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ANESTHETICS ALTER OUTER MEMBRANE ARCHITECTURE AND TEMPERATURE RANGE OF GROWTH OF ESCHERICHIA COLI K12

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SUMMARY: The addition of local anesthetics procaine and 2-phenylethanol during cell growth and membrane isolation lowered the phase transition temperature of purified outer membranes of Escherichia coli. Furthermore, when added to growth media, these anesthetics lowered to an equal extent the maximum temperature of growth without affecting growth at low temperatures. The phase transition of the cytoplasmic membrane was not affected by the presence of the drugs. These data substantiate the hypothesis that the temperature range over which the cell can maintain the outer membrane in a mixed (gel + liquid crystalline) lipid state determines the temperature range over which growth can occur.

INTRODUCTION: Much attention has recently been focused on the role that membrane architecture plays in determining the temperature range of microbial growth (1, 2). Although conclusive evidence is lacking, a relationship seems to exist between the physical state of membrane lipids and the upper and lower temperature limits of growth in various bacteria (3, 4, 5). To investigate this relationship we have performed a series of experiments on spin-labelled and fluorescently labelled membranes and lipids isolated from Escherichia coli (6, 7). Our studies and ^{2}H-NMR studies (8, 9) have indicated that: a) both the outer and cytoplasmic membranes undergo broad thermotropic order-to-disorder transitions over which gel and liquid crystalline lipid domains clearly exist, and b) the midpoint of the phase transition of the outer membrane occurs at significantly higher temperatures

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than that of the cytoplasmic membrane. Further, our studies (6, 7) have revealed that the thermotropic transition in the outer membrane occurs at increasingly higher temperatures as the growth temperature is elevated. Since we find that the temperature range over which the outer membrane can adapt to remain in a mixed (gel + liquid crystalline) lipid state is identical to the temperature range over which the cell can adapt to grow (6, 7), we have proposed that the coexistence of gel and liquid crystalline domains in the outer membrane is a prerequisite for growth. If this hypothesis is correct, then agents which melt gel phase lipid and thus lower the phase transition of the outer membrane, should concomitantly lower the maximum temperature for growth. We report here that in fact low concentrations of anesthetics decrease the lipid order to disorder temperature of the outer membrane and, as predicted, decrease, to an equal extent, the maximum temperature for growth.

MATERIALS AND METHODS: The growth of E. coli strain W1485F$^-$, isolation and characterization of membranes, and electron spin resonance spectroscopy was as previously described (6), except for the addition of either phenylethanol (PEA)[5] or procaine (0.1% or 10mM final concentration respectively) to the growth media and to all buffers. The degree of purity of cytoplasmic and outer membranes equalled or exceeded that described earlier (6). As judged by membrane protein concentration and by sodium dodecyl sulfate polyacrylamide slab gel electrophoresis (10), neither anesthetic affected the incorporation of major outer membrane proteins. The experimental curves (hyperfine splitting parameter vs temperature and order parameter vs temperature) were analyzed in terms of linear components by fitting regression lines to appropriate sections using the method of least squares. This method has been shown to allow the determination of break points in both model systems and membranes (6, 7, 11, 12). For each growth condition duplicate membrane samples were isolated and each isolate was fully characterized.

To assess the possibility that growth in PEA or procaine leads to compensatory changes in membrane architecture, outer membranes isolated from cells grown in the absence of anesthetics were washed and resuspended in either procaine (10mM) or PEA (0.1%) prior to spin labelling. These membranes exhibited identical transitions compared to those grown and isolated in the presence of anesthetics.

RESULTS AND DISCUSSION: Electron spin resonance spectroscopy of spin labelled outer membranes isolated from cells grown in the presence of either PEA or procaine revealed differences in structural transitions compared to those of control outer membranes from cells grown at the same temperatures.

[5]Abbreviation: PEA, 2-phenylethanol

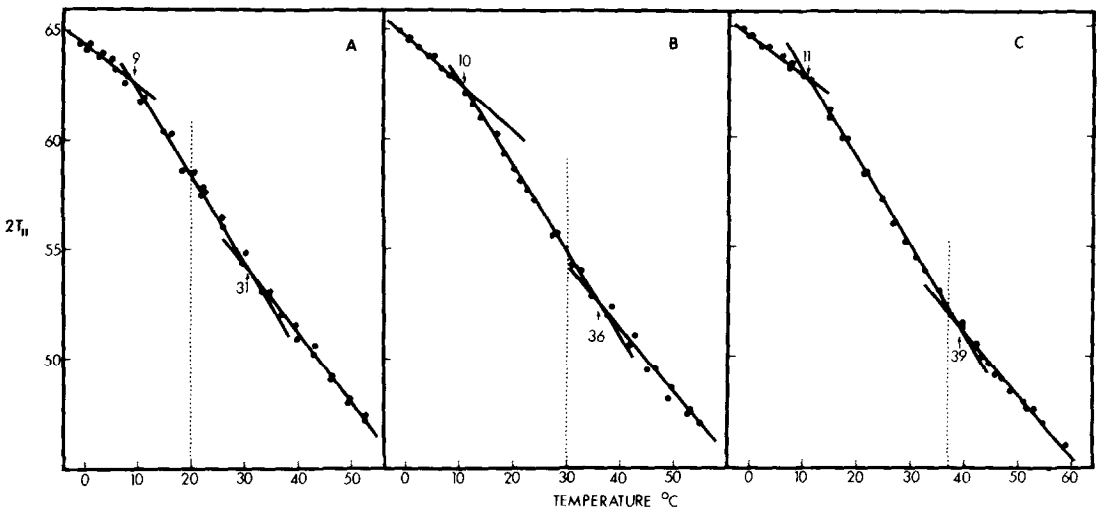

Figure 1. Hyperfine splitting parameter, $2T_{\parallel}$ (Gauss), as a function of temperature in E. coli outer membranes labelled with 5-doxyl stearate. The hyperfine splitting parameter is related to the rotational mobility of the spin label and therefore reports the local fluidity of the membrane lipids. High values of $2T_{\parallel}$ reflect low fluidity. Breaks in the temperature dependence of $2T_{\parallel}$ have been correlated with lipid phase separations or lipid phase transitions (6). Membranes were isolated from cultures of E. coli W1485F grown at a) 20° b) 30° and c) 37°C in M9-glucose minimal medium supplemented with 0.1% PEA. The membranes were isolated as previously described except that 0.1% PEA was present in all buffers used during the membrane isolation. The vertical broken line indicates the growth temperature. The arrows indicate phase changes.

As shown in Figure 1, the low temperature (gel → gel + liquid crystalline) transitions in outer membranes from cells grown in the presence of PEA at 20, 30 and 37°C were nearly identical, and were identical to those of control membranes (6). In contrast, the upper transition (gel + liquid crystalline → liquid crystalline) temperature changed with growth temperature (Fig. 1, Table 1) and occurred at lower temperatures than in control outer membranes from cells grown in the absence of anesthetic. As can be seen in Figures 1 and 2, the difference between the upper transition temperature, T_U and the growth temperature, T_G, decreased linearly with increasing growth temperature. For outer membranes isolated from cells grown in the presence of anesthetic, this difference approached zero at a growth temperature of 41°C. Thus, outer membranes from cells grown in the presence

TABLE 1. Upper Transition Temperature (^{o}C) of outer membranes from cells grown in PEA.

GROWTH TEMPERATURE	$2T_{\parallel}$ [a]	S [b]
20	31.1	30.9
30	35.8	36.1
37	39.2	39.4

a. Determined using the hyperfine splitting parameter, $2T_{\parallel}$
b. Determined using the order parameter, S

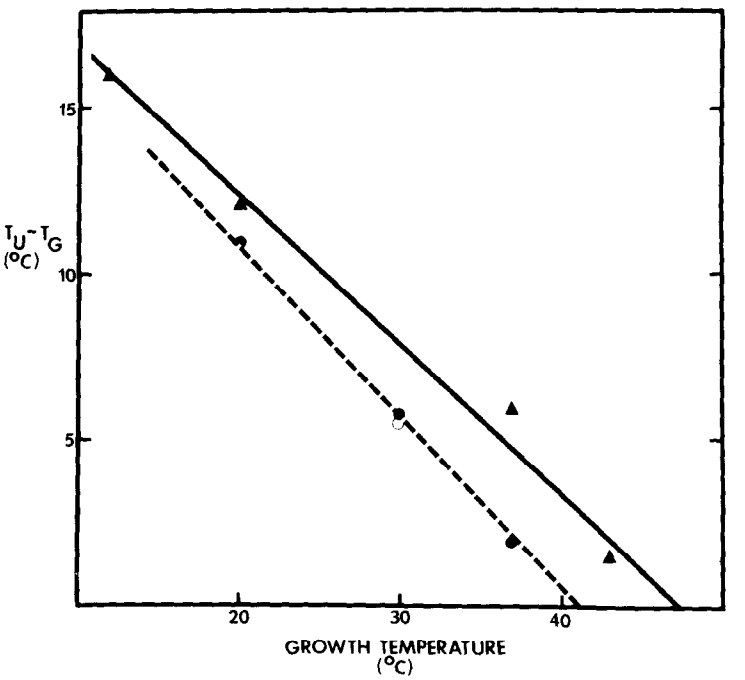

Figure 2. The difference between the growth temperature, T_G, and the upper transition, T_U, (T_U-T_G) in outer membrane isolates as a function of growth temperature. ▲——▲ outer membranes from cells grown in M9 glucose minimal medium, ---- outer membranes from cells grown in M9 glucose medium containing either 0.1% PEA (●) or 10mM procaine (o).

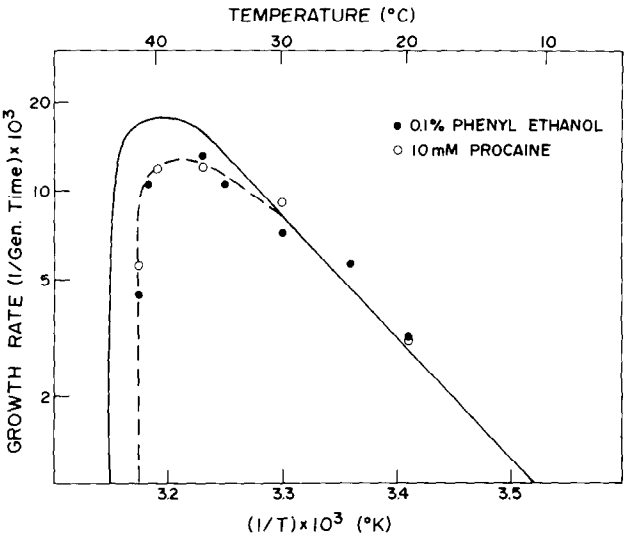

Figure 3. Growth rates of cultures of E. coli W1485 incubated at various temperatures were determined by monitoring the optical density of the cultures of 560nm. ———Cultures growing in M9 minimal medium containing 0.4% glucose. ---Cultures growing in M9 minimal-glucose medium supplemented either with 0.1% PEA (•) or 10mM procaine (o).

of PEA or procaine are maintained in a mixed lipid state, up to a growth temperature of approximately 41°C. Above this temperature the membrane would be expected to exist in a completely liquid crystalline state. In contrast, control outer membranes are maintained in a mixed lipid state up to 47°C.

As predicted, the decrease in T_U of the outer membrane in the presence of anesthetics is mirrored by a decrease in the maximum temperature of growth (Fig. 3). As cells are grown above 30°C in the presence of anesthetic, growth is slower and is completely inhibited at 42°C. This temperature is essentially identical to the upper limit that the cells can maintain the outer membrane within its transition when anesthetic is present. The concurrent decrease in the upper temperature limit of growth and in the high temperature limit of the transition of the outer membrane upon addition of anesthetic renders further evidence that the temperature range over which the outer membrane can exist within its transition deter-

mines the temperature range over which growth can occur. The growth rates of cells incubated at low temperatures (<30°C) in the presence of anesthetic were essentially identical to those of cultures without perturbant. This is consistent with our model since at these lower growth temperatures the outer membrane should exist well within its transition, and the melting of small amounts of gel phase lipids should be of little consequence.

Structural transitions of spin labelled cytoplasmic membranes were practically identical for membranes isolated from cells grown either in the presence or absence of perturbants. These findings suggest that the outer membrane is more sensitive to low concentrations of anesthetics than is the cytoplasmic membrane. At concentrations of PEA higher than those used here, the synthesis of outer membrane proteins is inhibited (13) presumably through the involvement of cytoplasmic membrane function.

The differential sensitivity of the outer membrane to PEA and procaine may reflect a complex supramolecular structure. Recently a model has been put forth which describes the outer membrane in terms of polymer domains (14). Microdomains which exist within this network apparently cannot be detected by x-ray diffraction (15) but participate in the membrane phase transition(8, 9, 16). Other evidence suggests that membrane lipid domains are created by long range lipid-protein interactions (17). Such a "polymer-network" model of the outer membrane could explain increased sensitivity to anesthetics in terms of the disruption of lipid-protein interactions as has been described by Mazzanti et al. (18).

Although the structural transition of the outer membrane changes as function of growth temperature (6), these changes have been shown to require components other than the phospholipid (7). Lipopolysaccharides derived from cells grown at increasingly higher temperatures exhibit elevated transition temperatures (19). That spin labels which partition only into domains of the outer membrane devoid of lipopolysaccharide (20-22) may be detecting these changes (6) gives further evidence to the complexity of outer membrane structure.

The results reported here indicate that low concentrations of local anesthetics inhibit growth in E. coli by disrupting the mixed (gel + liquid crystalline) lipid state in the outer membrane. This effect is reversed at lower growth temperatures consistent with a mechanism involving the restoration of gel phase lipid. The maintenance of the outer membrane in a mixed lipid phase may be critical for its synthesis or function. The outer membrane proteins which associate in a specific manner to form pores (23, 24) may require a specific lipid structure. In the mixed lipid state the resulting high lateral compressibility should enable the membrane to accommodate newly synthesized protein without large volume changes and should more readily allow for conformational changes (25) thus favoring pore formation. In summary our results indicate that the growth limits of Gram negative bacteria can be altered and restricted by compounds which preferentially alter the structure of the outer membrane.

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