Neosynthesis of Cardiolipin in *Rhodobacter sphaeroides* under Osmotic Stress†

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ABSTRACT: The phospholipid composition of *Rhodobacter sphaeroides* cells resuspended in various hypertonic solutions has been examined by thin-layer chromatography and ESI mass spectrometry. *R. sphaeroides* responds to hyperosmotic stress by increasing the amount of cardiolipin in the membranes; this phenomenon occurs in spheroplasts also. Cardiolipin increases quickly and continuously during the time when the cells are resuspended in hypertonic medium. The optimum of stimulation of the neosynthesis of cardiolipin during osmotic stress was found to be at external 1 osm. ESI-MS analyses allowed the identification of two different cardiolipins in *R. sphaeroides*: the tetravaccenylcardiolipin ($[M - H]^-$, *m*/*z* 1456.9) and the trivaccenylmonopalmitoylcardiolipin ($[M - H]^-$, *m*/*z* 1430.0).

Anionic phospholipids play an important role in energy conversion, solute transport, protein transduction, and motility through both their contribution to the physical properties of the lipid phase of the membrane and their interaction with proteins (*1*−*3*).

Among anionic phospholipids, cardiolipin or bisphosphatidylglycerol (BPG)[1] is unique, having a dimeric structure with four acyl chains and two negative charges. It is typically present in membranes able to generate an electrochemical potential for substrate transport and ATP synthesis.

All of the proteins participating to the oxidative phosphorylation establish interactions with cardiolipin; for example, the removal of the cardiolipin from the respiratory protein cytochrome *c* oxidase renders this complex inactive (*4*). On the other hand, it is well-known that the addition of exogenous cardiolipin is essential to preserve the stability and functions of mitochondrial carrier proteins in the course of isolation and purification procedures (*5*).

Cardiolipin not only establishes specific interactions with all integral membrane proteins involved in the oxidative phosphorylation and mitochondrial carriers, but also is well documented in its interaction with the reaction center of the photosynthetic bacteria. Recently, the relevance of cardiolipin on structure and function of the reaction center of *Rhodobacter sphaeroides* and on its thermal stability has been pointed out by Jones and Allen (*6*, *7*) and Fyfe (*8*), respectively.

Cardiolipin represents about 20% of mitochondrial phospholipids, while in general it is less abundant in bacteria. However, looking carefully in the literature, discrepancies in the cardiolipin content of bacteria can be found; in some cases the amount of cardiolipin recovered in the lipid extract of eubacteria represented 80% of total phospholipids (*9*). The amount of cardiolipin found in some bacteria apparently improves by performing the lipid extraction after cell sonication or disruption (*10*).

In previous studies, the lipid composition of the purple non-sulfur photosynthetic bacteria has been described in detail; the cardiolipin content in the lipid extract of these bacteria ranges from about 2% to 14% (*11*, *12*).

In the present study we have performed investigations on the cardiolipin content of the photosynthetic bacterium *R. sphaeroides*, whose membrane contains as major polar lipids phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and sulfoquinovosyldiacylglycerol (SQDG). By comparison of the lipid composition of cells and chromatophores from *R. sphaeroides* it has been previously found that the cardiolipin content is higher in chromatophores than in the whole cells (*12*). Interestingly, we found that the exposition of *R. sphaeroides* cells to hyperosmotic stress induces an increase in the cardiolipin content; the possible role of this lipid is discussed.

MATERIALS AND METHODS

Materials. Lysozyme was obtained from Sigma. All organic solvents used were commercially distilled and of the highest available purity (Sigma-Aldrich). TLC plates (silica gel 60A), obtained from Merck, were washed twice with chloroform/methanol (1:1 v/v) and activated at 120 °C before use.

Microorganism Cultures. R. sphaeroides R-26 and wild-type 2.4.1 strains were grown in saturating light conditions in liquid medium containing peptone from lactalbumin (Fluka), as previously described (*13*, *14*), at room temperature. The cells were collected in the early stationary state by centrifugation and resuspended in 20 mM Tris-HCl, pH 7.5 (buffer A or control buffer).

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[1] Abbreviations: BPG, bisphosphatidylglycerol; ESI, electrospray ionization; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; SQDG, sulfoquinovosyldiacylglycerol; TLC, thin-layer chromatography.

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When not specified, the results are referred to the *R. sphaeroides* R-26 strain.

Preparation of Spheroplasts. Spheroplasts were prepared as previously described (*15*). Cells were centrifuged at 9000*g* for 10 min, and the pellet obtained was resuspended (20 mL/g wet weight) in 120 mM potassium phosphate (pH 8.0) containing 20 mM EDTA and 400 μg of lysozyme/mL. After 15 min of incubation at room temperature, the suspension was diluted 1:1 with water. The spheroplast suspension was centrifuged again at 7000*g* for 10 min, and the pellet was resuspended in 50 mM HEPES (pH 7.8) with 5 mM $MgSO_4$ (buffer B).

Study of the Effect of High External Osmolarity on the Lipid Composition. Equivalent aliquots of cells (about 1.4 g wet weight) were suspended in 40 mL of buffer A containing respectively 500 mM NaCl, 500 mM KCl, and 1 M sucrose. The cell suspensions were incubated in an open flask under gentle stirring for 2 h at room temperature (23−25 °C); lipids were extracted from each sample by the standard procedure (see below).

Study of the Effect of Increasing Osmolarity on the Cardiolipin Level in the Membranes of R. sphaeroides. Aliquots of cells (about 1.4 g wet weight) were suspended in 40 mL of buffer A containing 100, 200, 400, and 600 mM and 1 M NaCl, respectively, and incubated for 2 h at room temperature; then the lipids were extracted from each aliquot by the standard procedure (see below).

Time Course of Cardiolipin Increase during the Exposition of R. sphaeroides Cells to High External Osmolarity. Aliquots of cells (about 1.4 g wet weight) were suspended in 40 mL of buffer A containing 400 mM NaCl for different incubation times (15, 30, 60, and 180 min and 20 h) at room temperature; for each incubation time lipids were extracted from equivalent aliquots by the standard procedure (see below).

Lipid Extraction. Total lipids were extracted using the Bligh and Dyer method (*16*); the extracts were carefully dried under N_2 before weighting.

Thin-Layer Chromatography. Total lipid extracts were analyzed by TLC on silica gel (20 × 10 cm, layer thickness 0.2 mm). Lipids were eluted with the solvent chloroform/methanol/acetic acid/water, 85:15:10:3.5 (v/v), and detected by spraying with 5% sulfuric acid in water, followed by charring at 120 °C for 30 min.

Quantitative analyses of the cardiolipin content were performed by video densitometry using the software Image J (http://rsb.info.nih.gov/ij). The lipid standard curves were linear in the concentration range 0.2−3 μg.

Isolation and Purification of Cardiolipin. Preparative TLC of the total lipid extract was carried out on silica gel 60A plates (Merck, 20 × 20 cm, 0.5 mm thick layer) in the solvent chloroform/methanol/acetic acid/water, 85:15:10:3.5 (v/v). After scraping the silica in the band of cardiolipin from the plate, the lipid was extracted from the silica five times with chloroform/methanol, 1:1 (v/v). After centrifugation, the supernatants were combined and dried under a stream of N_2.

Mass Spectrometry. For negative ion mass spectrometry, lipids were converted in the ammonium salt form as described by Kates (*16*). Typically, 4.5 mL of 0.2 N HCl was added to a solution containing about 10 mg of lipids in 5 mL of chloroform/methanol (1:1 v/v). The biphasic system was mixed and centrifuged. After removal of the upper phase, the lower chloroform phase was washed twice with 4 mL of methanol/water (10:1 v/v). The chloroform phase (containing the free acid form of lipids) was immediately neutralized by addition of ammonium hydroxide to pH 7−8 and brought to dryness. The residue was dissolved in 0.5 mL of chloroform/methanol (1:1 v/v) and diluted with 10 mL of acetone. After being cooled at −20 °C overnight, lipids in the ammonium salt form were collected by centrifugation and dried. Dried samples were dissolved in chloroform/methanol (1:1 v/v).

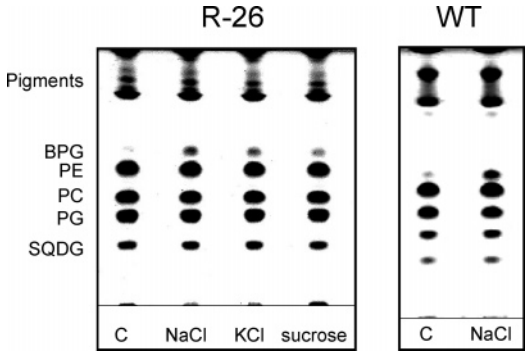

FIGURE 1: Effect of high external osmolarity on the lipid composition of *R. sphaeroides*. TLC separation of polar lipids isolated from *R. sphaeroides* R-26 (R-26) and 2.4.1 wild-type (WT) strains. Lipid extracts were obtained from equivalent aliquots of *R. sphaeroides* cells kept for 2 h at room temperature in buffer A (control) and buffer A containing 500 mM NaCl (NaCl) or 500 mM KCl (KCl) or 1 M sucrose (sucrose). 30 μg of each lipid extract has been loaded on the plate; after the run lipids were stained by spraying with 5% H_2SO_4 followed by incubation at 120 °C for 30 min.

Electrospray ionization mass spectra (ESI-MS) were obtained with a QSTAR hybrid Qq-TOF mass spectrometer (Applied Biosystems/MSD Sciex) equipped with a turbo ion spray interface. MS-MS measurements were carried out by fragmenting the target ions at proper collision energy (usually −80 eV).

Statistical Analysis. The GraphPad InStat software (Sigma, St. Louis, MO) was used to process the data by analysis of variance (ANOVA) to indicate statistically significant differences between means (one-way ANOVA with post-hoc Tukey test, $p < 0.05$).

All reported data represent mean values ± standard deviation obtained from four replicates.

RESULTS

Effect of High External Osmolarity on the Lipid Composition of R. sphaeroides Cells. In this study we have investigated the effects of hyperosmotic stress on the lipid composition of *R. sphaeroides* membranes and, in particular, on the cardiolipin content.

Initially, to evaluate the effects of high external osmolarity on the lipid composition of *R. sphaeroides*, we have analyzed by TLC the lipid extracts obtained from control cells and from cells exposed at high concentrations of different osmolites. Control cells were resuspended in 20 mM Tris-HCl (buffer A), while stressed cells were resuspended in the same buffer containing in addition 1 M sucrose or 500 mM NaCl or KCl.

Figure 1 shows the lipid profiles of the cells before and after osmotic shock of *R. sphaeroides* R-26 and 2.4.1 wild-type strains. Individual lipid components present in the

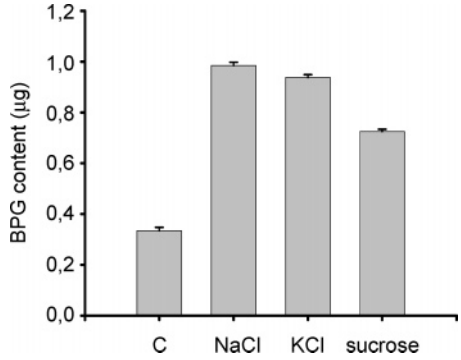

FIGURE 2: Video densitometric analyses of cardiolipin (BPG) content in *R. sphaeroides* in the presence of different osmolites obtained from TLC profiles.

extracts have been identified by comparison of their R_f values with those of authentic standard markers. The main lipid components of *R. sphaeroides* cells were identified (in R_f order) as SQDG, PG, PC, and PE, while cardiolipin or bisphosphatidylglycerol appears to be only a minor component; the pigments run with the solvent front. By comparison of the TLC profiles relative to *R. sphaeroides* R-26 (R-26), it is evident that the extracts obtained from cells incubated in hyperosmotic media contain relative amounts of SQDG, PG, PC, and PE similar to those of the cells suspended in isotonic medium (control cells), while the cardiolipin content is clearly increased. Similar results have been observed also for the *R. sphaeroides* 2.4.1 wild-type strain (WT).

The bar graph in Figure 2 illustrates the cardiolipin content of *R. sphaeroides* after incubation of cells in various hypertonic media. Data in Figure 2 indicate that the exposition of *R. sphaeroides* cells to high external osmolarity (1 osm) gives rise to an increase of cardiolipin levels for all of the osmolites tested. This increase is significantly ($p < 0.001$) higher in the presence of NaCl than that found at the same osmolarity of KCl and sucrose.

Cardiolipin represents only about 1% of the total lipid extract of control cells, while after osmotic shock it becomes two or three times higher.

Identification of Cardiolipin of R. sphaeroides. The lipid extract of *R. sphaeroides* has also been analyzed by ESI-MS; for this purpose, to avoid the interference of the abundant pigments, the polar lipids of the extracts have been precipitated in ice-cold acetone before mass spectrometry analyses. Figure 3A reports the ESI-MS (negative ions) spectrum of the polar lipid fraction of the extract of *R. sphaeroides* cells resuspended in 500 mM NaCl (see Materials and Methods).

In the high-mass range of the spectrum the molecular ions corresponding to the main membrane phospholipids and glycolipids of *R. sphaeroides* present are SQDG (36:1) at *m*/*z* 847.6, SQDG (34:0) at *m*/*z* 821.5, PG (36:2) at *m*/*z* 773.5, and PG (34:1) at *m*/*z* 747.5. A minor peak at *m*/*z* 742.5 has been attributed to the molecular ion of PE (36:2); obviously, the molecular ion of PC is absent because this lipid is very difficult to ionize in the negative ion mode. Furthermore, two bicharged peaks of about the same intensities at *m*/*z* 728.5 and *m*/*z* 714.5 are also present in the spectrum and correspond to the molecular ions, $[M - H]^{2-}$, of two different cardiolipins. These two peaks were not detectable in the ESI-MS spectrum of the polar lipids of control cells (not shown).

The lipid component identified as cardiolipin in the TLC of Figure 1 was isolated and purified by preparative TLC and analyzed by mass spectrometry. The ESI-MS(−) spectrum (not shown) of purified cardiolipin showed molecular ions at *m*/*z* 1456.9 and *m*/*z* 1430.0 $[M - H]^-$ and bicharged peaks at *m*/*z* 728.5 and 714.5 of about the same intensity. Although we have not performed an ESI-MS quantitative analysis, this last finding suggests that both cardiolipins are synthesized during osmotic stress.

To obtain information on the fatty acid composition of cardiolipins, we have also acquired the product ion spectrum of the ions at *m*/*z* 714.5 and 728.5 (Figure 3B,C). In Figure 3C an intense peak at *m*/*z* 281.3 indicates the presence of the C18:1 fatty acid in the molecule while in Figure 3B two peaks in the fatty acid region at *m*/*z* 255.2 and 281.3 corresponding to the carboxylate ion of C16:0 and C18:1,

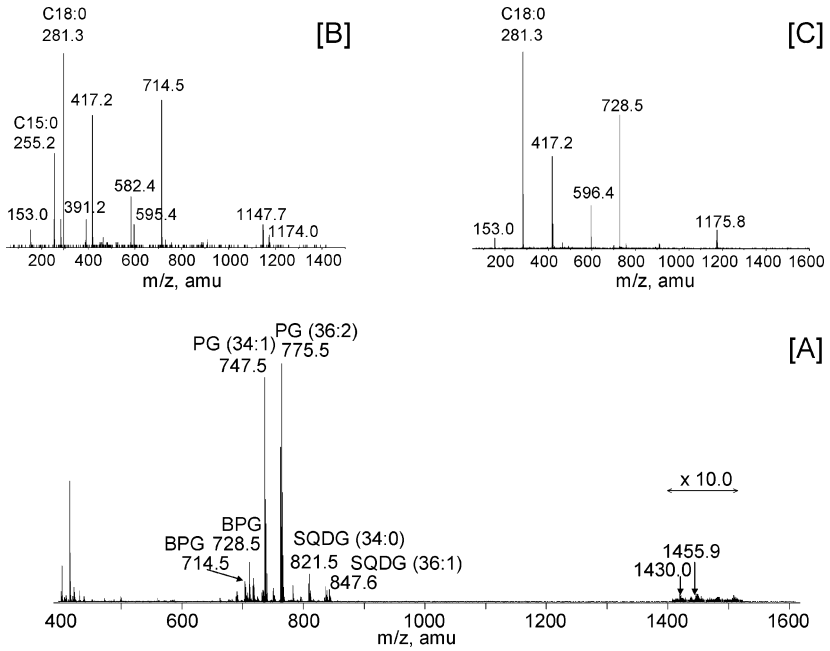

FIGURE 3: (A) ESI-MS (negative ions) of the total lipid extract of *R. sphaeroides* cells resuspended in buffer A containing 500 mM NaCl and product ion scan of the bicharged ions at *m*/*z* 714.5 (B) and *m*/*z* 728.5 (C).

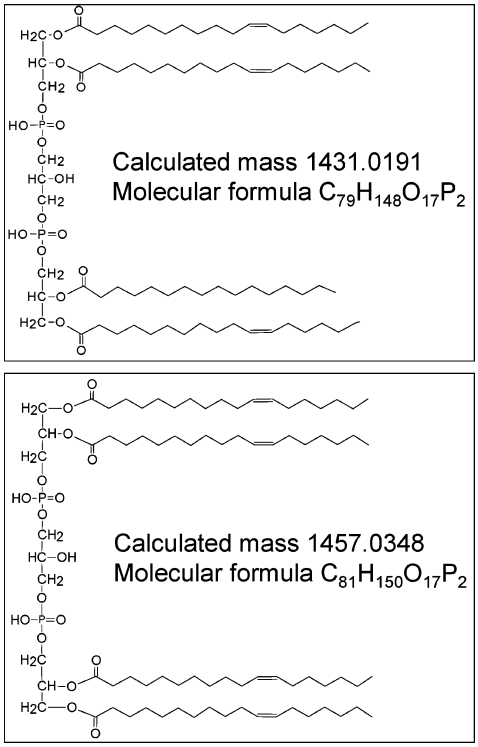

FIGURE 4: Structures of the two cardiolipins of *R. sphaeroides*.

respectively, are evident. On the basis of the work of others (*11*) we assume that the C18:1 tail corresponds to the vaccenic acid. On the basis of the above ESI-MS data the molecular structures of the two cardiolipins of *R. sphaeroides* appear to be those illustrated in Figure 4. The two cardiolipins result in being dimeric forms of the two different phosphatidylglycerol molecules present in the spectrum of the total lipid extract (Figure 3A) (*17*). As the two cardiolipins are clearly not distinguishable on TLC, in the following TLC data of this report we will use the term cardiolipin to indicate a mixture of the two different molecular species at about the same proportions.

Effect of Increasing Osmolarity on the Cardiolipin Level in the Membranes of R. sphaeroides. To shed light on the effect of increasing osmolarity on the cardiolipin level in the membranes of *R. sphaeroides*, the cells have been incubated in the presence of different external concentrations of NaCl. The lipid extract of cells in the various experimental conditions has been analyzed by TLC. Figure 5 illustrates the different cardiolipin amount present in the lipid extracts of cells exposed to increasing NaCl concentrations, determined by video densitometry. Interestingly, up to external 500 mM NaCl the cardiolipin content in the lipid extract significantly ($p < 0.001$) increases with increasing osmolarity, while at higher NaCl concentrations, the cardiolipin content decreases.

This finding suggests that the bacterial cardiolipin synthase activity is stimulated under hyperosmotic conditions, being the optimum of the enzyme activity at the external osmolarity of about 1 osm.

Time Course of the Cardiolipin Increase in Response to Osmotic Stress. To follow and quantitate the changes in cardiolipin content in *R. sphaeroides* membranes during the exposition to high external osmolarity, the cells were resuspended in hyperosmotic medium, and lipids were

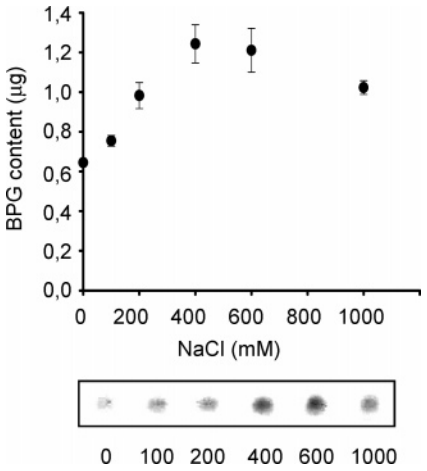

FIGURE 5: Effect of increasing osmolarity on the cardiolipin level in the membranes of *R. sphaeroides*. Values of cardiolipin (BPG) content (μg) on the *y* axis have been estimated by video densitometry. Lipids were extracted from equivalent cell aliquots incubated for 2 h in media with different NaCl concentrations (see Materials and Methods). 30 μg of each extract has been loaded on the plate; staining was as in Figure 1.

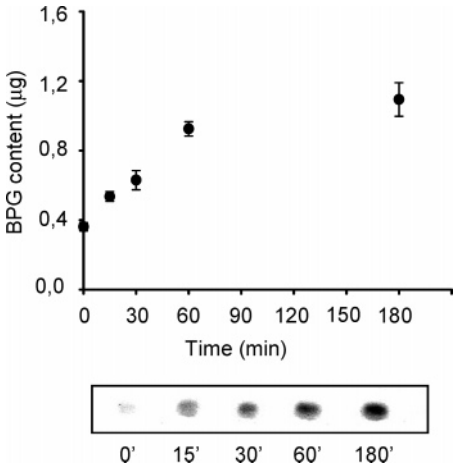

FIGURE 6: Time course of the increase of the cardiolipin (BPG) content of *R. sphaeroides* during the exposition to high external osmolarity. Cells were resuspended in buffer A containing 400 mM NaCl. Lipid extracts were obtained from equivalent cell aliquots at different incubation times (0, 15, 30, 60, and 180 min and 20 h) in the high-salt medium. The cardiolipin content has been estimated by video densitometry. 30 μg of lipid extracts has been loaded on the plate; staining was as in Figure 1.

extracted from equivalent aliquots of cells taken at different time intervals from the start of hyperosmotic shock; then the different lipid extracts were analyzed by TLC. Figure 6 shows the time course of cardiolipin increase during the osmotic shock obtained by video densitometric analysis. As can be seen, the cardiolipin amount increases continuously in the cells resuspended in hyperosmotic medium; in particular, we have estimated that 3 h after the osmotic shock a significant ($p < 0.001$) amount of cardiolipin (0.9 ± 0.1 μg over 30.0 ± 0.5 μg of total lipids) was newly formed in the cells. After 20 h of incubation, the amount of newly formed cardiolipin is even higher, being about 1.2 μg (not shown).

Response of Spheroplasts to Osmotic Stress. Previous results demonstrated that in some microorganisms the removal of the cell wall affects the composition of the plasma membrane; in particular, in *Staphylococcus aureus* the

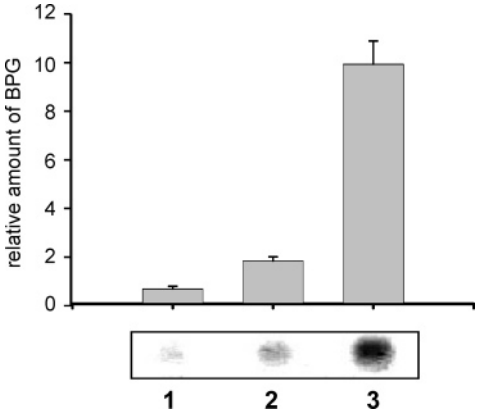

FIGURE 7: Cardiolipin enrichment in spheroplasts after incubation in hypertonic medium. Cardiolipin (BPG) content in (1) *R. sphaeroides* cells resuspended in buffer A (control), (2) spheroplasts prepared as described in the Materials and Methods section and resuspended in buffer B, and (3) spheroplasts after incubation for 1 h in buffer B containing in 400 mM NaCl. 30 μg of lipid extracts has been loaded on the plate; staining was as in Figure 1. The bar graph illustrates the results of video densitometric analyses; BPG in samples 2 and 3 has been normalized to the amount of BPG in control cells.

removal of the cell wall by lytic enzymes caused an increase in cardiolipin content (*18*). Therefore, we have compared the lipid composition of spheroplasts with that of intact cells; in addition, we have also analyzed the lipid composition of spheroplasts exposed to hyperosmotic stress.

The TLC in Figure 7 compares the lipid extract of spheroplasts with that of intact cells and, furthermore, illustrates the composition of the lipid extract of spheroplasts resuspended in 500 mM NaCl. It can be clearly seen that (a) the cardiolipin content of spheroplasts is higher ($p < 0.05$) than that of intact cells and (b) the cardiolipin level in spheroplasts significantly ($p < 0.001$) increases after osmotic stress. It is concluded that the neosynthesis of cardiolipin induced by osmotic stress is only in part related to the cell wall lysis and that this phenomenon can also occur in spheroplasts. Quantitative estimation of cardiolipin present in the different samples of Figure 7 revealed that after osmotic shock cardiolipin of spheroplasts becomes 10 times higher. Therefore, the extent of cardiolipin increase in spheroplasts in the presence of external 500 mM NaCl is higher than that observed in intact cells, suggesting that the ability of *R. sphaeroides* to neosynthesize cardiolipin appears to be increased after removal of the cell wall.

DISCUSSION

The capacity of organisms to respond to fluctuations in their osmotic environments is an important physiological process that determines their abilities to thrive in a variety of habitats. When bacteria encounter unfavorable conditions, they activate their stress-response mechanism, which enables them to survive in a hostile environment. One of the important physiological processes in all cells is the ability to maintain the internal osmolarity at a relatively invariant level in face of fluctuations in the osmolarity of the environment. To survive osmotic stresses, the cells of most organisms need to adapt by accumulating specific solutes under hyperosmotic conditions and releasing them under hypoosmotic conditions. These solutes are called "compatible solutes" because they can be accumulated to high levels by de novo synthesis or transport without interfering with cellular processes. Many low-molecular-weight, relatively nontoxic, water-soluble organic compounds, such as sugars, polyols, betaines, and amino acid derivatives, serve to maintain the proper balance between the external and internal osmolarity.

Bacteria use ion-motive-force driven transporters of the ATP binding cassette to accumulate compatible solutes (*19*). It has been reported that two strains of halophilic photosynthetic bacteria, *Rhodovulum* sp. CP-10 and *Rhodobacter* sp. RS-1, cultured in different salt concentrations, accumulated three particular solutes, identified as ectoine, glycine betaine, and glutamic acid (*20*).

Moreover, a number of studies have shown that microorganisms alter their membrane lipid composition during osmotic shock. An increase in the amount of glycolipids was observed in *Bacillus subtilis* (*21*) and in *Saccharomyces cerevisiae* (*22*) growing in hypertonic NaCl medium. Interestingly, with regard to the phospholipids, when *B. subtilis* cells were grown in hypertonic medium, an increase in the cardiolipin content at the expense of PG was observed (*21*). A rise in the cardiolipin content was also found in *Escherichia coli* (*23*) and *S. aureus* (*18*) cultured in or exposed to hypersaline medium.

Changes in the lipid composition also occur when microorganisms living in a hypersaline environment are subjected to hypotonic stress. Upon osmotic downshift the cells of a halophilic archaeon of the genus *Halorubrum* synthesize archaeal cardiolipin; by comparison of the quantities of various lipid components in the lipid extracts of swelling cells, it has been shown that, in correspondence of the archaeal cardiolipin increase, a decrease of archaeal PG occurs (*24*). In the case of *Halobacterium salinarum* the archaeal glycocardiolipin is a nonabundant phospholipid whose levels increase in response to hyperosmotic stress; the glycocardiolipin increase in *H. salinarum* induced by osmotic stress occurs at the expense of its precursor, the glycolipid S-TGD-1 (*25*).

It has been suggested that the cardiolipin increase occurring in cells during swelling or shrinkage may represent a general physiological response of microorganisms to osmotic stress and that a higher cardiolipin cell content could protect the cell from lysis, confering an increased resistance of the membrane to the rupture.

Data in the present report are consistent with this novel role for cardiolipin in osmoadaptation.

We have shown that cardiolipin levels in *R. sphaeroides* cells increase as a consequence of osmotic stress. Since cardiolipin is synthesized at the expense of phosphatidylglycerol, a decrease of this precursor should be expected; however, due to the high amount of phosphatidylglycerol in the lipid extract of control cells it is difficult to detect the small decrease. Besides cardiolipin increase, no other changes in lipid composition have been found. ESI-MS analyses allowed the identification of two different cardiolipins, whose structure results in being the dimeric form of two different PG molecules present in this microorganism. The tetravaccenylcardiolipin ($[M - H]^-$, *m*/*z* 1456.9) has been previously found to be associated with the photosynthetic reaction center (*7*), while this paper reports for the first time the presence of the trivaccenylmonopalmitoylcardiolipin ($[M - H]^-$, *m*/*z* 1430.0) in *R. sphaeroides*. Although a heterogeneity in the

cardiolipin molecular species was also found in mitochondrial membranes (*26*), it cannot be excluded that the two cardiolipins might be asymmetrically distributed in the different membrane domains of *R. sphaeroides*.

Our results suggest that the bacterial cardiolipin synthase activity is stimulated under hyperosmotic conditions, being the optimum of the enzyme activity in intact cells at the external osmolarity of 1 osm. Interestingly, in spheroplasts, i.e., after the cell wall removal by lysozyme, the neosynthesis of cardiolipin induced by osmotic shock still occurs and appears to be even more considerable.

Many different signals may activate membrane proteins during osmotic stress; for example, during osmotic upshift, water will flow out of the cell, and the turgor will decrease, causing an alteration in the protein−lipid interactions, which could directly affect the activity of cardiolipin synthase.

The modifications in lipid composition following the cardiolipin synthase activation could affect the physicochemical properties of the membrane such as bilayer tickness, membrane fluidity, and transport properties.

It should be considered that the conversion of two PG molecules to cardiolipin plus glycerol increases the order on the membrane surface and that cardiolipin may be engaged in the regulation of ionic lateral conduction by the plasma membrane, because it is well-known that the glycerol moiety of the cardiolipin molecule takes part in the lateral conduction of protons through H-bonded networks (*27*). The increase in cardiolipin concentration might behave as a barrier against the high ionic level or contribute to the active transport (*28*). In addition, the free glycerol, a well-known osmoprotectant, could contribute to the resistance of the cell to osmotic stress. Crystallographic data available both on cytochrome *c* oxidase and on the bacterial reaction center have shown that cardiolipin mediates the interactions between different subunits in these integral protein systems (*8*). Cardiolipin, due to its extraordinary protein affinity and to the ability to establish bridging contacts between different units in the quaternary structure of protein systems involved in the bioenergetic processes of the cell, would help in keeping these systems active even when the membrane is under mechanical tension.

In conclusion, the increased cardiolipin levels in the bacterial membranes would help to sustain the cell bioenergetic systems under osmotic stress conditions. Excess or scarcity of solutes in the extracellular environment should induce an increase of the rate of ATP consumption. The neosynthesized cardiolipin in the membrane could contribute to render the ATP synthesis more efficient.

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