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# Membrane fluidity of *Escherichia coli* during heat-shock

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**Abstract**

The excimer-forming fluorophore dipyrenylpropane has been used to measure the relative fluidity of total membranes isolated from *Escherichia coli* grown at 30 or 45°C, or exposed to a heat-shock from 30 to 45°C for various periods of time. Parallel experiments were performed using [$^{35}$S]methionine pulse-labeling of cells, to study the induction of heat-shock proteins (HSPs) at different times after the sudden change in *E. coli* growth-temperature from 30 to 45°C. Results suggest that upon an abrupt temperature upshift from 30 to 45°C, membrane fluidity adjustment to the steady-state level at the high temperature, takes place during the *E. coli* heat-shock response.

*Keywords:* Membrane fluidity; Fluorescence; Heat shock; (*E. coli*)

## 1. Introduction

The effect of temperature on the physiology of bacteria has received considerable attention from quite different perspectives [1–13]. It is well known for instance, that *Escherichia coli* membranes exhibit a variable composition of acyl-substituents in phospholipids depending on the growth temperature [1–5,7]. Based on the findings that the higher the growth temperature, the lower the ratio between unsaturated and saturated fatty acyl chains [1], and considering the regulatory effect of this change in composition on membrane fluidity, Sinensky [2] proposed that bacteria cope with thermal variations through a homeoviscous adaptive response. A number of subsequent studies have confirmed that this hypothesis is essentially correct for *E. coli* [8,9] and for several other species of bacteria [10–12]. However, not all bacterial species exhibit fully efficacious homeoviscous adaption mechanisms [6]. In this regard, Cossins et al. [13] introduced the concept of a variable homeoviscous efficacy that allows a quantitative estimation of the tendency of organisms to show a more or less ideal membrane homeostatic response when challenged by a certain temperature change.

Most data on the effect of variations of growth-temperature on bacterial membranes, come from studies of systems in steady-state conditions. However, a sudden change of temperature should have an almost instantaneous physical effect on bilayers [14] which could possibly be followed by a bacterial response detectable at the membrane level. At cellular level, there is abundant information on the stress produced by an abrupt change of temperature. It is well known that a temperature upshift gives rise to the heat-shock response consisting of a transitory increase in the synthesis of the heat-shock proteins (HSPs) [15–22]. Most of the heat-shock genes are positively regulated by sigma-32 which in turn, is under the control of another sigma factor, sigma-E [21,22]. The amount of sigma-32 increases rapidly after the temperature upshift both by an increase in the transcription and translation of gene *rpoH* which encodes for sigma-32, and by an increase in the stability of the protein itself [21].

In the present study, the excimer-forming fluorophore dipyrenylpropane [23–27] is used to study the relative fluidity of total membranes isolated from *E. coli* grown at 30 or 45°C, or exposed to a temperature upshift from 30 to 45°C for various periods of time. The temporal modification of membrane fluidity is compared with the heat-shock response of *E. coli* as judged by the induction of two HSPs, DnaK and GroEL [20], followed by radioactive-pulse labeling, at different times after the temperature upshift.

Abbreviations: DPyP, 1,3-di(1-pyrenyl)propane; HSPs, heat-shock proteins.

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The aim is to examine the evolution of fluidity during heat-shock and to determine whether the membrane changes occur in a similar time-scale as the cellular response to thermal stress. It should be mentioned that the heat-shock condition chosen for this study represents a considerable temperature stress that can be expected to induce an important modification of the membrane fluidity. However, 45°C is not a lethal temperature and, as will be shown in this work, the heat-shock response at this temperature is still transient for W3110, the *E. coli* strain used in the present work. The lethal temperature for this strain in Luria broth [28], the culture medium employed here, is approx. 50°C.

## 2. Materials and methods

The fluorescent monitor 1,3-di(1-pyrenyl)propane was purchased from Molecular Probes. Spectrophotometrically transparent liquid paraffin was obtained from Merck, Darmstadt, Germany (Paraffin viscous, Merck 7160, B.P., U.S.P.). Viscosity standards were from Cannon. [$^{35}$S]Methionine (1000 Ci/mmol) was from Amersham. All other reagents were analytical grade. Triple distilled water was used throughout.

Kinematic viscosities of spectrophotometrically transparent liquid paraffin at different temperatures were determined using a series of Cannon viscometers previously calibrated using ASTM standard oils [29]. The viscosity in centipoise (cp) was calculated from the kinematic viscosity and the paraffin density as determined with a pycnometer.

The prototroph W3110 *Escherichia coli* K12 strain, was used throughout. Cultures employed for experiments were prepared by diluting an overnight culture 1:50 into 10 ml pre-warmed Luria broth [28]. Cells were grown in 125 ml Erlenmeyer flasks with shaking (180 rpm) at the desired temperature, 30 or 45°C, to mid-log phase ($OD_{550}$ of 0.4, approx. $1 \cdot 10^8$ cells $ml^{-1}$). For the heat-shock experiments, cultures grown at 30°C were directly transferred to another water-bath shaker at 45°C and maintained at this temperature for different times, as indicated in each particular experiment. Total membranes of *E. coli* cells grown at 30 or 45°C, or exposed to a heat-shock from 30 to 45°C during different times as described above, were obtained by a procedure previously described [30] based on the method by Osborn et al. [31]. Phospholipid content of membrane vesicles was calculated from phosphorus determinations performed according to the method by Bartlett [32]. Induction, [$^{35}$S]methionine-labeling, SDS-PAGE separation and autoradiographic detection of heat-shock proteins, were performed as previously described [20]. To verify that a similar concentration of proteins was loaded on each well, protein bands in the gel were stained with Coomassie blue before obtaining the corresponding autoradiograph. Autoradiographs were scanned using a computer controlled densitometer (UltroScan XL, Pharmacia LKB).

Labeling of membranes with DPyP was performed by a procedure essentially similar to that described by Almeida et al. [24]. Fluorescence measurements were performed in a LS50 Perkin Elmer spectrofluorometer equipped with a four-position thermostated sample chamber and a magnetic stirrer. The selected temperature of membrane dispersions (30 or 45°C), was approached upwards and maintained constant, at least during 5 min, before recording a fluorescence value. Loss of reproducibility of fluorescence readings was observed for membrane dispersions incubated near 60°C. Samples were excited at 329 nm and the excimer ($I_e$) and monomer ($I_m$) fluorescence intensities were read at 379 and 480 nm, respectively.

## 3. Results and discussion

Intramolecular excimer formation [23] by fluorophores such as 1,3-di(1-pyrenyl)propane (DPyP), has proved useful in the estimation of microviscosities or relative fluidities of model bilayers or natural membranes [23–27]. DPyP is an extremely hydrophobic probe which partitions into the membrane lipid bilayer and is able to give monomer ($I_m$) and excimer ($I_e$) fluorescence [25]. Since intramolecular excimers originate in a monomolecular process, they can be observed at very low ratios of fluorophore to matrix (< 1:500) [24,25,27,33]. Excimer formation was initially characterized for the fluorophore dissolved in liquid paraffin at different temperatures. The corresponding viscosities had been previously determined. Fig. 1 shows the excimer to monomer fluorescence intensity ratio ($I_e/I_m$) of the monitor as a function of the quotient between the kelvin temperature ($T$) and viscosity ($\eta$) of the medium. This kind of plot renders a calibration curve as shown by

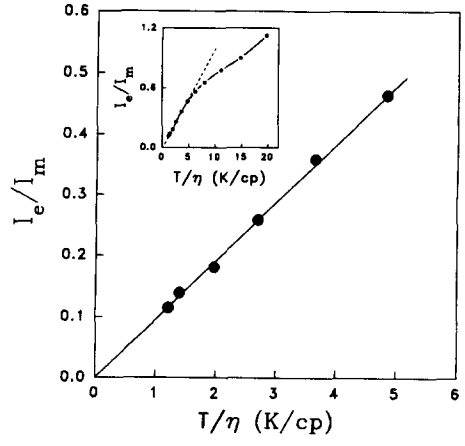


Fig. 1. Excimer to monomer fluorescence intensity ratio ($I_e/I_m$) of DPyP dissolved in liquid paraffin as function of the quotient between the kelvin temperature ($T$) and viscosity ($\eta$) of the medium. Samples containing 0.5 $\mu$M DPyP were excited at 329 nm and the excimer ($I_e$) and monomer ($I_m$) fluorescence intensities read at 379 and 480 nm. Viscosities were previously determined as described in Section 2. For $I_e/I_m <$ 0.5, the plot is linear such that Eqs. (1) and (2) are obeyed.

Almeida et al. [22]. For $(I_e/I_m) < 0.5$, the plot is linear with the straight line extrapolating to an intersect equal to zero. At higher $I_e/I_m$ values, linearity is lost although excimerization still increases with $T/\eta$. The linear region of Fig. 1 can be described by the following empirical equation:

$$\frac{I_e}{I_m} = kT_F\frac{1}{\eta} \quad (1)$$

where $I_e/I_m$ is the ration between the fluorescence intensities of the excimer and monomer, respectively; $k$ is a proportionality constant; $T_F$ is the kelvin assay temperature and $\eta$ is the viscosity (in cp). Interpolation in the linear region of Fig. 1 of a certain $I_e/I_m$ ratio determined at a temperature $T_F$ for DPyP incorporated into a membrane, allows to obtain its apparent viscosity and therefore, the inverse of this parameter, the fluidity ($F$) [24]. However, there is a well known conceptual problem in trying to establish an analogy between the macroscopic properties of a fluid with those of a two-dimensional system such as a membrane [14]. In the present work, the calibration curve of Fig. 1 is used only to determine the range in which $I_e/I_m$ varies proportionally to $T/\eta$. Within this range, different $I_e/I_m$ ratios from membrane samples under a number of experimental conditions can be compared to obtain relative viscosity or fluidity values. Since the $I_e/I_m$ values obtained for DPyP incorporated into the bacterial membranes under the experimental conditions of the present study (not shown) correspond to this linear region, the expression, based on Eq. (1), for comparing the fluidities of two membrane systems, A and B, is as follows:

$$\frac{F_B}{F_A} = \frac{\frac{1}{T_F^B}\left(\frac{I_e}{I_m}\right)_B}{\frac{1}{T_F^A}\left(\frac{I_e}{I_m}\right)_A} \quad (2)$$

where $F_A$ and $F_B$ are the fluidities $(1/\eta)$, $T_F^A$ and $T_F^B$, the kelvin measurement temperatures, and $(I_e/I_m)_A$ and $(I_e/I_m)_B$, the excimer to monomer fluorescence ratios of systems A and B, respectively. Thus, the relevant parameter for fluidity comparison purposes is $((1/T_F)\cdot(I_e/I_m))$, similar to that occasionally employed by other authors [34]. However, if $T_F^A = T_F^B$, Eq. (2) reduces to a ratios of the $I_e/I_m$ values, i.e., of the simplified form of the fluidity parameter values for systems A and B, respectively. To estimate relative membrane fluidities, an arbitrary system can be taken as a reference state of fluidity equal to unity in Eq. 2. In the present case, we have chosen as reference state the membranes from *E. coli* cells grown at 30°C for which the empirical parameter $((1/T_F)\cdot(I_e/I_m))$, as determined from DPyP fluorescence, is also measured at 30°C.

Fig. 2 depicts the fluidity measured at 30 and 45°C of membranes from cells grown at 30°C as well as the fluidity measured at 45°C of membranes from cultures at this same high temperature. It can be observed that DPyP clearly differentiates between membranes isolated from cells grown at 30 or 45°C, the fluidity of the former being greater, when measured at constant temperature (B and C). Thus, the monitor not only distinguishes between both membrane types, but it also reveals the existence of a

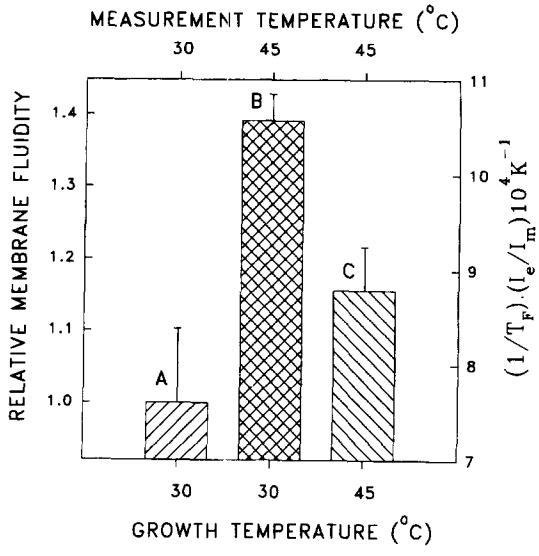


Fig. 2. Relative membrane fluidities of *E. coli* under several conditions. (A) measured at 30°C in membranes from cells grown at the same temperature; (B) measured at 45°C in membranes from cells grown at 30°C and (C) measured at 45°C in membranes from cells grown at the same temperature. The molar ratio of DPyP to membrane phospholipid was 1:1400. Relative fluidities were calculated according to Eq. (2) by taking as reference state of fluidity equal to unity, the membranes from *E. coli* cells grown at 30°C for which the empirical fluidity parameter $((1/T_F)\cdot(I_e/I_m))$ shown in the right-hand scale, was measured also at 30°C. Results are the means ± S.D. of four experiments.

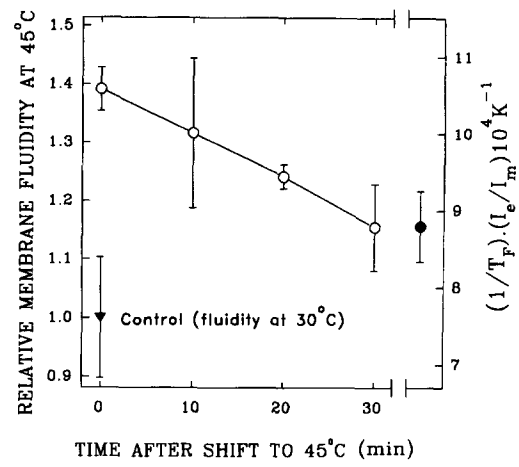


Fig. 3. Relative fluidity at 45°C of *E. coli* membranes, as a function of time after a temperature upshift from 30 to 45°C (○). The control (▼) corresponds to the fluidity value at 30°C of membranes from cells grown at this same temperature, which is also taken as reference state of fluidity equal to unity in Eq. (2). The rest of experimental conditions are as in Fig. 2. For comparison, the relative fluidity value of membranes from cells growing in steady-state at 45°C (●) is included. Results are the means ± S.D. of four experiments.

regulatory mechanism that modifies the membrane fluidity during growth at different temperatures. The higher fluidity detected at constant temperature for membranes from cells grown at 30°C with respect to those from cultures at 45°C is consistent with the reported increase in the proportion of *cis*-vaccenic to palmitic acid substituents in bacterial phospholipids, as *E. coli* growth temperature is lowered [1–3,7]. The figure also shows that fluidity at 45°C of membranes from cells grown at that temperature (C), is greater than fluidity at 30°C of membranes from cultures at 30°C (A). If a perfect homeostatic regulation were operative, one would expect to obtain, according to Sinensky's proposal [2], the same fluidity value in (A) and (C). Taken together, data from Fig. 2 confirm the operation of a homeostatic mechanism that opposes the direct physical effect of a higher temperature on membrane viscosity, although regulation, under the experimental conditions of this work, does not appear to be as precise as proposed by Sinensky [2]. A quantitative evaluation of these results can be done in terms of the 'homeoviscous efficacy' which we define in a somewhat modified form with respect to that proposed by Cossins et al. [13]. On the one hand, the increase in fluidity induced by the direct physical effect of the temperature upshift (PE) can be determined from the difference between the fluidity at 45 and 30°C of membranes from cell grown at 30°C (B minus A). On the other hand, the compensatory response (CR) attributable to an apparent thermoadaptive mechanism, can be obtained from the difference in fluidity at 45°C between membranes from cells grown at 30 or 45°C (B minus C). The homeoviscous efficacy (HE) will result from the ratio between the magnitudes of the compensatory response and the physical effect of temperature upshift (CR/PE). In this way we obtain an efficacy of 0.60 for the fluidity adjustment mechanism when temperature is raised from 30 to 45°C. It is possible, however, that cells subjected to a milder stress of temperature could respond with a more effective homeoviscous adjustment. Besides, it should be kept in mind that the estimate obtained from our data must be taken only as indicative of the tendency of *E. coli* to maintain membrane fluidity within certain range. As is well known, homeoviscous efficacy values reflect not only the physiology of a particular organism, but also the methodology used to measure fluidity [12].

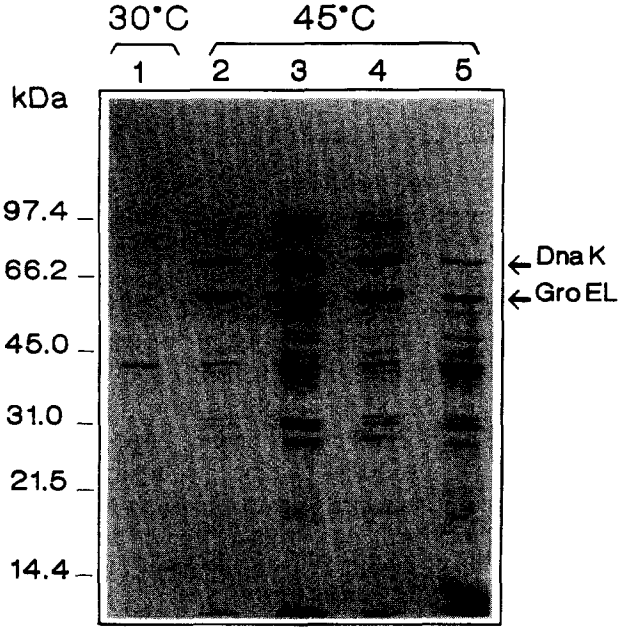


Fig. 4. Autoradiograph of 11% SDS-PAGE displaying L-[$^{35}$S]methionine-labeled proteins during cell exposure to 45°C. Exponentially growing *E. coli* cells at 30°C in Luria broth were shifted to 45°C for different times: 10, 20 or 30 min. During the last 5 min of the indicated time-period of the heat-shock, the cells were pulse-labeled with 96 μCi/ml [$^{35}$S]methionine (1000 Ci/mmol). Control experiments were performed by exposing cells in steady-state growth at 30 or 45°C to a similar labeling procedure. Lane 1: steady-state growth at 30°C; lanes 2, 3, 4 : 10, 20 and 30 min heat-shock at 45°C, respectively; lane 5: steady-state growth at 45°C. Assignment of bands to DnaK and GroEL proteins, was done as previously described [20].

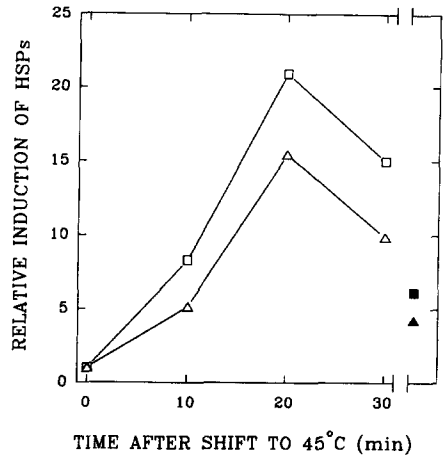


Fig. 5. Relative induction of the HSPs GroEL (□) and DnaK (△) by a heat-shock from 30 to 45°C, as a function of time after the temperature upshift. Relative induction values are estimated from the ratios between the amounts of [$^{35}$S]methionine incorporated into the individual proteins during 5 min-pulses, at various times after the temperature upshift from 30 to 45°C, and in steady-state at 30°C, as measured by the densitometric scan of the autoradiograph of Fig. 2. For comparison, relative induction values corresponding to GroEL (■) and DnaK (▲) from cells growing in steady-state at 45°C, are also included.

To determine the time-dependence of the membrane fluidity change after the temperature upshift from 30 to 45°C, we performed the experiments shown in Fig. 3. The studies were done on membranes obtained from cells grown at 30°C and subjected to heat-shock at 45°C for different periods of time. We have found that fluidity, which is initially raised by the temperature upshift, decreases gradually with time reaching after 30 min, the value corresponding to membranes from steady-state cultures at 45°C. Should this time-course of membrane fluidity change be qualified as fast or slow in terms of bacterial physiology? In this regard, an obvious term of comparison is the heat-shock response as detected by the transitory

increment in the synthesis of heat-shock proteins induced by a sudden temperature upshift [15–20].

The heat-shock response of *E. coli* was followed by studying the protein synthesis pattern of cells that had been pulse-labeled with [$^{35}$S]methionine during steady-state growth at 30 and 45°C or at various times after a temperature upshift from 30 to 45°C [20]. Radiolabeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiograph. As shown in Fig. 4 the pattern of protein synthesis is dramatically modified by the temperature upshift. The most marked changes are increases in the syntheses of DnaK and GroEL proteins, which can be taken as markers of the heat-shock response [20,35–38]. As previously described [20], the criteria for assignment of protein bands to DnaK and GroEL were: (1) the increase in their syntheses induced by a temperature upshift; (2) their migration in gels similar to proteins overproduced by cells carrying plasmids containing the corresponding genes; (3) their apparent molecular weights as deduced from their mobilities; and (4) the similarity between the pattern of heat-induced proteins shown by cells used in this work, and patterns already published by other groups [35–38]. Fig. 5 shows the time dependence of the relative induction of DnaK and GroEL proteins after the temperature upshift, as obtained from the densitometric scanning of Fig. 4. Values plotted correspond to the ratio of the apparent synthesis rate at various times after the temperature change from 30 to 45°C, and the apparent synthesis rate in steady-state at 30°C. In the experiment shown in Figs. 4 and 5, as well as in duplicate experiments, the maximum intensity of the response occurs approx. 20 min after the temperature upshift. At 30 min, although synthesis of HSPs has decreased, it has not yet reached the level corresponding to cells growing in steady-state at 45°C. The transient response to a heat-shock of 45°C presents a maximum rate of synthesis of HSPs, at a time-point later than that corresponding to a heat-shock of 42°C. Strain W3110, as reported for other strains [17,39], presents the maximum rate of HSPs synthesis 5–10 min after a temperature upshift to 42°C but shows a sustained heat-shock response when temperature is raised to 50°C (data not shown). Therefore, it is possible that the maximum rate of HSPs synthesis occurs at different times depending on the temperature of the stress.

Comparison of Figs. 3 and 5 shows that upon the temperature upshift, a dramatic increase of membrane fluidity precedes the increase in synthesis of HSPs. Besides, the homeoviscous response, which results in the membrane fluidity relaxation to the level characterizing membranes from cells in steady-state growth at the high temperature, occurs during the *E. coli* heat-shock response. Fluidity changes seem to play important roles in many physiological processes including the cell cycle in eukaryotes [14]. In prokaryotes, membrane fluidity has been recognized as an essential factor in DNA replication of *E. coli* cells due to the membrane attachment of the chromosome at the initiation point [30,40,41]. Present results suggest that membrane fluidity changes might also be considered as part of the complex cellular events that characterize the heat-shock response. Although there is evidence for the role of proteins as sensors and misfolded proteins as signals, this does not exclude the participation of other sensors/signals such as nucleic acids, ribosomes and membranes, in the induction of the heat-shock response [21,42]. In support of a possible role of the membrane in the heat-shock response, there are data showing that ethanol and local anesthetics can trigger this response in *E. coli* [17,43,44]. In addition, it has recently been reported that the level of sigma-E is proportional to the concentration of outer-membrane protein precursors in the periplasm [22], suggesting a relationship between changes in the membrane and induction of the sigma-E factor which in turn induces the transcription of *rpoH*, the gene encoding for sigma-32, the main positive regulator of the heat-shock response.

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