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The effect of salt on the lipid composition of *Ectothiorhodospira*

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Abstract. Major components of polar lipids of halophilic phototrophic *Ectothiorhodospira* species were PG, CL, PC and PE. PA was only present in minor amounts. According to ^{14}C-incorporation, polar lipids approximated to 75%–93% of the total lipid carbon. With increasing salinity, a strong increase in the portion of PG and a decrease in that of PE (especially in *Ectothiorhodospira mobilis* BN 9903) and CL (especially in *E. halophila* strains) were observed. Moreover, there was a significant increase in the excess negative charges of phospholipids upon increasing medium salinity. This increase was most dramatic in the slightly halophilic *E. mobilis* BN 9903, but quantitatively less important in both strains of *E. halophila* which had, however, a higher percentage of negative charges of their lipids. During salt-shift experiments, *E. halophila* BN 9630 responded to suddenly increased salinity by promoting the biosynthesis of PG and decreasing that of PC, CL and PE. Upon dilution stress, responses were reversed and resulted in a strong increase in PE biosynthesis. The effects of lipid charges and bilayer forming forces in stabilizing the membranes of *Ectothiorhodospira* species during salt stress are discussed.

Key words: *Ectothiorhodospira* – Phospholipids – Salt adaptation

Halophilic bacteria are adapted to best growth conditions at some optimum salt concentration, and conditions of stress are only found at salt concentrations above the optimum values. These and also halotolerant bacteria have developed mechanisms to cope with this stress. Adaptational mechanisms include selective accumulation of compatible solutes (Imhoff 1986; Csonka 1989; Trüper and Galinski 1989, 1990) as well as structural changes (Kushner and Onishi 1966; Kennedy and Rumley 1988) to maintain integrity of macromolecules and membranes under conditions of varying sailinities. One important aspect of structural adaptation are changes in the composition of cell envelopes and membranes. Well known examples are the highly negatively polarized cell envelopes of extremely halophilic archaeobacteria, the Halobacteriaceae (Lanyi 1974; Brown 1963). Structural adaptation of membranes mainly involves alterations in the composition of proteins, lipids and fatty acids. Adaptational changes in the lipid composition of membranes of several eubacteria revealed principal differences in the reactions of gram-negative and gram-positive halotolerant bacteria (see Kates 1986). Typical of the former group is an increase in the portions of PG at the expense of PE, while in the latter one there are increasing portions of CL while those of PG decrease at higher salinities. All known examples have in common a resultant increase in the negative polarity of membrane lipids which is primarily interpreted in terms of enhanced charge screening (Ohno et al. 1979; Hiramatsu et al. 1980) and altered membrane permeability for ionic solutes (Kanemasa et al. 1972; Ohno et al. 1976, 1979).

Anoxygenic phototrophic *Ectothiorhodospira* species represent a group of halophilic bacteria which require alkaline and saline growth conditions (Imhoff 1989). They are gram-negative and have a lipid composition similar to other gram-negative bacteria (Asselineau and Trüper 1982; Imhoff et al. 1982). For the first time, comparative analyses of the salinity-dependent lipid composition of slightly, moderately and extremely halophilic bacteria belonging to one and the same genus have been performed, which we report of in the present communication.

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Abbreviations: PC, phosphatidylcholine; PG, PG-1, PG-2, phosphatidylglycerol; CL, CL-1, Cl-2, cardiolipin; PE, phosphatidylethanolamine; PA, phosphatidic acid; NL, nonpolar lipids; ori, origin; TLC, thin layer chromatography

Materials and methods

Strains and culture conditions

The *Ectothiorhodospira* strains investigated were *E. mobilis* strain BN 9903 (salt optimum 5% w/v total salts), *E. halophila* strain BN 9630 (salt optimum 15%) and *E. halophila* strain BN 9626 (salt optimum 25%). These strains were isolated from the Wadi Natrun in Egypt (Imhoff et al. 1979; Imhoff, unpublished results) and kept in the collection of anoxygenic phototrophic bacteria in our institute. For growth, the modified medium for *Ectothiorhodospira* species, as described by Imhoff (1988), was used (amounts per liter): 1 ml of trace element solution SLA (Imhoff and Trüper 1977), 0.8 g KH_2PO_4, 2.0 g Na-acetate, 1.0 g $Na_2S_2O_3 \cdot 5\ H_2O$, varying amounts of NaCl (to obtain the desired salinity which is approximately 2% w/v without added NaCl). The components were dissolved in 600 ml of distilled water. Then 200 ml 1 M Na-carbonate buffer (pH 9) was added and the solution autoclaved after volume adjustment. The following solutions were sterilized and added separately: 2% $MgCl_2 \cdot 7\ H_2O$ (5 ml/l), 1% $CaCl_2 \cdot 2\ H_2O$ (5 ml/l), 5% $Na_2S \cdot 9\ H_2O$ (5–10 ml/l), 20% NH_4Cl (4 ml/l). Total salt concentrations were adjusted in steps of 2.5% from 2.5% to 15% for *E. mobilis* BN 9903, from 10% to 25% for *E. halophila* BN 9630 and from 15% to 30% for *E. halophila* BN 9626.

Cultures for lipid analyses were grown in 50-ml screw capped bottles, inoculated with 10% of a fresh preculture and incubated at 37° C under illumination of 12000 lux until grown to late exponential phase. For shift experiments with *E. halophila* BN 9630, a 100 ml fermentation vessel was used allowing growth and sampling under nitrogen flux at a constant temperature of 37° C; illumination was adjsuted to 25000 lux. All cultures were grown over four transfers under identical conditions prior to incubation with radioactive tracers for lipid analyses.

Extraction of lipids

Lipids were extracted by a modification of the Bligh and Dyer method (1959) as described by Kates (1972). Cells were harvested by centrifugation (Sorvall RC-5, SS34 rotor, 15000 rpm), resuspended in distilled water and chloroform/methanol 1:2 (v/v) was added to the extraction mixture to obtain a ratio of chloroform/methanol/water of 1:2:0.8 (v/v/v). Cell debris was removed by centrifugation as specified above and the pellet reextracted until supernatant and pellet became colourless. By adding chloroform and water to supernatants, the ratios of chloroform/methanol/water were brought to 1:1:0.9 (v/v/v). For separation and collection of the lipid containing lower chloroform phases, mixtures were centrifuged in 30 ml Corex tubes for 20 min at 8000 rpm. Small amounts of benzene and ethanol were added to the chloroform phases, which were then brought to dryness in a rotary evaporator. Finally, lipids were redissolved in a small volume of chloroform/methanol 4:1 (v/v) and this solution, containing the total lipid fraction, was used for further analyses.

Analyses of lipids

Separation of the total lipid fraction was performed by two dimensional thin layer chromatography. Aliquots of the lipid extracts were spotted on heat-activated silica TLC plates (Sil G25, UV_{254}, Macherey and Nagel, Düren, FRG), which were developed in two dimensions by using chloroform/methanol/water 85:25:4 (v/v/v) and chloroform/methanol/acetic acid/water 85:15:10:3.5 (v/v/v/v) as solvent systems for the first and second dimension, respectively. TLC plates were always carefully dried between the two runs. For detection and identification of lipids standards of PE, PG, CL, PA, PC lyso-PE, lyso-PC (all from Sigma, Deisenhofen, FRG) and various group specific spray reagents were applied to the plates. The spray reagents were: ninhydrin for primary amino groups (Kates 1972), the Dragendorff reagent for quarternary ammonia compounds (Stahl 1967), alpha-naphtol for glycolipids (Stahl 1967), the periodate-Schiff reagent for both glycolipids and other vicinal hydroxyl group containing lipids (Kates 1972) and the phosphate reagent (Vaskovsky and Kostetsky 1968).

Radiochemical analyses and quantification of lipids

Tracer incorporation of either ^{14}C-acetate or $^{32}PO_4^{3-}$ (Amersham, Buckinghamshire, UK) during growth was used to label the lipids. The amount of the carbon- and phosphate tracers, added together with inocula to 50-ml bottles, were 1201 µmol (spec. activity 33 µCi/mmol) and 294 µmol (spec. activity 221 µCi/mmol), respectively. For shift experiments with *Ectothiorhodospira halophila* BN 9630, 305 µmol of ^{14}C-acetate (spec. activity 656 µCi/mmol) and 73 µmol of $^{32}PO_4^{3-}$ (spec. activity 2725 µCi/mmol) were applied. In shift-up experiments, 40-ml cultures were grown in media of 15% salinity and shifted to 25% salinity by adding appropriate volumes of 30% saline medium (containing the tracers). In shift-down experiments, 50-ml cultures of 25% total salinity were shifted to 15% by the addition of medium containing 5% total salts. Appropriate dilution experiments under isoosmotic conditions were used as control experiments. Time courses of lipid adaptation in correlation to growth under conditions of sudden osmotic stress were investigated; 10-ml portions of cell suspension were removed in time intervals after the shifts, lipids were extracted, separated by two dimensional TLC and tracer incorporation into main phospholipids examined. Lipid spots on TLC plates were detected by autoradiography on Hyperfilm MP (Amersham) with sheet sizes of 18 × 20 cm. On the basis of autoradiographs lipid spots were marked on the TLC plates and then directly scraped off and transferred into liquid scintillation vials, supplied with 5 ml of Lumagel scintillation cocktail (Baker, Deventer, NL) and counted in a Beckman LS 230 scintillation counter using the external standard method. All experiments were done in duplicate.

Protein determination

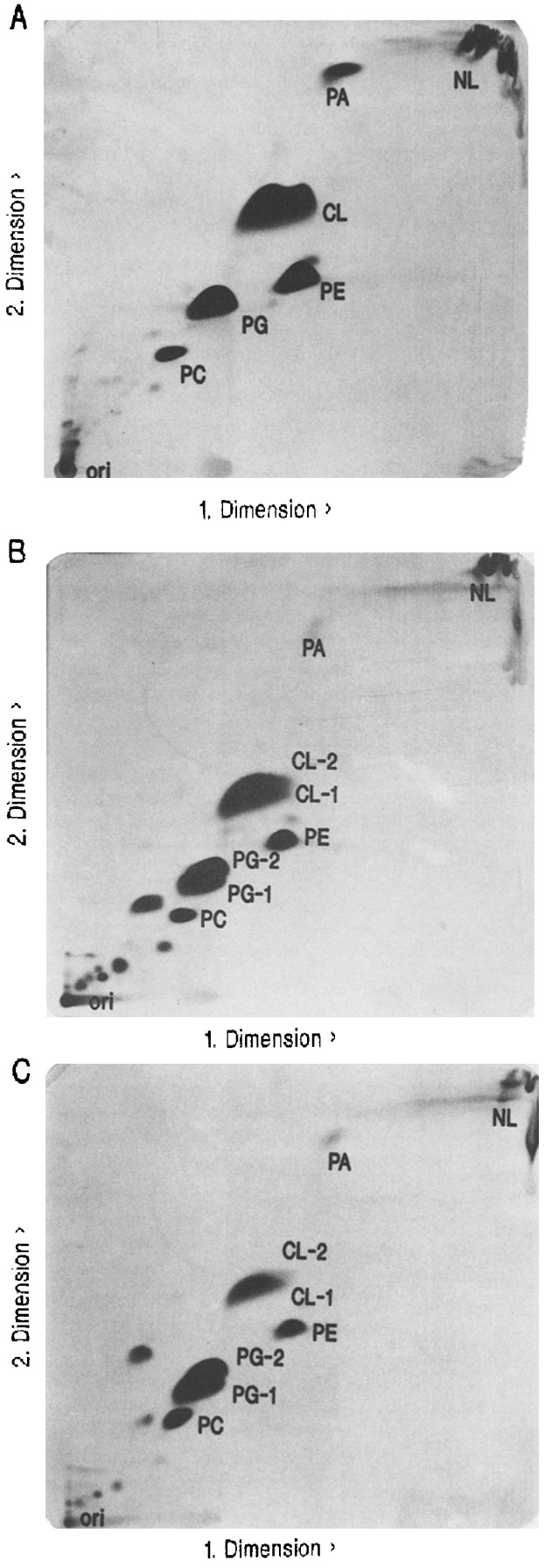
The Lowry method (Lowry et al. 1951) involving destaining and protein precipitation of whole cells by acetone/methanol 7:2 (v/v) extraction was used to monitor growth.

Results

Patterns of lipid composition

Major phospholipids of all three strains were PC, PG, PE and CL (Fig. 1) but only minor amounts of PA could be detected. Lyso-PE and lyso-PC as well as residual organic components were present each in amounts of less than 1% of the total polar lipids. These minor components proved to be insignificant for the process of salt adaptation because of their small contents and only insignificant changes with varying salinities.

The phospholipids PG-2 and CL-2, which were only present in strains of *Ectothiorhodospira halophila*, deserved special attention. On thin layer plates both move very close to the main components PG-1 and CL-1, respectively. PG-2, like PG-1 also stains with Schiff's reagent. Fatty acid analyses for PG-2 and CL-2 cf. PG-1 and CL-1 yielded high contents of C 18:1 and C 14:0



while those of C 16:0, C 16:1 and C 18:0 were significantly lower (Table 1). These differences obviously led to separation on TLC plates. We assume them to be fatty acid variants of the respective main components PG-1 and CL-1. Because of headgroup identity, the sum of both was included into the calculations of salinity-dependent phospholipid changes.

Salt dependence of lipid composition

The total lipid content of the cells was largely constant and independent of the salinity, with 0.3 to 0.4 mg lipid/mg protein for *E. mobilis* BN 9903 and both strains of *E. halophila*. Based on ^{14}C-incorporation, portions of polar lipids and photosynthetic pigment containing nonpolar lipids showed only small variations without clearly salt-dependent trends. On the average the polar lipid content was 88.3% (range: 87% to 90%), 82.4% (range: 75% to 87%) and 91.3% (range: 89% to 94%) of total lipids for *E. mobilis* BN 9903, *E. halophila* BN 9630 and *E. halophila* BN 9626, respectively. At identical salinities portions of main phospholipids were similar among the strains of *E. halophila*, whereas both clearly differed from respective portions of *E. mobilis* BN 9903 (Table 2). With ^{14}C-acetate as well as $^{32}PO_4^{3-}$ (Table 2 cf. Fig. 2), major changes in the phospholipid compositions on increasing salt concentrations were observed in the portions of PG, PE and CL without significant tracer specific differences

Table 1. Fatty acid composition of PG-1, PG-2, CL-1 and CL-2 of *Ectothiorhodospira halophila* BN 9630 and BN 9626 (*in brackets*) grown at optimum salinities (in percent of the total fatty acids)

	PG-1	PG-2	CL-1	CL-2
C 12:0	0.1	tr	0.1	tr
	(0.1	tr	tr	0.5)
C 14:0	0.3	0.7	0.7	2.0
	(0.7	2.6	2.2	3.3)
C 16:0	19.2	6.1	19.0	7.1
	(22.5	8.2	22.8	6.3)
C 16:1	0.6	tr	0.9	tr
	(tr	tr	tr	tr)
C 18:0	21.3	10.0	21.4	14.0
	(18.9	tr	19.5	7.9)
C 18:1	54.6	81.7	53.3	69.5
	(49.5	66.3	29.5	37.6)
C 19(cy)	1.8	tr	1.8	1.8
	(2.6	4.0	3.0	tr)
C 20:0	1.4	0.7	1.6	2.3
	(1.9	2.7	4.8	3.6)

tr, trace amounts of less than 0.05%

Fig. 1A–C. Autoradiographs of total lipid fractions (labeled with ^{14}C-acetate) separated by two dimensional thin layer chromatography. Lipids of *Ectothiorhodospira mobilis* BN 9903 (**A**), *E. halophila* BN 9630 (**B**) and *E. halophila* BN 9626 (**C**) grown at optimum salinities of 5%, 15% and 25%, respectively

Table 2. Salt dependence of phospholipid composition after ^{14}C-incorporation (in percent of the respective phospholipids) of *Ectothiorhodospira mobilis* BN 9903 (*A*), *E. halophila* BN 9630 (*B*) and *E. halophila* BN 9626 (*C*)

A

	Salinity (% w/v)					
	2.5	5.0	7.5	10.0	12.5	15.0
PC	6.5	4.9	5.1	6.6	4.5	5.0
PG	39.9	46.6	51.6	53.1	57.3	56.3
PE	38.0	35.8	28.6	24.6	24.8	23.8
CL	12.9	9.9	12.5	12.9	9.6	9.3
PA	4.0	2.9	2.3	2.8	3.8	5.6
PENC[a]	25.2	28.6	45.2	50.5	51.0	51.7
% BL[b]	46.4	51.4	56.6	59.7	61.8	61.3

B

	Salinity (% w/v)						
	10.0	12.5	15.0	17.5	20.0	22.5	25.0
PC	9.5	10.9	8.8	8.8	11.8	10.6	10.8
PG	55.7	66.6	68.3	72.5	74.4	77.5	76.7
PE	16.8	10.5	12.7	11.6	7.5	8.4	7.1
CL	17.3	11.7	10.0	7.0	6.1	3.4	2.4
PA	0.9	0.3	0.4	0.3	0.2	0.1	3.0
PENC	64.9	68.9	67.2	66.4	67.5	65.4	66.6
% BL	65.0	77.5	77.1	81.1	86.2	88.1	87.5

C

	Salinity (% w/v)						
	15.0	17.5	20.0	22.5	25.0	27.5	30.0
PC	10.6	10.3	14.0	12.2	13.3	11.4	9.7
PG	65.9	71.1	74.5	72.4	77.4	80.8	82.2
PE	11.1	9.1	6.1	7.6	5.9	5.7	6.8
CL	12.0	9.2	5.8	4.5	3.0	0.8	0.7
PA	0.3	0.3	0.1	3.3	0.4	1.3	0.6
PENC	68.5	70.4	66.1	64.9	64.6	66.6	67.7
% BL	76.6	81.4	88.5	84.6	90.7	92.2	91.9

[a] Percentual excess of negative charges: %(PG + 2 CL + PA) − % (PE + PC)
[b] Percent of bilayer stabilizing lipids: % (PG + PC)

covering the general trend. A general tendency was an increase in the portions of PG accompanied by decreases in the portions of both, PE and CL. Highest contents and strongest decreases of PE were found in *E. mobilis* BN 9903. While in this strain decreases in PE content completely predominated over those in CL content, decreases in portions of CL dominated in the strains of *E. halophila*, most significantly in *E. halophila* BN 9626. On the other hand, contents of PC and PA showed no clear trends. Portions of PC were higher in the more halophilic strains, increasing in the order *E. mobilis* BN 9903, *E. halophila* BN 9630 and *E. halophila* BN 9626. Portions of PA, however, were significantly lower in the *E. halophila* strains compared to *E. mobilis* BN 9903.

The importance of changes in portions of PG is demonstrated by increases in all three strains, by the size of the changes and by a strongly increased basic portion e. g. at the lowest growth supporting salinity (or at the salt optimum) again in the order of increasing halophilicity.

The dominance of antagonistic changes of anionic PG and zwitterionic PE in *E. mobilis* BN 9903 led to a strongly increasing excess of negative charges. In this strain the percentual excess of negative charges doubled over the entire salt range from 2.5% to 15% total salts (Table 2). Values for both *E. halophila* strains, however, showed only small variations. Obviously, this is largely due to the stronger decrease in portions of double anionic CL accompanied by comparatively low changes of portions of neutral PE.

Salt-shift experiments

These experiments were made with *E. halophila* BN 9630 by shifting the salintiy of the medium from 15% to 25% and vice versa. Control experiments were made by simply diluting the cultures with medium without changing the salt concentrations. Phases of initial adaptive responses

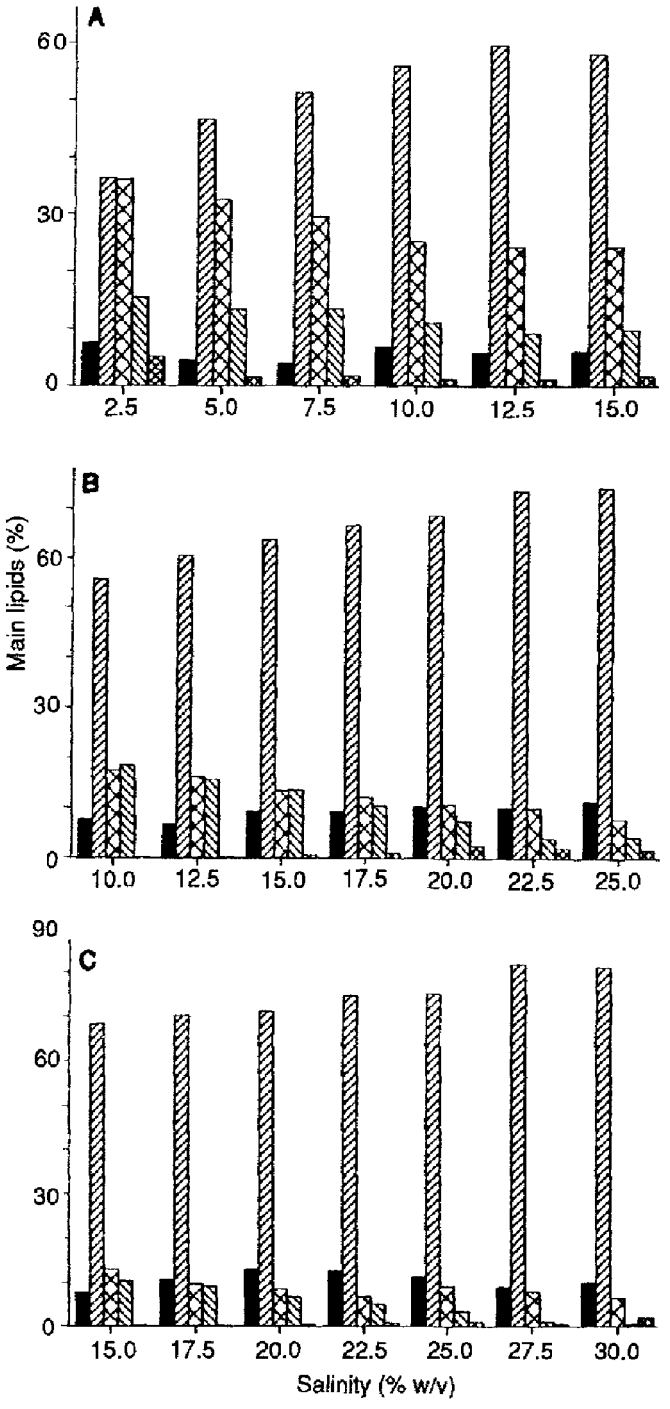


Fig. 2. Portions of the main phospholipids PC (■), PG (▨), PE (⊠), CL (▧) and PA (▩) of *Ectothiorhodospira mobilis* BN 9903 (A), *E. halophila* BN 9630 (B) and *E. halophila* BN 9626 (C) grown at varying salinities. Cultures were grown in the presence of $^{32}PO_4^{3-}$ and portions of individual main phospholipids were determined after tracer incorporation (in percent of the total incorporation of $^{32}PO_4^{3-}$ into the main phospholipid fraction)

(during the lag interval after the salt-shift) could be distinguished from subsequent intervals of adjustment, when growth was resumed. According to the lipid composition, absolute and relative biosynthetic rates of PG remained principally highest under all conditions, those of PE and CL at intermediate and those of PC and PA at lowest levels.

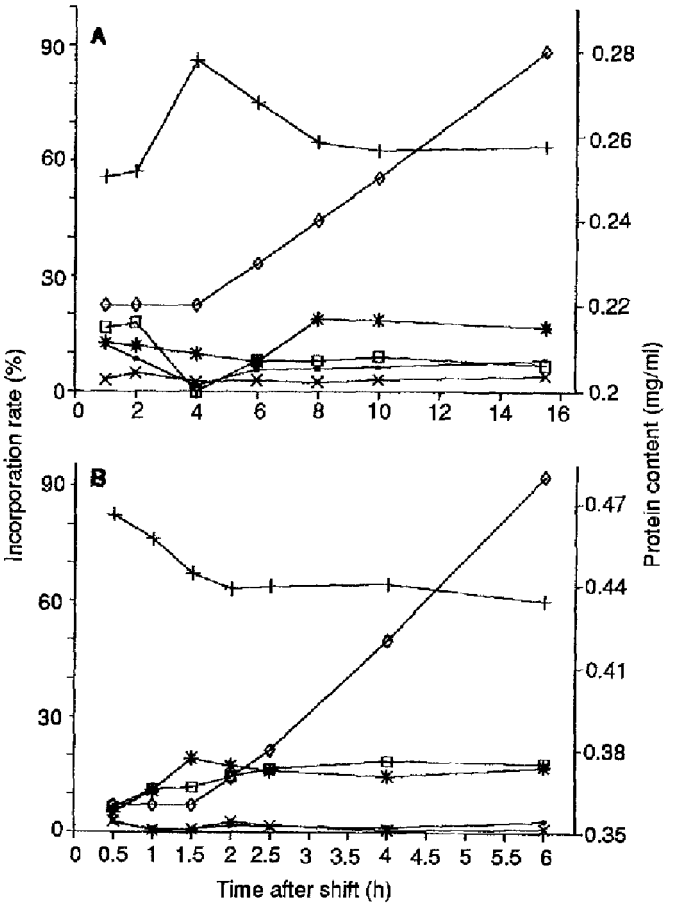


Fig. 3. Incorporation of ^{14}C-acetate into phospholipids of *Ectothiorhodospira halophila* BN 9630. Cultures were pregrown at 15% salinity which was either adjusted to 25% (A, shift-up) or held constant (B, control). ^{14}C-acetate was added at the moment of shift. Incorporation rates into phospholipids are given in percent of the total incorporation · mg protein^{-1} · h^{-1} during the considered time interval. *Symbols*: (-■-) PC, (+) PG, (*) PE, (⊖) CL, (-*-) PA, (-◈-) protein (mg/ml)

Shift-up. The absolute incorporation rates remained low during the time of adaptation before growth was resumed 4 h after the shift-up. At this time, cells started to grow and the rate of total lipid biosynthesis strongly increased (incorporation of ^{14}C-acetate: from 41 to 188 dpm · mg protein^{-1} · h^{-1} · 10^3 between the fourth and sixth hour after shift-up), and a level was reached which remained high until the end of the epxeriment. During the initial lag phase incorporation rates were particular low within the first hour, had a maximum during the second hour (incorporation of ^{14}C-acetate: 99 dpm · mg protein^{-1} · h^{-1} · 10^3) and decreased thereafter until growth started. In order to relate the biosynthetic rates of individual phospholipids to the lipid composition, in the following we will consider biosynthetic rates of individual phospholipids relative to total lipid biosynthesis.

During the first 2 h after increasing the salinity from 15% to 25%, relative incorporation rates of ^{14}C-acetate into the different lipids remained nearly constant (Fig. 3 A). Thereafter, relative to the biosynthesis of other lipids, the biosynthetic rate of PG drastically increased while those of PC, PE and CL decreased. This effect was

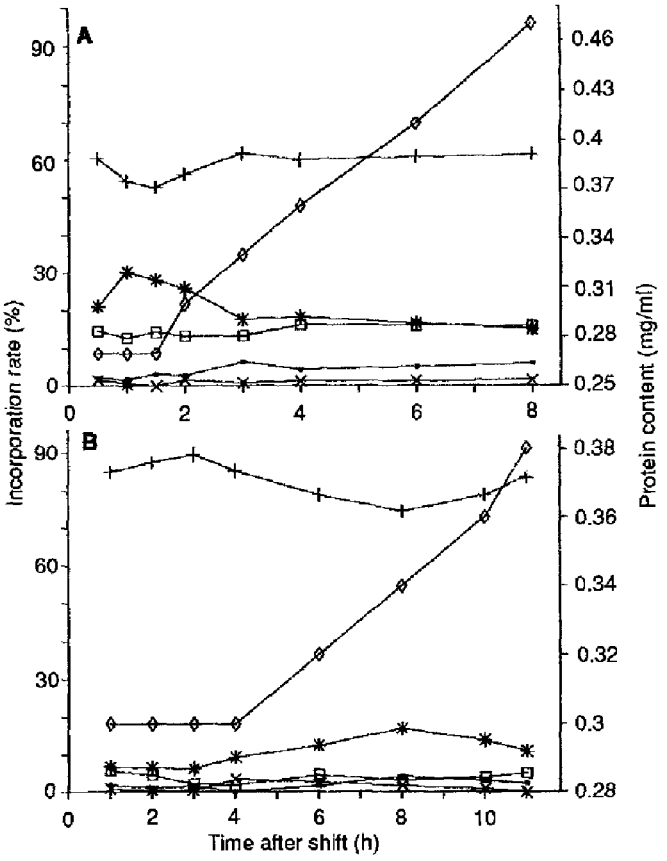


Fig. 4. Incorporation of ^{14}C-acetate into phospholipids of *Ectothiorhodospira halophila* BN 9630. Cultures were pre-grown at 25% salinity which was either adjusted to 15% (**A**, shift-down) or held constant (**B**, control). ^{14}C-acetate was added at the moment of shift (data recording as described in Fig. 3)

correlated with a remarkable drop in absolute biosynthetic rates for the latter three phospholipids from 8 to 1, from 12 to 9 and from 18 to 0 dpm · mg protein^{-1} · h^{-1} · 10^3 until the fourth hour after shift-up while that of PG only decreased much less from 57 to 37 dpm · mg protein^{-1} · h^{-1} · 10^3. The low relative biosynthetic rate of PA remained quite constant. During the initial time after growth was resumed (fourth to sixth hour), the relative biosynthetic rate of PG gradually decreased while those of PE, CL and PC increased. Conditions became steady after 8 h, when the initially increased relative biosynthetic rate of PG adjusted to a lower steady level and also relative biosynthetic rates of other phospholipids (rising after the lowest level 4 h after the shift) ultimately became constant. During this time the strongest increase was found for PE. When relative steady-state biosynthetic conditions were reached for the time scale of the experiment, absolute biosynthetic rates of ^{14}C-acetate for PC, PG, PE, CL and PA were at 11, 130, 35, 15 and 5 dpm · mg protein^{-1} · h^{-1} · 10^3 and showed only small variations in the following course.

In the control culture (Fig. 3B) growth already was resumed after 1.5 h. Before reaching steady-state biosynthetic conditions after 2 h, the relative biosynthetic rate of PG slightly declined while those of PE and CL increased. Both effects were opposite to the initial response after shift-up. This was due to a comparative strong increase in absolute biosynthetic rates of PE and CL in the interval from 0.5 to 2 h (^{14}C-acetate incorporation: from 10 to 147 and 13 to 124 dpm · mg protein^{-1} · h^{-1} · 10^3, respectively), while those of PG, PC and PA only increased three- to fourfold (^{14}C-acetate incorporation: from 157 to 530, 5 to 16 and 6 to 25 dpm · mg protein^{-1} · h^{-1} · 10^3).

Shift-down. There were more rapid responses during shift-down of the salt concentration from 25% to 15% (Fig. 4A) than after shift-up. Growth was resumed already after 1.5 h and a steady-state of lipid biosynthetic rates relative to each other was achieved after 3 h. The most significant initial response was a drastic increase in the relative biosynthetic rate of PE, while in parallel that of PG declined. This was due to the dominating increase in the absolute biosynthetic rate of PE (incorporation of ^{14}C-acetate: from 19 to 53 dpm · mg protein^{-1} · h^{-1} · 10^3) between 0.5 and 1.5 h compared to smaller increases of biosynthetic rates of other lipids (incorporation of ^{14}C-acetate: from 2 to 6 for PC, from 54 to 99 for PG, from 13 to 27 for CL, and constant at 1 dpm · mg protein^{-1} · h^{-1} · 10^3 for PA). Because of its absolute height, this entailed an increase in the relative biosynthesis of PE while that of PG decreased and those of other lipids remained rather constant. After starting growth, initially decreased relative biosynthesis of PG slightly rose again while that of PE declined to a steady level which was achieved after 3 h. These relations were based on an increase in the absolute biosynthetic rate of PG during the first 3 h (^{14}C-acetate incorporation increased to 111 dpm · mg protein^{-1} · h^{-1} · 10^3) while only that of PE declined (^{14}C-acetate incorporation decreased to 32 dpm · mg protein^{-1} · h^{-1} · 10^3). For the following period of the experiment, ^{14}C-acetate incorporation rates of PC, CL and PA were adjusted in ranges from 12 to 14, from 24 to 36 and from 2 to 3 dpm · mg protein^{-1} · h^{-1} · 10^3, respectively, entailing steady biosynthetic relations.

In the control experiment (Fig. 4B) the lag interval after dilution was clearly extended compared to shift-down and the only response of significance was a slight increase in the relative biosynthesis of PG accompanied by decreasing relative biosynthetic rates of CL and rather constant ones of other lipids. This again was opposite to the initial responses after shift-down. After starting growth, relative biosynthetic rates of all lipids remained constant.

Discussion

PC, PG, PE and CL (with only minor amounts of PA) were found to be the main phospholipids of the three *Ectothiorhodospira* strains investigated, thus largely confirming results of Imhoff et al. (1982) and Asselineau and Trüper (1982). However, portions of individual phospholipids deflected with respect to the latter reference. This could be due to differences in media compositions, sensi-

tivity of the methods for lipid quantification or growth stages of the analyzed cells.

A general tendency of changes in the composition of phospholipids (in salt-adapted cells) was a significant increase in the portions of PG with increasing salinity, while portions of PE and CL decreased. Lipid adaptation in *E. mobilis* BN 9903 (containing high portions of PE), approached to known phenomena of gram-negative eubacteria with salinity-dependent PG/PE antagonisms, as has been found for *Vibrio costicola* (Hanna et al. 1984), *Pseudomonas halosaccharolytica* (Ohno et al. 1979; Hiramatsu et al. 1980) or even for the non-halophilic *Escherichia coli* (Munro and Bell 1973; McGarrity and Armstrong 1975). Although decreasing portions of CL already occurred in *E. mobilis* BN 9903 at higher salt concentrations, this effect became dominating in the *E. halophila* strains. Such responses so far were not reported to occur during salt adaptation of eubacteria. Opposite responses, however, increasing portions of CL at the expense of PG, appear to be typical of halotolerant and halophilic gram-positive bacteria, i.e. the halotolerant staphylococci (Kanemasa er al. 1972; Komaratat and Kates 1975; Hurst et al. 1984) and *Planococcus citreus* (Thirkell and Summerfield 1977).

Intensive research on the dynamics of salinity-dependent phospholipid adaptation has been done with *V. costicola* (Adams et al. 1987; Kogut and Russell 1984; Russell et al. 1985, 1986). This bacterium adapts to salt stress by a three stage mechanism in the variation of absolute phospholipid biosynthetic rates: as an immediate response after shift-up (phase one, approx. 10 min) there was an inhibition of the biosyntheses of all phospholipids. In phase two, PG biosynthesis was intensively promoted compared to that of PE and in phase three, when the culture adopts a growth rate according to the final salinity, phospholipid biosynthetic rates became steady and were adapted to each other. After dilution stress, time courses were comparatively shorter and adaptive mechanisms nearly reversed.

Sudden conditions of salt stress or dilution stress imposed on *E. halophila* BN 9630 entailed a temporal two step response on adaptive phospholipid biosynthesis with similar alterations: during the lag phase after the change in salinity, absolute biosynthetic rates were low and relative biosynthetic rates of individual lipids diverged; during the subsequent adjustment (when cultures started to grow) relative biosynthetic rates became steady and initial overshoots were balanced. After shift-up, there was a resultant increase in the relative biosynthesis of PG, because absolute biosynthetic rates always were significantly higher than those of other lipids and in addition decreased much less than those of PC, PE and CL. After shift-down, there was a resultant increase in the relative biosynthesis of PE because of its high absolute biosynthetic rate and the strong increases in this rate, even though that of PG also increased and always remained highest.

Major differences of adaptive phospholipid biosyntheses between *V. costicola* and *E. halophila* BN 9630 were: i) phase one of the salt response of *V. costicola* which is an inhibition of the biosynthesis of all lipids could not be observed in *E. halophila* because samples were not taken during the very early response after the shifts; ii) biosynthetic rates of PG always remained highest in *E. halophila* even after dilution stress; iii) after shift-up, there was no interval of "adjustment in growth" in *E. halophila* BN 9630. During this phase, Russell et al. (1986) found adaptation of phospholipid biosynthetic rates and growth rates in *V. costicola*, while growth rates of *E. halophila* remained constant from the beginning of the growth phase, although biosynthetic rates of lipids still changed and steady biosynthesis was reached hours later. Moreover, lag phases after the shifts were always extended compared to *V. costicola*.

Salt adaptation phenomena of cell surfaces offer different aspects for interpretation which are ultimately connected with problems of membrane stabilization under conditions of high ionic strength.

i) One aspect refers to the regulatory adjustment of the permeability properties of the membrane under conditions of high ionic strength (Kanemasa et al. 1972; Ohno et al. 1976, 1979). Despite the fact that selective cationic permeability of negatively charged bilayer membranes has been demonstrated in artificial systems such as liposomes (Hopfer et al. 1970), it is uncertain whether these effects are of importance in membranes of living cells.

ii) Far more important, interest has primarily been focused on membrane lipid polarity because increasing polarity of membrane lipids at higher salt concentrations was typical of the gram-positive and gram-negative eubacteria mentioned above. Our results as well show an increase in negative membrane lipid polarity in parallel to increasing halophilism of the investigated *Ectothiorhodospira* strains. Because lipid polarities in all investigated eubacteria increased in parallel to increasing salt concentrations, a plausible explanation was that alterations of cellular membranes and membrane lipids are based on charge interactions. One major effect could be the stabilization of the membrane by charge screening. Consequently, this was often discussed in connection with salt-dependent lipid compositions (see Kates 1986), although Lanyi (1974) stated that charge screening is complete already at a few tenth of molarity. We have found, however, that the percentual excess of negative charges only increased in *E. mobilis* BN 9903 whereas in both *E. halophila* strains this value was fairly constant though principally higher compared to *E. mobilis*. Due to this, in *E. mobilis* BN 9903 increasing amounts of cations were bound to the membrane surface with increasing salinity, whereas in *E. halophila* strains this amount was largely constant though principally higher (Ditandy and Imhoff, unpublished results).

iii) According to our results, the most important aspect for the interpretation of changing lipid compositions under high ionic strength appears to be the bilayer stabilization. An inherent property of phospholipids is their capability to adopt non-lamellar structures such as hexagonal-II phases (Melchior 1982). The tendency to form non-lamellar phases has been measured with pure lipid-phases. Under these conditions, PE, CL, and PA were found as potential non-bilayer formers, whereas PG

and PC formed stable bilayers (Cullis and de Kruijff 1979; de Kruijff et al. 1979; Rand and Sengupta 1972). Little attention has been paid to this aspect in the relevant literature concerned with eubacterial salt adaptation of the lipid composition (Hanna et al. 1984; Ohno et al. 1979; Hiramatsu et al. 1980; Kanemasa et al. 1972; Kates 1986; Komaratat and Kates 1975; Hurst et al. 1984; Thirkell and Summerfield 1977). In both strains of *E. halophila*, the most halophilic eubacteria whose lipid compositions have been studied in dependence on the salt concentration, the percentual excess of negative charges was high but showed only small variations. Portions of bilayer forming lipids, however, steadily increased with the salinity (Table 2). This was also true for *E. mobilis* BN 9903, where both charged lipids and bilayer stabilizing ones increased with the salinity. Our results are quite conclusively interpreted in terms of salt dependent alterations of phospholipid compositions to decrease the contents of bilayer destabilizing lipids such as PE and CL. From the lowest to the highest salinities, portions of potential non-bilayer forming phospholipids were reduced from 53.6% to 38.7%, from 35% to 12.5% and from 23.4% to 8.1% in *E. mobilis* BN 9903, *E. halophila* BN 9630 and *E. halophila* BN 9626, respectively. In his excellent review on non-bilayer structures, Seddon (1990) states that CL above 1.5 M NaCl turns into inverted micellar structures due to enhanced headgroup dehydration. Bilayer destabilizing effects of high ionic strength could be counteracted by increasing incorporation of bilayer forming lipids into the membranes.

Whether or not salinity-dependent lipid adaptation in phototrophic *Ectothiorhodospira* altogether reflects specific ways of direct salt influences on individual, possibly membrane bound biosynthetic enzymes or a more complicated interplay of comprehensive regulatory phenomena, remains to be elucidated.

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