Glycinebetaine stimulates, but NaCl inhibits, fatty acid biosynthesis in the moderately halophilic eubacterium HX

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Abstract. Total fatty acid synthetase (FAS) and cyclopropane fatty acid synthetase (CFAS) activities in cell-free lysates of the moderately-halophilic eubacterium HX, have been determined using radiolabelled malonyl-CoA and S-adenosylmethionine respectively as the precursor. The activities of FAS and CFAS were extremely low in vitro in 100 mM buffers, but were stimulated up to 100-fold by exogenous addition of the compatible-solute glycinebetaine to lysates; optimum activities of FAS and CFAS in vitro were obtained in 2-3 M concentrations of this compatible solute. In contrast, NaCl added to the lysate assay system was strongly inhibitory: CFAS was 97% inhibited by 1 M NaCl whereas FAS was less sensitive with 3 M NaCl giving 82% inhibition. When the culture medium salinity was raised from 1 to 3 M NaCl, the endogenous activity of CFAS measured in vitro in lysates without additional compatible solute was approximately doubled. This increase in CFAS activity is enough to account for the known increase in CFA content which occurs when culture medium salinity is raised, and the data are discussed in the context of the role of intracellular compatible solutes during haloadaptation of membrane lipid composition.

Key words: Halophilic eubacteria — Fatty acid synthesis — Cyclopropane fatty acid — Salinity — Haloadaptation — Osmoregulation — Compatible solute — Glycinebetaine — Sodium chloride

Two of the major biochemical changes which occur during haloadaptation in moderately-halophilic eubacteria are the accumulation of intracellular compatible solutes and the alteration of membrane lipid composition (Csonka 1989; Russell 1989a). The process of haloadaptation is a special case of osmoregulation in which some of the effects are particularly pronounced because of the high external solute concentrations which can be tolerated by this group of eubacteria. Therefore, they are a particularly good choice of organism in which to study osmoregulatory phenomena.

Aerobic moderate halophiles generally accumulate amino acids (e.g. glutamate and proline) and related compounds such as glycinebetaine as their major compatible solutes when grown on complex media (Imhoff 1986; Wohlfarth et al. 1990; Severin et al. 1992). The substances are either taken up directly from the medium or made from immediate precursors. In contrast, when cultures are grown in defined media the major compatible solutes are commonly the tetrahydropyrimidines, ectoine and hydroxyectoine, which are made by a de novo biosynthetic process. The moderately-halophilic eubacterium designated HX is typical in this respect, in that it accumulates mainly glycinebetaine (with smaller amounts of glutamate and ectoine) as the major compatible solute in proteose-peptone/tryptone broth medium but makes mainly ectoine when grown in defined medium (N.J. Russell and E. A. Galinski, unpublished results).

The most frequently-observed salinity-dependent change in lipid composition of this group of eubacteria (as well as a number of non-halophiles) is an increase in the proportion of anionic phospholipids (mainly phosphatidylglycerol) which also contain higher proportions of cyclopropane fatty acyl groups (Russell 1989a; 1992). The membrane-lipid response of HX to a rise in external salinity from 1 to 4 M NaCl is also typical in that there is a 1.5-fold increase in the proportion of phosphatidylglycerol, which contains 2.1-fold more cyclopropane fatty acyl chains (Adams 1988; Adams et al. 1990).

Another reason for selecting HX in order to study the effect of NaCl and compatible solutes on fatty acid biosynthesis, is that it not only grows over a wide salinity range (0.4-4.5 M NaCl) with specific growth rates (μ) at 30 °C of 0.62 and 0.25 in complex broth medium containing 1.0 and 4.0 M NaCl respectively (Adams 1988), but also adapts rapidly to a sudden shift-up in salinity from 1 to 4 M NaCl (Adams et al. 1990). It is known that the alterations in phospholipid composition

Abbreviations: FAS, fatty acid synthetase; CFA, cyclopropane fatty acid; CFAS, cyclopropane fatty acid synthetase

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are a mechanism for preserving the integrity of the membrane lipid bilayer in the face of high external salinity (Sutton et al. 1990, 1991). In contrast, neither the general function of cyclopropane fatty acyl chains in membrane lipids (Russell 1989b) nor their specific function in haloadaptation (or osmoregulation) is understood (Russell 1989a). This paper reports the effect of NaCl and a compatible solute on the biosynthesis of cyclopropane fatty acids in the moderately-halophilic eubacterium HX, and compares that data with solute effects on total fatty acid biosynthesis, as part of a wider study of the environmental regulation of membrane lipid biosynthesis in eubacteria.

Materials and methods

Organism and culture conditions

The bacterium HX was designated originally as Vibrio HX, but it has been excluded from this genus and others such as Aeromonas and Halomonas (see Adams et al. 1990 for a discussion). Since its true taxonomic status is unknown, it is referred to here simply as HX.

For experimental purposes, batch cultures were grown at 30 °C in a liquid medium (PPTM) containing 1.0% (w/v) proteose peptone (Difco), 1.6% (w/v) tryptone (Difco) and 2.0 mg% (w/v) MgCl₂, containing the appropriate amount of NaCl (Analytic) to give a final concentration of 1 or 3 M. Other details of cultural conditions are given in Adams et al. (1990).

Preparation of cell-free lysates

Liquid cultures were grown to the late-logarithmic stage (A660 = 0.6) and the bacteria collected by centrifugation at approx 7000 × g for 10 min. The bacterial pellets were resuspended in a volume of 100 mM-K-phosphate buffer, pH 7.6, which was equivalent to 1/20th of the original culture volume. Compatible solutes were predissolved at the appropriate concentration in this buffer solution as required, rather than being added subsequently to lysates prepared in buffer alone, because the latter method did not give reproducible data for the fatty acid biosynthesis enzymes.

The bacteria were broken by 2 or 3 passages through a French pressure cell (Aminco, Silver Springs, Md., USA) at 44 MPa pressure. Cellular breakage was estimated by phase-contrast light microscopy to be > 99%. It was not necessary to remove the residual intact bacteria because they are impermeable to the radioactive precursors used to assay total or cyclopropane fatty acid synthetase activities (vide infra). The lysates were used the same day for determination of synthetase activities.

Assay of total fatty acid synthetase

Total fatty acid synthetase (FAS) activity was measured by incubating lysates with [2-¹⁴C]-methylmalonyl-CoA (final concn 3.7 kBq · mL⁻¹, Amersham International, specific radioactivity 2.22 GBq · mmol⁻¹) with shaking at 30 °C. Reactions were started by adding the radioactive precursor in a minimum volume (1-10 μl) of water to 1 ml of lysate, containing added cofactors at final concentrations as follows: acetyl-CoA (100 μM), NADPH (1.0 mM), ATP (500 μM) and Aciyl Carrier Protein (200 μg per assay, from Escherichia coli). The reaction was stopped by mixing with an equal volume of KOH (40%, w/v). This mixture was heated at 70 °C for 1 h which releases the fatty acids by saponification. After cooling, an equal volume of water was added, and the solution was acidified to pH < 2 using approx. 6 N-HCl. The fatty acids were extracted with three aliquots of light petroleum (6:40-60 °C) and the combined extracts placed in plastic scintillation vials. The solvent was evaporated and 10 ml scintillant (FisherFluor 1', Fisher, Loughborough, UK) added and the radioactivity determined using a LKB Rackbeta liquid-scintillation counter programmed with an external-standard channels-ratio facility for the estimation of counting efficiency. The (CFAS activity was expressed as a/d min · ¹⁴C activity incorporated · (mg lysate protein)⁻¹. Although there were differences in the activity of individual lysates prepared from separate cultures, the variation in replicate determinations for any one lysate was < 5% and the trends observed (e.g. in the effects of compatible solutes) were consistently reproducible. Protein concentration was measured using the modification of the Lowry test described by Markwell et al. (1978).

Assay of cyclopropane fatty acid synthetase

Cyclopropane fatty acid synthetase (CFAS) activity was measured by incubating lysates with S-adenosyl-L-[methyl-¹⁴C]methionine (Amersham International, specific radioactivity 2.22 GBq · mmol⁻¹) with shaking at 30 °C. The final concentration of S-adenosyl-L-[methyl-¹⁴C]methionine was 14.4 kBq · mL⁻¹ in all experiments, except those reported in Fig. 4 when a 10-fold higher concentration was used. Reactions were started by adding the radioactive precursor in a minimum volume (10-20 μl) of water to 1 ml of lysate. The reaction was stopped, and the fatty acids extracted and counted as described above for FAS. Radio-GC analysis of the products of CFAS incubations demonstrated that the radioactivity was incorporated selectively in CFA (data not shown).

Results

Effect of glycinebetaine on the activities of total fatty acid synthetase and cyclopropane fatty acid synthetase

Cultures of HX were grown in complex medium (PPTM) containing 1 M NaCl and harvested at the end of the logarithmic phase for the determination of total fatty acid

![Fig. 1. The effect of glycinebetaine on cyclopropane fatty acid synthetase activity in cell-free lysates of bacterium HX prepared from cultures grown in medium containing 1 M NaCl. Enzyme activities in lysates prepared in buffer alone (○) or containing 1 (●), 2 (▲), 3 (▼), or 4 (□) M glycinebetaine are expressed as a/d min · ¹⁴C activity incorporated · (mg lysate protein)⁻¹ from S-adenosyl-L-[methyl-¹⁴C]methionine in a minimum volume of water to give a final concentration of 14.4 kBq · mL⁻¹.](image)