the two alkalophile strains (Table I). Notably, while efflux of ions from alkalophile lipid vesicles was faster than from those prepared from commercial lipids, the rate of efflux of each ion was much slower from vesicles prepared from strain OF4 lipids than from those prepared from lipids of *B. firmus* RAB. Chloride ion efflux was the most rapid of the three ions examined in both strains, with ionophore-mediated rubidium efflux distinctly faster than calcium efflux in vesicles

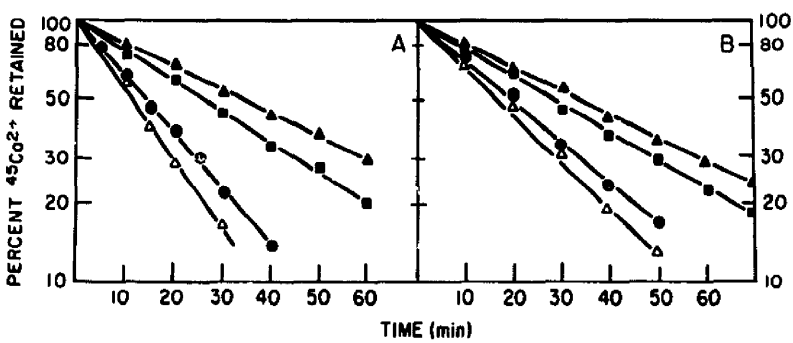

Fig. 2. A23187-mediated $^{45}Ca^{2+}$ efflux from vesicles prepared from membrane lipids from obligately and facultatively alkalophilic *B. firmus*. The symbols and panels are exactly as described in the legend to Fig. 1, except that $^{45}Ca^{2+}$-loaded vesicles that were treated with A23187 were employed.

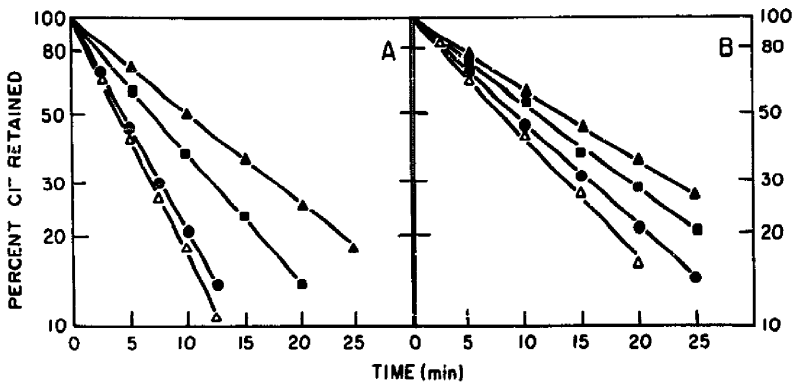

Fig. 3. Chloride efflux from vesicles prepared from membrane lipids from obligately and facultatively alkalophilic *B. firmus*. The symbols and panels are exactly as described in the legend to Fig. 1, except that chloride-loaded vesicles were employed, and efflux was monitored with a chloride-sensitive electrode as described under Materials and Methods.

from *B. firmus* RAB lipids and only marginally faster than calcium efflux in vesicles from strain OF4 lipids.

For each ion whose efflux was examined, in vesicles from both alkalophile lipid preparations, efflux was more rapid from vesicles prepared from total membrane lipid than from just the phospholipid fraction (Figs. 1–3, Table I). Inclusion of diacylglycerol in the phospholipid fraction increased the rate of ion efflux, for each ion in both strains, beyond the rate observed with total lipids. Conversely, inclusion of isoprenoids in the phospholipid vesicles decreased the rate of efflux, for each ion in both strains, to a rate that was lower

TABLE I

FIRST ORDER RATE CONSTANTS OF VALINOMYCIN-MEDIATED $^{86}Rb^+$, A23187-MEDIATED $^{45}Ca^{2+}$ AND SPONTANEOUS Cl^- EFFLUX FROM VESICLES PREPARED FROM MEMBRANE LIPIDS OF ALKALOPHILIC BACTERIA

Efflux of ions was conducted as described under Materials and Methods at pH 8.0, 25°C. The rate constants were determined from first order plots such as those shown in Figs. 1–3. A value for total internal ion was obtained by release of trapped ion with 0.2% Triton X-100. Values shown are means ± S.D.

Lipid source for vesicles	Ion efflux assayed	Efflux rate constants, $k(10^{-2}\ min^{-1})$, observed with vesicles containing			
		total lipid	phospholipid (PL)	PL + diacylglycerol [b]	PL + isoprenoid [c]
Commercial lipids [a]	$^{86}Rb^+$	–	2.4 ± 0.2	–	2.8 ± 0.2
	$^{45}Ca^{2+}$	–	1.3 ± 0.1	–	1.2 ± 0.1
	Cl^-	–	5.8 ± 0.2	–	6.9 ± 0.3
B. firmus RAB (obligate)	$^{86}Rb^+$	7.7 ± 0.4	5.0 ± 0.2	9.7 ± 0.6	3.6 ± 0.2
	$^{45}Ca^{2+}$	4.9 ± 0.2	2.7 ± 0.2	5.8 ± 0.2	2.1 ± 0.1
	Cl^-	15.4 ± 1.1	8.9 ± 0.5	17.3 ± 1.3	6.9 ± 0.5
B. firmus OF4 (facultative)	$^{86}Rb^+$	3.9 ± 0.2	3.2 ± 0.2	4.6 ± 0.5	2.5 ± 0.2
	$^{45}Ca^{2+}$	3.6 ± 0.2	2.5 ± 0.1	4.1 ± 0.2	2.1 ± 0.2
	Cl^-	7.7 ± 0.4	6.3 ± 0.3	8.7 ± 1.0	5.3 ± 0.3

[a] Vesicles were prepared from commercially obtained egg phosphatidylglycerol (60%), egg phosphatidylethanolamine (20%), and bovine heart cardiolipin (20%).

[b] Vesicles were prepared from membrane phospholipids (90%) and homologous 1,2-diacylglycerol (10%).

[c] Vesicles were prepared from membrane phospholipids (94%) and homologous isoprenoids (6%). Isoprenoids from *B. firmus* RAB were used in experiments with vesicles made from commercial lipids.

TABLE II

HALF-TIME OF VALINOMYCIN-MEDIATED $^{86}Rb^+$ EFFLUX FROM VESICLES PREPARED FROM MEMBRANE LIPID FRACTIONS OF *BACILLUS FIRMUS* RAB AND OF4

$^{86}Rb^+$ efflux from pre-loaded vesicles was assayed in the presence of valinomycin at the indicated pH values. Values for $t_{1/2}$ were calculated by extrapolation of initial rates of efflux in plots of % retention vs. time.

Vesicles prepared from	*B. firmus* strain	$t_{1/2}$ (min) of $^{86}Rb^+$ efflux at		
		pH 7.2	pH 8.0	pH 9.0
Total membrane lipid	RAB	8.4	9.0	10.2
	OF4	22.4	18.0	18.0
Membrane phospholipid (PL)	RAB	12.8	14.1	16.3
	OF4	23.9	22.1	21.4
PL + diacylglycerol (10%)	RAB	6.8	7.0	8.1
	OF4	17.1	15.0	13.6
PL + isoprenoid (6%)	RAB	23.3	19.2	21.3
	OF4	32.7	27.9	24.4

than that observed for the phospholipids alone. By contrast, addition of isoprenoid to liposomes prepared from commercial phospholipids did not significantly change the rates of ion efflux (Table I).

The finding that efflux of each ion, from every type of preparation, was faster in vesicles from the obligate alkalophile (*B. firmus* RAB) than from the facultative strain (*B. firmus* OF4) supported the earlier suggestion that the membrane lipids of the facultative strain allowed the maintenance of a tighter barrier structure. Were that property to be exacerbated as the pH was lowered from alkaline to near-neutral, it would further support the notion that a basis for obligate alkalophily could be inadequate membrane barrier structure at near neutral pH for the obligate strains. Accordingly, ionophore-mediated $^{86}Rb^+$ efflux was examined in various vesicle preparations from the two strains at three different pH values. As shown in Table II, cation efflux from vesicles from OF4 was consistently slower than for comparable preparations of *B. firmus* RAB. More importantly, for vesicles prepared from total lipids, phospholipids, or phospholipids plus homologous diacylglycerol, the rate of valinomycin-mediated efflux of rubidium was unchanged or slightly diminished in OF4 liposomes as the pH was reduced from 9.0 to 7.2, whereas efflux from RAB liposomes was more rapid. Only in preparations of phospholipids to which isoprenoids were added did both strains exhibit at least as slow an efflux rate at pH 7.2 as at pH 9.0 (Table II).

Reflection coefficients for acetamide penetration are higher for both OF4 cells and vesicles than for corresponding preparations of B. firmus RAB, and the respective effects of isoprenoids and diacylglycerol parallel those found on ion efflux

As shown in Table III, both whole cells and vesicles prepared from the lipids (either total membrane lipids or phospholipids) of strain OF4 exhibited higher reflection coefficients than did comparable preparations from *B. firmus* RAB. The higher values represent a relatively greater discrimination between the solute, acetamide, and the aqueous solvent on the part of preparations from OF4. Additions of either diacylglycerol or isoprenoids to the vesicles of each strain affected the reflection coefficients in the same way as they had affected ion efflux. That is, addition of homologous isoprenoids to vesicles prepared from

TABLE III

REFLECTION COEFFICIENTS FOR ACETAMIDE PENETRATION IN INTACT CELLS AND VESICLES PREPARED FROM MEMBRANE LIPIDS FROM ALKALOPHILIC *BACILLUS FIRMUS* STRAINS

Cells or vesicles (at total lipid concentrations of 2 mM) were dispersed in 30 mM KCl and mixed with acetamide solution. Reflection coefficients were determined from initial rates of volume change as described under Materials and Methods. Values shown are means ± S.D.

Preparation	*B. firmus* strain	Reflection coefficient
Cells [a]	RAB	0.38 ± 0.02
	OF4	0.55 ± 0.09
Vesicles		
Total lipids	RAB	0.24 ± 0.01
	OF4	0.40 ± 0.03
Phospholipids (PL)	RAB	0.30 ± 0.02
	OF4	0.45 ± 0.04
PL + isoprenoids (6%)	RAB	0.44 ± 0.04
	OF4	0.60 ± 0.08
PL + 1,2-diacylglycerol (10%)	RAB	0.22 ± 0.02
	OF4	0.34 ± 0.05

[a] Washed cells that were grown at pH 10.5 and harvested in late log phase.

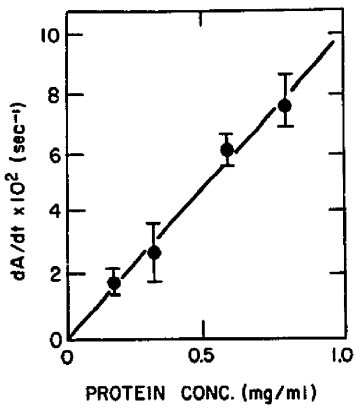

Fig. 4. The dependence upon the rate of absorbance changes during shifts in osmolarity on the concentration of bacterial cells. Data are shown for changes in absorbance at 600 nm with time (dA/dt) for cells of *B. firmus* RAB, at the indicated concentrations of cell protein, subjected to a change in osmolarity of 0.1 osM.

phospholipids from either strain resulted in a higher reflection coefficient. Conversely, the addition of homologous diacylglycerol to phospholipid vesicles prepared from either strain resulted in a lower reflection coefficient.

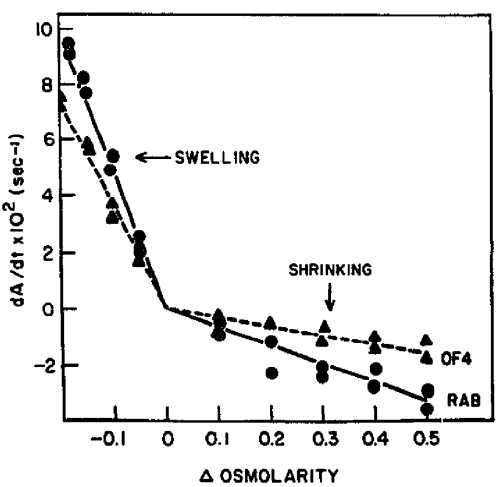

Fig. 5. The rates of cell volume change upon imposed changes in osmolarity. Cells of *B. firmus* RAB (●) or of strain OF4 (▲) were suspended in phosphate buffered salt solution and mixed with an equal volume of similarly buffered salt solution (at pH 8.0) of the desired osmolarity. Osmolarity was varied by decreasing or increasing the concentration of NaCl in the solution for swelling or shrinking experiments, respectively. The rate of cell volume change was monitored, as described under Materials and Methods, as changes in absorbance at 600 nm and were normalized to a protein concentration of 1 mg/ml.

Osmotic permeability parallels other differences in permeability between the obligately and facultatively alkalophilic strains

As shown in Fig. 4 for strain RAB, there was a direct proportionality between absorbance changes and changes in cell volume upon sudden imposed changes in osmotic pressure that was linear over a range of cell protein concentrations. In Fig. 5, the rates of response of the two strains to changes in osmolarity of various magnitudes are shown. For both swelling and shrinking, cells of OF4 exhibited a slower rate of change than did cells of *B. firmus* RAB.

Discussion

A relationship between the membrane lipid composition and the permeability properties of the membrane has long been recognized and has been examined via ion efflux studies such as those presented here (see, for example, Ref. 18). We had previously shown that the major, consistent difference between several facultatively alkalophilic bacilli and two obligately alkalophilic bacilli was an almost total absence of unsaturated fatty acids and a diminution in the bulkier branched chain fatty acids in the facultative strains [2]. The current study suggests that membranes of an obligate alkalophile are considerably more permeable to solutes than are those of a closely related, facultative strain. The use of ionophore-mediated ion efflux for two of the solute species examined represents a non-physiological assay, and all of the determinations were conducted in a lipid vesicle setting that differs from the actual cytoplasmic membrane. Accordingly, the differences between preparations cannot be assumed to reflect phenomena that are necessarily correlated with physiological consequences. Nonetheless, the patterns are of interest in the context of earlier physiological observations. Most strikingly, the functional differences between the vesicles from the obligate vs. facultative alkalophile become somewhat more pronounced as the pH is lowered from highly alkaline to near-neutral, i.e., the rate of passive or ionophore-mediated ion efflux from the

obligate alkalophile increases disproportionately relative to the facultative strain at near-neutral pH. These findings are thus consistent with our current hypothesis that properties of the membrane, leading to inadequate barrier function at near-neutral pH, are the basis for obligate alkalophily. Several earlier findings are also accommodated by this hypothesis: a loss of membrane integrity by obligately alkalophilic *B. alcalophilus* and *B. firmus* RAB at near-neutral pH [4]; and the low protonmotive forces [19] and H^+/O ratios [20] exhibited by obligately alkalophilic *B. firmus* RAB at near neutral vs. alkaline pH. Enhanced membrane leakiness at near-neutral pH would lead to the low H^+/O values even if respiratory chain function were comparable over the entire pH range, as our most recent data suggest (see, for example, Refs. 21, 22).

The rates of solute efflux, especially the rate of chloride efflux, are faster than those observed in similar experiments with lipids from other membranes; observations of slower rates (see, for example Ref. 23) generally involved model systems that included phosphatidylcholine. Still, it is notable that vesicles prepared from alkalophile membrane lipids are generally more permeable to solutes than liposomes prepared from commercial phospholipids in approximately similar ratios. A relationship between this property and the ability to grow at very high pH may exist, but the nature of such a possible connection is presently obscure. It is similarly unclear what the physical basis might be for the increase in permeability, as the pH declines to near-neutral, specifically in the membrane lipids from the obligate strain.

The other major finding in the current study was the modulatory effects of the two major components of the membrane neutral lipid fraction on permeability of homologous phospholipid liposomes from the alkalophiles. The isoprenoid fraction consistently increased the apparent tightness of the membrane in measurements of both ion efflux and reflection coefficients, whereas the diacylglycerol fraction had the opposite effect. Hopanoids, and acyclic tri- and tetraterpenoid compounds have been found in a variety of bacterial membranes [24], including at least some organisms that grow in environments in which there is a pH or temperature stress. It has been suggested that these membrane compounds serve a sterol-like, rigidifying function in the bacterial membranes [25–29]. Most recently, Lazrak et al. [30] prepared vesicles from the total lipids of a *Halobacterium*; the rigidity of these vesicles was increased and their water permeability was decreased by the inclusion of homologous bacterioruberins. While alkalophile membranes have not been found to contain hopanoids or highly polar acyclic terpenoids, they do contain bacterioruberins. There may be further compounds among the unidentified fraction of the substantial alkalophile isoprenoid component that can function similarly.

A precedent for the effects of diacylglycerol that were observed here is suggested by studies such as those of Epand [31] and of Das and Rand [32] in which diacylglycerol was found to alter the temperature of transition of phosphatidylethanolamine-containing bilayers from lamellar to hexagonal phase forms. The presence of high concentrations of phospholipid species that have been associated with hexagonal phase forms in the alkalophile membranes has already been noted [2]. The basis for a diacylglycerol-induced increase in permeability, its relationship to the isoprenoid effect, and its possible physiological significance have yet to be elucidated.

Acknowledgements

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