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CORRELATION BETWEEN TEMPERATURE RANGE OF GROWTH AND STRUCTURAL TRANSITIONS IN MEMBRANES AND LIPIDS OF *ESCHERICHIA COLI* K12

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Summary

Purified cytoplasmic and outer membranes isolated from cells of wild-type *Escherichia coli* grown at different temperatures were labelled with 1,6-diphenyl-1,3,5-hexatriene and anlyzed using fluorescence polarization techniques. Lipids extracted from the membranes were similarly analyzed using fluorescence polarization. The thermotropic structural transition in outer membranes changed as a function of growth temperature. The structural transition in cytoplasmic membranes and lipids extracted from either cytoplasmic or outer membranes did not change with growth temperature. These data suggest that adaptive changes which occur in the outer membrane determine the temperature range of growth of *E. coli*. These changes apparently require alterations in outer membrane components other than phospholipids.

The factors which limit bacterial growth at extreme temperatures are not known. In the case of Gram-positive thermophiles, it has been suggested that the existence of two lipid phases in the membrane may be necessary for survival and cellular division. These organisms adapt to different environmental temperatures presumably by changing their lipid composition. Maximal and minimal growth temperatures are thus thought to be defined by the beginning and end of the membrane lipid phase separation of the lipid composition present [1,2].

Although the structure and function of the Gram-negative cell envelope has recently been under intensive investigation, the situation remains complex

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Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid.

due to the existence of both an outer and cytoplasmic membrane. The cytoplasmic membrane has been shown to be a typical bilayer containing protein and phospholipid [3] while the outer membrane contains, in addition, substantial amounts of lipopolysaccharide [4]. We report here that for *Escherichia coli* K12 , a typical Gram-negative mesophile, adaptive changes which occur in the outer, not the cytoplasmic, membrane appear to determine the temperature range of growth. These adaptive changes apparently require alterations in outer membrane components other than phospholipids. This represents the first evidence to suggest that a thermotropic membrane structural transition can be modulated in response to growth temperature by components other than phospholipids.

Membranes from cultures of *E. coli* strain W1485F$^-$ were isolated and characterized as described previously [5]. The degree of purity of cytoplasmic and outer membrane isolates equalled or exceeded that described earlier [5]. Lipids were extracted from the purified membranes by using the method of Folch et al. [6] and resuspended in 10 mM Hepes, pH 7.5. The extracts were assayed for protein [7], ketodeoxyoctanoic acid [8] (a component of lipopolysaccharide) and phosphate [9] and were shown to contain less than 4.0% of the protein and less than 1.0% of the ketodeoxyoctanoic acid found in the isolated membranes. Prior to incorporation of the fluorescent probe, lipid extracts were dispersed in a bath sonicator. The dispersions cleared upon sonication indicating the formation of stable structures. Absorbance values of lipid dispersions or membranes were matched to insure equal light scattering.

1,6-Diphenyl-1,3,5-hexatriene (Sigma Chemical Co.) was incorporated into membranes and lipids by methods to be described elswhere (Rouslin, W., MacGee, J. and Gupte, S., unpublished results). The molar ratio of probe:lipid was less than 1:100. Fluorescence polarization of the samples (membranes at approx. 1 mg/ml protein, extracted lipids at approx. 10 mg/ml phospholipid) as a function of temperature was measured using a Perkin-Elmer MPF 44A fluorescence spectrometer in the ratio mode with the polarizer accessory. The probe was excited at 358 nm and its fluorescence was detected at 428 nm. In all preparations the fluorescence intensity was nearly equal. For each growth temperature two independently isolated membrane and lipid samples were characterized. Inflection points in the temperature dependence of fluorescence polarization were determined as previously described [5]. The figures presented represent typical experiments.

Data presented in Fig. 1A show the temperature dependence of label fluorescence polarization (which reflects membrane fluidity) in outer membrane fractions isolated from cells grown at 20 and 37°C. Inflection points are interpreted to represent the beginning and the end of a broad phase separation or gel to liquid crystalline phase change based on studies in well defined model systems [10,11]. As can be seen, the end of the phase change shifted dramatically as a function of growth temperature so that the outer membrane always existed within its broad phase change, in a heterogeneous lipid state, at the temperature of growth. These data support earlier electron spin resonance (ESR) experiments which suggested that the temperature range over which the outer membrane can maintain a mixed lipid state correlates with the

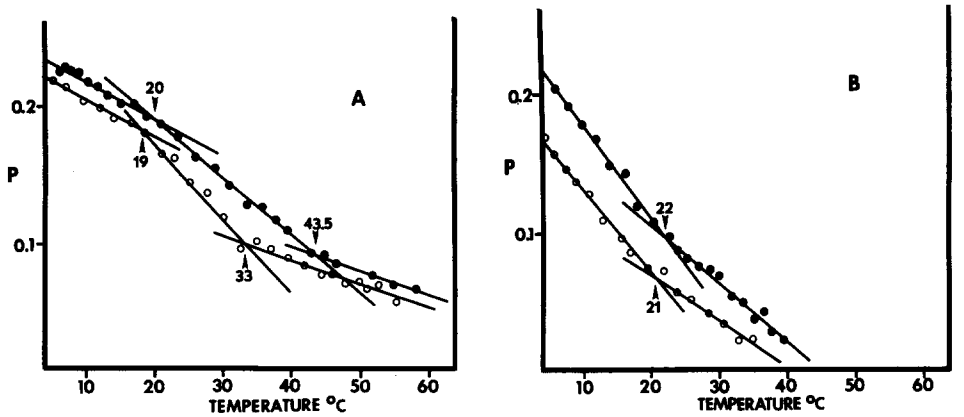

Fig. 1. Temperature dependence of 1,6-diphenyl-1,3,5-hexatriene polarization in outer membranes (A) and lipids extracted from outer membranes (B) grown at 20° C (open circles) and 37° C (closed circles). Arrows indicate transition temperatures. The lower transition in the lipids extracts apparently occurs below approx. 4° C and could not be detected.

temperature range over which growth occurs [5]. In fact, identical high-temperature transitions were observed in experiments using fatty acid spin probes and polarization of 1,6-diphenyl-1,3,5,-hexatriene. Whilst ESR probing indicated that the phase change began at 9° C, fluorescence polarization shows that a phase change begins at 19° C. The discrepancy may arise from the possible partitioning of the fluorescence probe into both monolayers of the outer membrane. The spin label used in the previous ESR experiments partitions only into the phospholipid inner monolayer and not the lipopolysaccharide outer monolayer [12—14].

As shown in Fig. 1B, lipids extracted from outer membranes did not exhibit transition points similar to outer membranes nor did transition points change as a function of growth temperature. Cytoplasmic membranes and their extracted lipids from cells grown at 20 and 37° C all exhibited transitions at 21—22° C (data not shown). Thus, lipids extracted from both outer membranes and from cytoplasmic membranes exhibited break points identical to those exhibited by intact cytoplasmic membranes regardless of growth temperature. Previous ESR experiments also indicated that the cytoplasmic membrane did not shift its (approx. 20° C) phase transition as a function of growth temperature [5]. Thus, the structural transition of the outer membrane, but not the cytoplasmic membrane, changes as a function of growth temperature and this change apparently requires components other than phospholipids. Whilst it is known that the fatty acid composition of the phospholipids in the outer membrane changes as a function of growth temperature [15, 16], these changes do not appear to directly alter the structural transitions shown here (Fig. 1B). To our knowledge this is the first demonstration that changes in membrane architecture which are dependent upon non-phospholipid components may provide a mechanism for environmental adaption. We do not rule out the possibility that changes in membrane structure could arise in connection with lipid changes due to altered interactions of phospholipid with other membrane components.

It is understandable that the adaptability of the outer membrane may

be crucial for the cell's ability to grow over a broad range of temperatures. Outer membrane proteins presumably associate in a specific manner to form pores or transmembrane channels [17, 18]. Such pore structure may require a specific lipid state. In this regard we find intriguing the recent evidence that induced increases or decreases in the fluidity of the outer membrane affect the processing and assembly of outer membrane proteins [19—21].

Lastly, it should be noted that experiments reported here confirm our earlier ESR observations [5] which suggested that the phase transition in the outer membrane of *E. coli* always occurs at higher temperatures than in the cytoplasmic membrane. Similar differences in the phase transition of cytoplasmic and outer membranes have recently been detected by ^{2}H-NMR quadrupolar echo spectroscopy [22].

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