Effect of Growth Temperature on Cold Osmotic Shock in *Escherichia coli* ML 30

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Several types of micro-organism, including Gram-negative bacteria (Heppel, 1967), fungi (Wiley, 1970) and yeast (Patching & Rose, 1971) are susceptible to cold osmotic shock. This is the name given to a procedure which involves suspending organisms in a hyperosmolar solution of a metabolically inert solute containing EDTA, and then in a dilute solution of MgCl_2 at 0° . Susceptible organisms then lose their ability to accumulate extracellular solutes but retain their viability (Heppel, 1967). Cold osmotic shock releases solute-binding proteins (Heppel, 1967; Wiley, 1970) and the technique is now widely used to extract these proteins from micro-organisms (Kaback, 1970). However, almost nothing is known of the physiological basis of cold osmotic shock and in particular of the role of the osmotic-, temperature- and EDTA-induced stresses which are involved.

The effects of cold stress may arise from the freezing of membrane lipids which facilitates release of membrane-bound transport proteins. Freezing of membrane lipids is thought to be important in the related phenomenon of cold shock in Gram-negative bacteria (Farrell & Rose, 1968). A decrease in growth temperature leads to synthesis of an increased proportion of unsaturated fatty-acid residues in microbial lipids (Farrell & Rose, 1967), which results in a lowering of the melting point of the lipids (Chapman, 1967). Such a lowering can be significant with only a few per cent increase in unsaturation (Lyons & Asmundson, 1965). This report shows that *Escherichia coli* growing at 15° rather than 37° synthesizes more unsaturated lipids, but this does not remove the susceptibility of the bacteria to cold osmotic shock, although organisms grown at the two temperatures respond to it differently.

METHODS

The strain of *Escherichia coli* ML30 used was provided by Dr J. L. Ingraham of the Department of Bacteriology, University of California, Davis, California, U.S.A. The bacterium was grown at 37° or 15° in 1 l. batches of medium containing (per l.): glucose (2 g.), KH_2PO_4 (3 g.), K_2HPO_4 (7 g.), $(\text{NH}_4)_2\text{SO}_4$ (5 g.), and $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ (0.5 g.); pH 7.1. Cultures were incubated as described by Patching & Rose (1969). Organisms were harvested from logarithmic-phase cultures containing 0.05 mg. dry wt organisms/ml. They were washed once with phosphate buffer (pH 7.0; 37°) and either used immediately in cold osmotic shock experiments or, after washing twice with cold (4°) water, freeze-dried prior to lipid extraction.

The complete cold osmotic shock treatment involved suspending organisms to a concentration of 0.5 mg. dry wt/ml. at 37° in tris buffer (pH 7.2) containing sucrose (0.5 M) and EDTA (0.1 mM) (stage A). Immediately after suspension, the organisms were removed by centrifugation and suspended to the same concentration in cold (0°) MgCl_2 (0.5 mM) (stage B). Modifications to the complete cold osmotic shock treatment are described in the text. Rates of accumulation of the nonmetabolizable sugar α -methylglucoside were measured

by suspending organisms in 0.1M-phosphate buffer (pH 7.0) containing 10 mM [$1\text{-}^{12}\text{C}$]- α -methylglucoside and [$1\text{-}^{14}\text{C}$]- α -methylglucoside to give a final activity of about 3.5×10^4 counts/min/ μ mole (Farrell & Rose, 1971). Rates of accumulation were calculated from the regression coefficients of the time-course plots for accumulation, and are quoted as μ moles α -methylglucoside accumulated/g. dry wt organism/h. Lipids were extracted from freeze-dried organisms with a mixture of CHCl_3 and CH_3OH (2:1, by vol.); the extract was washed using the procedure of Folch, Lees & Sloane-Stanley (1957). Lipid contents of organisms were estimated gravimetrically using the method of Rouser, Kritchevsky & Yamamoto (1967), and phosphorus contents of lipid extracts by the method of Bartlett (1959). Separations of phospholipids by thin-layer chromatography and of fatty-acid methyl esters by gas chromatography were carried out as described by Hunter & Rose (1972).

RESULTS

Organisms grown at 15° contained slightly more lipid (8.9% dry wt) than those grown at 37° (8.1%). Lipids extracted from organisms grown at either temperature contained the same amount (6.5% of dry wt) and types of phospholipids in about the same proportions. When the growth temperature was lowered from 37° to 15° , the major change in the fatty-acid composition of the lipids was an increase from 23% to 36% in the proportion of $\text{C}_{18:1}$ and a corresponding decrease in the proportion of $\text{C}_{16:0}$ acids.

Table 1 shows the effects of full and modified cold osmotic shock treatments on the ability of bacteria grown at 37° or 15° to accumulate α -methylglucoside. Rates of accumulation were about the same by untreated organisms grown at 37° or 15° . The full cold osmotic shock treatment or treatment omitting tris from the solution used in stage A eliminated accumulation in organisms grown at either temperature. Organisms grown at 37° or 15° retained their α -methylglucoside-accumulating ability when the MgCl_2 solution in stage B was used at 37° rather than 0° . Only organisms grown at 15° retained any accumulating ability if EDTA was omitted from stage A.

Table 1. *Effect of full and modified cold osmotic shock treatments on rates of accumulation of α -methylglucoside by Escherichia coli ML30*

Composition of suspending liquid in stage A			Composition of suspending liquid in stage B		Temperature of suspension in stage B ($^\circ\text{C}$)	Rate of accumulation of α -methylglucoside by bacteria grown at	
Sucrose (0.5M)	EDTA (0.1mM)	Tris buffer (pH 7.2)	MgCl_2 (0.5mM)	Sucrose (0.5M)		37°	15°
—	—	—	—	—	—	0.109	0.104
+	+	+	+	—	0	0.00	0.00
+	+	+	+	—	37	0.148	0.118
+	—	+	+	—	0	0.00	0.088
+	+	+	+	+	0	0.142	0.00
—	+	+	+	—	0	0.113	0.00
+	+	—	+	—	0	0.00	0.00

When sucrose (0.5M) was used in both stages A and B, or in neither stage, only organisms grown at 37° retained accumulating ability. Omitting tris from the solution used in stage A did not prevent the loss of solute-accumulating ability by bacteria grown at 37° or 15° .

DISCUSSION

The lowering of the melting point of membrane lipids caused by the increased synthesis of unsaturated fatty acids does not prevent the loss of ability to accumulate α -methylglucoside by *Escherichia coli* subjected to cold osmotic shock. Nevertheless, a cold stress seems to be essential in order to remove solute-transporting ability, as shown by the retention of this ability when the MgCl_2 solution in stage B was used at 37° rather than 0° . The most interesting finding from the data reported concerns the differences shown between organisms grown at 37° and 15° in their response to exposure to EDTA in stage A and to the osmotic stress imposed when organisms are transferred from stage A to stage B. Omitting EDTA from the solution used in stage A had no effect on the loss of α -methylglucoside-accumulating ability by organisms grown at 37° whereas it led to the retention of solute-accumulating ability by organisms grown at 15° . Since bacteria grown at either 37° or 15° contained the same amounts and types of phospholipid, the need for EDTA treatment by organisms grown at the lower temperature cannot be explained by a greater cation-binding capacity of the membrane lipids. Also the greater degree of unsaturation in the lipids in membranes of bacteria grown at 15° may lead to a less avid binding of divalent ions (Shah & Schulman, 1965), which would lessen rather than increase the need for EDTA treatment in order to affect membrane structure. The need by organisms grown at 37° , but not those grown at 15° , for an osmotic stress between stages A and B might be related to the greater mobility of the more unsaturated lipids in bacteria grown at the lower temperature, an explanation which was invoked to explain a greater rate of solute permeation in liposomes prepared from lipids of *E. coli* grown at low as compared with high temperatures (Haest, de Gier & Van Deenen, 1969). Our data suggest therefore that the loss of membrane-bound transport proteins when *E. coli* is subjected to cold osmotic shock is probably not due simply to the freezing of membrane lipids, although the degree of unsaturation in the membrane lipids may influence the response of bacteria to both the EDTA-induced and osmotic stresses involved in cold osmotic shock.

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