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The Growth and Phospholipid Composition of a Moderately Halophilic Bacterium during Adaptation to Changes in Salinity

Margot Kogut† and Nicholas J. Russell*

† Department of Biochemistry, King's College, University of London, Strand, London WC2R 2LS; and * Department of Biochemistry, University College, PO Box 78, Cardiff CF1 1XL, South Wales, UK

Abstract. The effect of a sudden change in NaCl concentration of the medium on the time course of alterations in growth rate and phospholipid composition of the moderately halophilic bacterium *Vibrio costicola* has been investigated. This organism and other moderate halophiles are known to contain a larger proportion of negatively charged phospholipids in their membranes when grown at higher salt concentrations. We show for the first time that the change in proportion of phosphatidylglycerol, relative to phosphatidylethanolamine, which occurs after a shift from 1 *M* to 3 *M* NaCl, or vice versa, is essentially completed during that period immediately following the salt shift when growth is zero or very slow, and before the cells have adopted the growth rate appropriate to the new salt concentration. It appears, therefore, that the alteration in membrane phospholipid composition may be a necessary physiological response for adaptation to change in salinity.

Moderately halophilic bacteria can grow over a wide range of salt concentrations (0.4–4.0 *M*) that overlap the maximum for nonhalophiles (~0.6 *M*) and the minimum for extreme halophiles (3.0 *M*) [6, 8]. In one such organism, *Vibrio costicola*, this versatility is due to phenotypic adaptation rather than selection of variants [2].

Studies on a number of moderate halophiles have shown that organisms grown at higher salinities have greater proportions of negatively charged phospholipids in their membranes [4, 7, 9, 10]. These changes have been interpreted as being a mechanism for counteracting the increase in Na^+ concentration at the membrane surface [4].

We are particularly interested in the process of adaptation that occurs in response to changing salinity and have chosen *V. costicola* for our investigations. In this organism phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin comprise 80% or more of the total phospholipids, and during growth in higher salt concentrations, membranes contain higher proportions of phosphatidylglycerol and cardiolipin, relative to phosphatidylethanolamine (K. Hanna, M. Kogut, C. Bengis-Garber, D. Kushner, and M. Kates, unpublished observations). In the present paper we show for the first time that these changes occur immediately after a change in salinity and are essentially complete by the time the growth rate appropriate to the new salt concentration has been established.

Materials and Methods

Vibrio costicola (NRC 3700, from D. J. Kushner) was grown at 30°C in a liquid medium containing 0.3% (wt/vol) tryptone (Oxoid Ltd., Basingstoke, Hampshire, England) and 0.3% (wt/vol) proteose peptone (Difco Laboratories, Detroit, MI) containing 1 *M* or 3 *M* NaCl (AnalaR grade) [2]. In "shift" experiments, the salt concentration was altered by sedimenting bacteria by centrifugation at 5000 *g* for 10 min and resuspending the pellet in prewarmed medium containing the new salt concentration. The growth of cultures was monitored by measurements of optical density at 500 nm. For experiments using protein synthesis inhibitors, chloramphenicol and tetracycline were purchased from Sigma (London) Chemical Co. Ltd., Poole, Dorset, England; the inhibitors were dissolved in water just prior to use.

For lipid extraction, aliquots of culture were centrifuged at 10,000 *g* for 10 min, and the bacterial pellet was extracted and washed using the method of Bligh and Dyer as detailed in Kates [5]. Phospholipids were separated by thin-layer chromatography [1] and quantitated by phosphorus analysis [11]. In some cases, analyses were confirmed by gas-liquid chromatography of the constituent fatty acids [12] using arachidic acid as internal standard; the results agreed to within 5%.

* To whom reprint requests should be addressed.

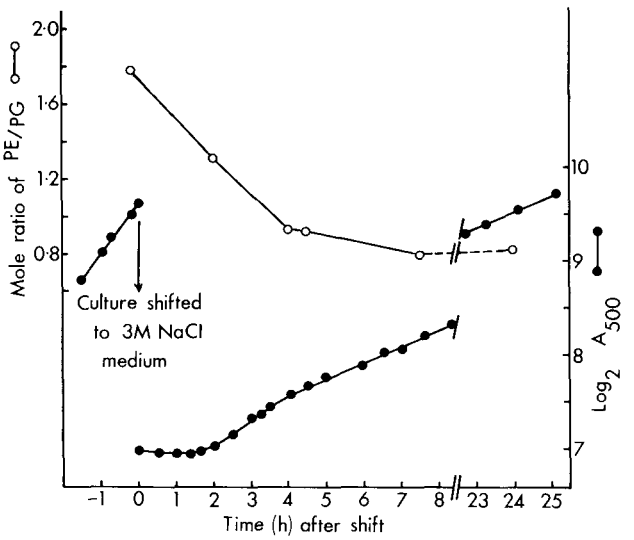

Fig. 1. The growth and change in phospholipid composition of *Vibrio costicola* during a shift-up from 1 *M* to 3 *M* NaCl. A culture growing exponentially in medium containing 1 *M* NaCl was centrifuged and resuspended in prewarmed medium containing 3 *M* NaCl. Growth was monitored by measuring OD_{500} and at intervals aliquots of culture were removed for lipid extraction and isolation of phosphatidylethanolamine (PE) and phosphatidylglycerol (PG); the phospholipid composition is expressed as the mole ratio PE/PG.

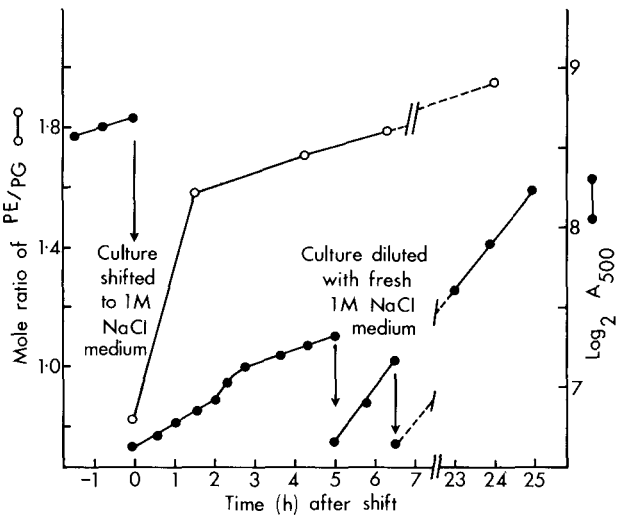

Fig. 2. The growth and change in phospholipid composition of *Vibrio costicola* during a shift-down from 3 *M* to 1 *M* NaCl. A culture growing exponentially in 3 *M* NaCl was shifted to 1 *M* NaCl; its growth was monitored and the phospholipids were isolated as described in Fig. 1. At intervals after the shift, the culture was diluted with fresh medium containing 1 *M* NaCl to ensure that exponential growth was maintained; control experiments showed that such dilution at constant salt concentrations has no effect on the mole ratio PE/PG.

Results

The NaCl concentration for optimum growth rate (μ = 0.6–0.7) of *Vibrio costicola* under the conditions used in these experiments was found to be 1 *M*. If the salt concentration is changed (shifted) during logarithmic growth (as detailed in *Materials and Methods*), the culture undergoes a period of adjustment in growth rate until this stabilizes at a rate characteristic of the new salt concentration. This is illustrated in Fig. 1, in which the salt concentration was suddenly increased from 1 *M* to 3 *M* (shift-up). There is first a lag period, followed by a burst of growth that is rapid compared to the final rate attained; the final rate is typical of control cultures grown in 3 *M* NaCl throughout, and it takes 6–7 h for this new rate (μ = 0.1–0.2) to be established.

Previous studies have shown that the proportions of the two major phospholipids, phosphatidylethanolamine (PE) and phosphatidylglycerol (PG), in *V. costicola* differ in cultures grown at different salt concentrations (K. Hanna, M. Kogut, C. Bengis-Garber, D. Kushner, and M. Kates, unpublished observations). The mole ratio PE/PG for control bacteria grown at a constant NaCl concentration of 1 *M* was 1.78 ± 0.33 (n = 6), compared with 0.97 ± 0.16 (n = 6) for bacteria grown at 3 *M* NaCl. We have now investigated the time course of changes in PE/PG mole ratio during a shift-up in salt concentration from 1 *M* to 3 *M* NaCl (Fig. 1). The change in PE/PG mole ratio occurs largely during the lag phase and the short burst of growth, and is essentially completed by the time the growth rate, typical of controls at 3 *M* NaCl, has been attained (Fig. 1).

We have also studied the effect of a reciprocal change in salt concentration (shift-down) from 3 *M* to 1 *M* on the growth and phospholipid composition of *V. costicola*. The result of such an experiment is illustrated in Fig. 2. In the shift-down experiments, growth does not cease completely, but the bacteria grow at a slower rate than that of control cultures in 1 *M* NaCl. During this phase of slow growth there is a burst of growth, similar to that seen at the end of the lag phase during a shift-up (Figs. 1 and 2). The PE/PG mole ratio alters rapidly following the lowering of the salt concentration, a large part of the change being accomplished within 2 h during the first part of the slow phase of growth (Fig. 2).

Large changes in the protein synthesis rates of *V. costicola* cultures after a salt shift have also been observed (M. Kogut, unpublished observations) and this may be accompanied by altered rates of protein turnover [3]. In an attempt to determine

whether enzyme induction may be involved in the adaptation process that follows a salt shift, we have used chloramphenicol and tetracycline to inhibit protein synthesis. The magnitude of the change in PE/PG mole ratio during a shift-up experiment was reduced markedly by both compounds. For example, in a shift-up experiment in which the PE/PG mole ratio in the control changed from 2.12 to 1.10 after 4.25 h in 3 *M* NaCl, the corresponding value in a culture to which tetracycline (30 μg/ml, sufficient to inhibit growth) had been added at the time of shift-up was 1.35. The interpretation of experiments using chloramphenicol is complicated by the fact that this inhibitor appears to affect the PE/PG ratio independently of a change in salt concentration.

Discussion

The moderate halophile *Vibrio costicola* exhibits phenotypic adaptation to changes in the salt concentration of its growth medium. This adaptation process involved changes in growth rate and in the membrane phospholipid composition, especially in the relative amounts of the two major phospholipids, phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). We have expressed the phospholipid composition as the mole ratio of PE/PG, since the changes in diphosphatidylglycerol (cardiolipin) are very small; furthermore, there is little conversion of phosphatidylglycerol to cardiolipin under the experimental conditions (i.e., utilizing a rich medium) of the shift experiments reported in this paper. This is not true when a synthetic medium is used, and the effects of growth medium and growth rate on phospholipid composition will be reported elsewhere.

Following a sudden increase or decrease in salt concentration there is a reduction in the growth rate, which is particularly pronounced during a shift-up when there is no growth for up to 2 h. The short burst of apparently rapid growth that occurs either at the end of shift-up (Fig. 1) or during shift-down (Fig. 2) may reflect some synchronous cell division induced by the sudden change in salt concentration. It is unlikely to be an optical artifact created by, for example, plasmolysis of the cells, because it occurs 2–3 h after the change in salt concentration; in some shift experiments, we have observed a sharp rise in culture turbidity immediately following resuspension of bacteria at a higher salt concentration, and we assume that this is due to plasmolysis.

Although differences in phospholipid composition associated with growth in different salt concentrations have been observed in several moderate halophiles [7, 9], including *V. costicola,* this is the first demonstration that these changes occur *before* the new growth rates are established. It has been suggested that the relative increase in negatively charged phospholipids in cultures grown in higher salt concentrations is necessary to balance the excess cationic charge (Na^+) at the membrane surface [4]. If this interpretation is correct, the time-course of the changes demonstrated by the present data suggests that the relative increase in phosphatidylglycerol may be a necessary physiological response for adaptation to increasing salinity in *V. costicola*. Only when the correct membrane composition has been achieved can the organism grow at the rate appropriate to the new salt concentration.

The shift experiments reported in this paper do not show whether the changes in PE/PG mole ratio are mediated by the increased synthesis or degradation of one phospholipid or the other. We are currently investigating this aspect by measuring the rate of phospholipid biosynthesis during salt shifts using radioactive precursors. Preliminary results indicate that the increase in phosphatidylglycerol relative to phosphatidylethanolamine during a shift-up is due to an increased rate of phosphatidylglycerol synthesis rather than phosphatidylethanolamine degradation. In addition, the present studies indicate that enzyme induction may be involved. It will be interesting to establish whether this is confirmed by further experiments, and also to determine the salt-sensitive enzyme(s) of phospholipid biosynthesis in this organism.

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Literature Cited

1. **Foot, M., Jeffcoat, R., Russell, N. J.** 1983. Some properties, including the substrate *in vivo*, of the Δ^9-desaturase in *Micrococcus cryophilus*. Biochemical Journal **209**:345–353.
2. **Forsyth, M. P., Kushner, D. J.** 1970. Nutrition and distribution of salt response in populations of moderately halophilic bacteria. Canadian Journal of Microbiology **16**:253–261.
3. **Hipkiss, A. R., Armstrong, D. W., Kushner, D. J.** 1980. Protein turnover in a moderately halophilic bacterium. Canadian Journal of Microbiology **26**:196–203.
4. **Hiramatsu, T., Ohno, Y., Hara, H., Yano, I., Masui, M.** 1980. Effects of NaCl concentration on the envelope compo-

nents in a moderately halophilic bacterium, *Pseudomonas halosaccharolytica*, pp. 189–199. In: Morisita, H., Masui, M. (eds.), Saline environments. Proceedings of the Japanese conference on halophilic microbiology. Kyoto, Japan: Nakanishi Printing Co., Ltd.

5. **Kates, M.** 1972. Techniques of lipidology: isolation, analysis and identification of lipids. Amsterdam: North-Holland.
6. **Kogut, M.** 1980. Strategies of microbial adaptability. Trends in Biochemical Sciences **5**:47–50.
7. **Komaratat, P., Kates, M.** 1975. The lipid composition of a halotolerant species of *Staphylococcus epidermis*. Biochimica et Biophysica Acta **398**:464–484.
8. **Kushner, D. J.** 1978. Life in high salt and solute concentrations: halophilic bacteria, pp. 318–368. In: Kushner, D. J. (ed.), Microbial life in extreme environments. London: Academic Press.
9. **Ohno, Y., Yano, I., Hiramatsu, T., Masui, M.** 1976. Lipids and fatty acids of a moderately halophilic bacterium, No. 101. Biochimica et Biophysica Acta **424**:337–350.
10. **Ohno, Y., Yano, I., Masui, M.** 1979. Effect of NaCl concentration and temperature on the phospholipid and fatty acid composition of a moderately halophilic bacterium, *Pseudomonas halosaccharolytica*. Journal of Biochemistry **85**:413–421.
11. **Russell, N. J.** 1974. The lipid composition of the psychrophilic bacterium *Micrococcus cryophilus*. Journal of General Microbiology **80**:217–225.
12. **Russell, N. J., Volkman, J. K.** 1980. The effect of growth temperature on wax ester composition in the psychrophilic bacterium *Micrococcus cryophilus* ATCC 15174. Journal of General Microbiology **118**:131–141.