Conditioning of the membrane fatty acid profile of *Escherichia coli* during periodic temperature cycling

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The membrane fatty acid composition of *Escherichia coli* becomes conditioned during periodic temperature cycling between 37 and 8 °C. After several cycles of temperature change, the bacteria become locked into a low-temperature physiology. Even after a prolonged incubation at high temperature the membrane fatty acid composition of conditioned cells was similar to that of cold-stressed cells.

INTRODUCTION

Micro-organisms must constantly adapt to a variety of changing conditions and stresses in their local environment to survive and multiply. In the natural environment, periods of constant temperature or conditions where temperature is up- or downshifted only once, as in a typical cold- or heat-shock experiment, are rare. The driving question of this study is how bacteria adapt their membrane fatty acid composition during periodic temperature cycling. Although the effect of fluctuating temperatures on bacterial growth rate and survival has been demonstrated (Mitchell *et al.*, 1994), there appears to have been no systematic study of how changing temperature during bacterial growth affects membrane composition. In this work we challenge the generally accepted homeoviscous model, which states that membranes consistently adapt to low temperature by increasing the ratio of the unsaturated fatty acids (UFAs) to saturated fatty acids (SFAs) (Sinensky, 1974).

METHODS

Bacterial growth. In all experiments *Escherichia coli* strain K-12 was grown in LB (Luria–Bertani) medium, consisting of 10 g tryptone l⁻¹, 5 g yeast extract l⁻¹ (both from Biolife Italiana Srl) and 10 g NaCl l⁻¹ (Merck), in rubber-stoppered tubes on a rotary shaker at 200 r.p.m. in the dark. Bacteria in control cultures were incubated at a constant temperature of 37 °C. In the experiments where temperature was cycled, bacteria were first incubated at 37 °C for 1 h; cells were then transferred to 8 °C for 1 h and so on to the end of incubation. In cold-shock

experiments, cells were grown at 37 °C for 2.5 h to the mid-exponential phase and were then transferred to 8 °C with no further temperature change. The temperature of the growth media was monitored every 5 min by a thermometer coupled to a data logger (Cole-Parmer). The OD₆₅₀ was measured every 30–60 min with a Multiskan Spectrum microtitre plate reader (Thermo-Fischer Scientific).

CO₂ and ATP measurements. Bacterial respiration was measured with a Hewlett Packard 5890 gas chromatograph equipped with a thermal conductivity detector as described previously (Odić *et al.*, 2007). Typically, a 0.25 ml gas sample was taken every 30–60 min during incubation using a 1 ml syringe with a hypodermic needle. The data were corrected for different amounts of CO₂ dissolved in the liquid phase at different temperatures. Intracellular content of ATP was measured with an ATP-dependent luciferin–luciferase system as described previously (Odić *et al.*, 2007).

Determination of cellular fatty acid composition by gas chromatography. Cells were harvested by centrifugation and pellets were resuspended in a sterile double-distilled water, frozen (–20 °C) and lyophilized. Lipids were transesterified using the HCl/methanol procedure (Dionisi *et al.*, 1999). Extracts of fatty acid methyl esters in hexane were analysed on a Shimadzu GC-14A gas chromatograph equipped with a flame-ionization detector. Relative proportions of fatty acids between C10:0 and C20:0 were calculated from peak areas. Identification was done either directly by comparison of retention times of unknown peaks with standard fatty acid calibration mixtures (BAME, MIDI; Sigma-Aldrich) or indirectly by calculation of equivalent chain-length (ECL) factors (Mjøs, 2003).

Statistical analysis. The data were analysed using Student's *t*-test for significant differences between the two sample means (*n*>3).

RESULTS AND DISCUSSION

The step-like growth of *E. coli* during temperature cycling is shown in Fig. 1(a). During temperature downshift from

Abbreviations: C16:0, palmitic acid; C16:1, palmitoleic acid; C17:0cyc, methylene hexadecanoic acid; C18:1, *cis*-vaccenic acid; SFA, saturated fatty acid; UFA, unsaturated fatty acid.

Fig. 1. Growth rate (a), CO₂ production (b), and intracellular concentration of ATP (c) of *E. coli* K-12 during constant incubation at 37 °C (□), cold shock (○), and periodic temperature cycling between 37 °C and 8 °C (△). Samples for OD₆₅₀, CO₂ production and ATP concentration were taken from the same flasks. Means ± SD are shown ($n \geq 6$). The temperature profile during oscillations is shown in (d).

37 to 8 °C bacterial growth stopped after approximately 30 min, when the temperature of the growth medium dropped to 15 °C or lower. Similarly during temperature upshift growth resumed approximately 30 min after the onset of temperature upshift, when the temperature in the

Fig. 2. Fatty acid content of *E. coli*: C18:1 (a), C16:1 (b), C16:0 (c), and C17:0cyc (d) during constant incubation at 37 °C (□), cold shock (○), and periodic temperature cycling between 37 °C and 8 °C (△). The dashed lines represent fatty acid composition after a prolonged incubation at 37 °C (for 3 h) following either three or five temperature cycles. Means ± SD are shown ($n \geq 3$).

growth medium reached 20 °C or higher. Production of CO₂ (Fig. 1b) correlated with the growth rate during temperature cycling, i.e. more CO₂ was produced when

cells were growing faster and less during slower growth. Unlike growth rate and CO₂ production, net ATP concentration (Fig. 1c) did not follow a 120 min period imposed by temperature oscillations. At the end of the experiment periodically fluctuated cells had fourfold higher net ATP concentration than cells constantly incubated at 37 °C. This may indicate that periodically cycled cells were under severe stress (Napolitano & Shain, 2005).

The response of the fatty acid composition to periodic temperature fluctuations is shown in Fig. 2. During the first two temperature cycles the fraction of unsaturated *cis*-vaccenic acid (C18:1) periodically increased with decreasing temperature and decreased with increasing temperature. With progressing number of temperature cycles ($n > 3$), however, the bacterial membrane lost its ability to change the fraction of C18:1 in response to temperature up- or downshift although cells were still able to grow. This is surprising since Garwin & Cronan (1980) demonstrated that unsaturation is a quick process, completed in less than 30 s, and does not require new protein synthesis. The lack of cell membrane response after three temperature cycles is not what homeoviscous adaptation predicts (Sinensky, 1974) and suggests that periodically oscillated cells maintain a homeostatic response to temperature fluctuations only when they are physiologically young. Not only UFAs but also SFAs changed during periodic temperature fluctuations. The fractions of both palmitic acid (C16:0) and C17:0 cyclopropane fatty acid in temperature-cycled cells were lower than in cells constantly incubated at 37 °C.

An unexpected finding of this study is that the fatty acid profile of periodically cycled cells was more similar to that of cold-shocked bacteria than to that of cells constantly incubated at 37 °C. One would expect either a membrane composition that is intermediate between cold or warm cells or a membrane composition that consistently fluctuates between the two extremes. Neither outcome was observed. It is, however, possible that cells become conditioned not to respond to high temperatures during temperature fluctuations. To test this we extended the time of incubation at 37 °C after cells had completed either three or five temperature cycles. There was no change of fatty acid composition with prolonged incubation at 37 °C,

when cells were already in the stationary phase (Fig. 2, dashed lines). This indicates that membrane fatty acid composition of stationary-phase cells is rather fixed and is not responsive to temperature fluctuations. Although the situation was slightly different when cells had completed three temperature cycles, the cells were still not able to revert to the membrane fatty acid composition of cells constantly incubated at 37 °C. This result suggests that the membrane fatty acid composition of *E. coli* becomes conditioned to cold during periodic temperature cycling.

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