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Homeoviscous Adaptation—A Homeostatic Process that Regulates the Viscosity of Membrane Lipids in *Escherichia coli*

(spin-labeling/phase transitions)

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ABSTRACT ***E. coli* incorporates increasing proportions of saturated and long-chain fatty acids into phospholipids as growth temperature is increased. It was found that this compositional variation results in the biosynthesis of phospholipids that have identical viscosities at the temperature of growth of the cells. This "homeoviscous adaptation" can also be observed in *E. coli* membrane preparations. Viscosities were determined by use of the electron spin resonance spin-label technique.**

Escherichia coli varies the fatty-acid composition of membrane lipids as a function of temperature of growth—as do many other organisms (1). As cells are grown at increasing temperatures, there is an increasing tendency to incorporate longer and more saturated fatty acids into phospholipids (2). One expected result of such a variation is that the phospholipids synthesized at progressively higher growth temperatures should exhibit progressively higher solid-to-liquid-crystalline phase transitions (3) and, indeed, an effect of this type has been reported (4). However, the true adaptive significance of the temperature-control phenomenon might be best revealed by examination of the physical state of the membrane lipids at the temperature of growth of the organism. In this report the fluidity of *E. coli* lipids and membranes is examined at the temperature of growth of the organism by use of an electron spin resonance (ESR) spin label. The results of this study are consistent with the hypothesis that variation of fatty-acid composition of membrane phospholipids serves in producing membranes whose lipids have a constant fluidity at the temperature of growth—a process dubbed "homeoviscous adaptation."

MATERIALS AND METHODS

Strains and Media. An *E. coli* K-12 strain, W3102, (a gift of M. Meselson) was used in all experiments. Cells were grown at various temperatures on Luria broth (5).

Description of ESR Equipment. Electron spin resonance spectra were recorded on a Varian Associates E-3 spectrometer equipped with a variable-temperature accessory.

Preparation of Spin-Labeled Samples. In all experiments the spin label used was methyl-12-nitroxylstearate prepared as described by Waggoner *et al.* (6). The spin label was stored as a 1×10^{-4} M solution in hexane, at 0° under nitrogen.

E. coli lipids were extracted by the method of Bligh and Dyer (7) and polar lipids were separated from neutral lipids by thin-layer chromatography on silicic acid as described by Overath (8). The polar lipid fraction contains the phospholipids and was used in all spin-labeled lipid preparations. The lipids were stored at 0° under nitrogen, in 2:1 methanol:chloroform at a concentration af 10 mg/ml.

Spin-labeled phospholipid samples were prepared by evaporating 0.4 ml of the spin label stock solution, giving a lipid-to-probe ratio in the sample of 67:1 (wt/wt).

The mix was applied to $0.1 \times 4 \times 60$-mm glass plates and the solvent allowed to evaporate. A second glass plate was then placed on top of the first and the edges were sealed with beeswax. The sample was then attached to a wooden applicator stick by means of Duco cement, providing the handle that was used to lower the sample into the ESR cavity.

E. coli membranes were prepared by the method of Kaback (9). Membranes were taken up in the minimum possible volume of 0.05 M potassium phosphate buffer, pH = 7.0. Membranes were spin-labeled by evaporating 100 μl of stock solution of spin probe in a test tube and then adding 50 μl of membrane suspension and vortexing for 2 min. Controls run with buffer alone gave no ESR signals.

Membranes were stored at 4° and were spin-labeled and measured within 24 hr of preparation.

Analysis of ESR Spectra. The line width of an ESR signal is, under ideal conditions, determined by the rates of tumbling of the paramagnetic molecules involved. This dependence of line width on molecular motion in solutions has been given an approximate theoretical treatment by Kivelson and McConnell and coworkers (10–12) in terms of the rotational correlation time, τ, for the system.* As used by Rich (14) for nitroxides, this equation takes the form:

$$\tau = (6.45 \times 10^{-10})\left[\sqrt{h(0)/h(-1)} + \sqrt{h(0)/h(1)} - 2\right] \Delta H(0) \quad [1]$$

where $h(0)$, $h(1)$, and $h(-1)$ are the amplitudes of the center, low-field and high-field lines, respectively, and $\Delta H(0)$ is the width of the center line in gauss.

This equation was used to calculate τ in these studies. Because of assumptions made in the derivation of Eq. 1, its validity is questionable at rotational correlation times longer than about 2 nsec. To extend the range of fluidities in which useful data can be obtained from Eq. 1, we used the relationship (15):

$$\tau = 4\pi\eta r^3/3kT \quad [2]$$

Abbreviation: ESR, electron spin resonance.

* The rotational correlation time (or relaxation time) has been defined by Debye (13) as "the time required for a system of oriented [molecules] to revert to a random distribution."

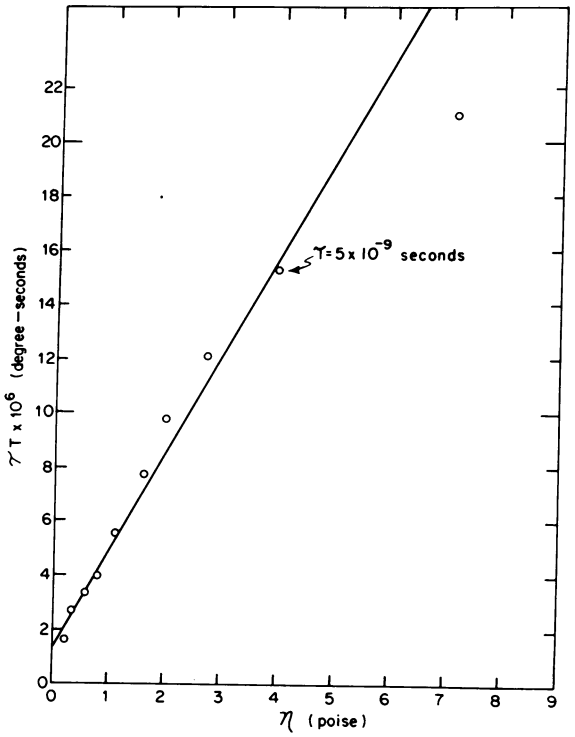

FIG. 1. The relationship between rotational correlation time (τ) calculated by Eq. 1 and the coefficient of viscosity (η) of the solvent at the temperature of measurement (T). Rotational correlation times for methyl-12-nitroxylstearate in castor oil obey Eq. 2, for values of τ equal to or less than 5 nsec.

where: η is the coefficient of viscosity, T is the absolute temperature, r is the radius of the equivalent hydrodynamic sphere, and k is the Boltzmann constant.

This equation states that $T\tau$ is proportional to η and hence, insofar as Eq. 1 generates valid rotational correlation times, the times so-calculated multiplied by the absolute temperature of measurement should be linear with the viscosity of the solvent. Fig. 1 shows a plot of $T\tau$ (τ calculated from Eq. 1) versus η for methyl-12-nitroxylstearate in castor oil. Viscosities of castor oil as a function of temperature can be obtained from several sources (16, 17). This result indicates that Eq. 1 is valid (at least for this spin probe in a lipid matrix) to rotational correlation times as long as 5 nsec. Furthermore, if one assumes that the molecular conformation of the spin probe is similar in the lipid samples used and in castor oil, Fig. 1 permits an accurate conversion of rotational correlation times to viscosities for these samples.

Since for long correlation times the large dependence of rotational correlation time on temperature changes is mediated primarily through viscosity changes (η shows an exponential dependence on temperature), it is possible to obtain approximate values of η from τ at values of τ (as calculated by Eq. 1) in excess of 5 nsec. Table 1 can be used to empirically relate such values of τ to viscosity.

Use of Eq. 1 also assumes isotropic motion of the spin label. Evidently, methyl-12-nitroxylstearate does move isotropically in a lipid matrix, since the hyperfine coupling constant of this probe in all experimental samples was identical to that in hexane (i.e. 15 G). Furthermore, there was no effect of orientation of the lipid samples in the magnetic field on the hyperfine coupling constant, in contrast to the orientation dependence exhibited by anisotropically moving fatty-acid spin-probes (18).

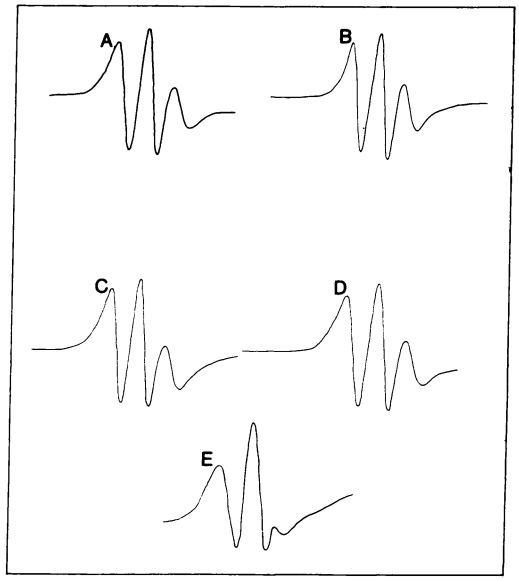

FIG. 2. Electron spin resonance spectra of methyl-12-nitroxylstearate in *E. coli* lipid extracts. Spectra A–D were taken at the growth temperatures (±2°) of the cells from which the lipid extracts were prepared. These were: *A*, 15°; *B*, 30°; *C*, 37°, and *D*, 43°. Spectrum *E* was taken at 15° of a lipid extract from cells grown at 43°.

RESULTS

Viscosity of E. coli Lipid Extracts. E. coli lipid extracts from cells grown at a variety of temperatures were spin-labeled, producing the spectra shown in Fig. 2. The similarity of spectra A through D shows that the motion of the spin label is nearly identical in lipid extracts from cells grown at different temperatures when neasured at the growth temperature. Spectrum E shows that the motion of the spin label in these extracts can, however, be dramatically altered by temperature.

In order to quantitate these observations, we calculated rotational correlation times (τ) from such spectra, as described in *Materials and Methods*. The rotational correlation times were converted to coefficients of viscosity (η) by means of Fig. 1 (for rotational correlation times less than 5 nsec) or Table 1 (for rotational correlation times greater than 5 nsec). The results of these experiments are shown in Table 2.

The results in Table 2 show that the variation in the viscosity of *E. coli* lipids measured at the temperature of growth of the cells from which the lipids are derived is about $^1/_{100}$ that of the variation in viscosity of lipid extracts from cells

TABLE 1. *Conversion table for rotational correlation times longer than 5 nsec and coefficients of viscosity*

τ (nsec)	η (poise)
67.1	37.6
32.1	22.1
14.1	16.6
13.5	13.3
9.0	10.3
7.1	7.7
5.0	3.9

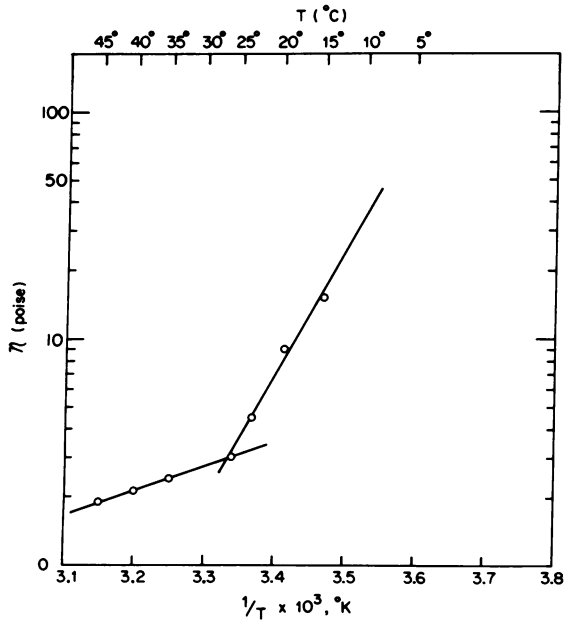

FIG. 3. A phase transition in an *E. coli* phospholipid extract. Lipids were extracted from cells grown at 43° and gave a phase transition at approximately 27°. Viscosities were determined as described in the *text*.

TABLE 2. *The viscosity of E. coli lipid extracts from cells grown at different temperatures*

Temperature of growth (°C)	Temperature of measurement (°C)	τ (nsec)	η (poise)
15	15	2.8	1.8
30	30	2.7	1.9
37	37	2.6	1.8
43	43	2.7	2.0
43	15	13.8	15

TABLE 3. *The viscosity of a lipid region of E. coli membranes*

Temperature of growth (°C)	Temperature of measurement (°C)	τ (nsec)	η (poise)
23	23	3.5	2.5
23	37	1.6	1.0
37	37	3.3	2.5

TABLE 4. *Phase transitions of E. coli lipid extracts from cells grown at various temperatures*

Temperature of Growth, T_G (°C)	Phase transition temperature, T_T (°C)	$T_G - T_T$ (°C)
15	−1 (±1)	16 ± 1
30	16 (±2)	14 ± 2
43	27 (±1)	16 ± 1

grown at a single temperature (43°) measured over the same temperature range (15°–43°). This phenomenon can be described as "homeoviscous adaptation."

Viscosity of a Lipid Region in E. coli membranes. The spin label used in this study would be expected to be incorporated into a lipid region of a membrane (19). Thus it is possible to perform an experiment similar to that described for *E. coli* lipid extracts on *E. coli* membranes. The results of such an experiment are described in Table 3. Membranes were prepared and spin-labeled as described in *Materials and Methods*. These data are consistent with those from the measurements on lipid extracts and show that *E. coli* lipids *in situ* likewise exhibit homeoviscous adaptation.

Phase Transitions of E. coli Lipid Extracts. Homeoviscous adaptation in *E. coli* appears to be due to variation in the fatty-acid composition of phospholipids. No variation in the types of polar head groups of phospholipids as a function of temperature has ever been recorded. Thus, as a consequence of homeoviscous adaptation, one might expect that the differences between the (chain melting) phase transition temperature of *E. coli* from cells grown at various temperatures and the temperature of growth would be similar.

To determine the phase transition temperatures of *E. coli* lipid extracts, we used the following equation, due to de Guzman Corrancio, which describes the dependence of the coefficient of viscosity on temperature:

$$\eta = A \exp(-\Delta E_{vis}/RT) \quad [3]$$

where A is a constant and ΔE_{vis} is an activation energy for viscous flow (20).

Thus a plot of log η versus $1/T$ should produce a straight line. An inflection in such a plot implies a phase transition at the inflection temperature.

Rotational correlation times were measured as a function of temperature for spin-labeled *E. coli* lipid extracts and these values were converted to viscosity coefficients by means of Fig. 1 and Table 1. An example of such a phase transition is shown in Fig. 3.†

Table 4 shows that these phase transition temperatures show consistent differences with respect to the growth temperature of the cells from which the lipid extracts were prepared.

DISCUSSION

Membrane lipids can be thought of as performing two physiological roles. They create a barrier to the free entry and exit of molecules into and out of the cell and provide a matrix in which (or on which) biochemical reactions take place.

One might expect that the rates of both transport processes and biochemical reactions would be affected by membrane lipid viscosity. Since the whole membrane is involved in uptake and retention processes, one would expect homeoviscous adaptation to be primarily a response to the barrier requirements of the membrane. Van Deenen and co-workers (22) have demonstrated that *E. coli* membrane lipid barrier properties do indeed depend on the temperature of growth of the cell from which the lipids were isolated.

† The report of C. D. Linden *et al.* (21), which appeared after this work was completed, suggests that the inflection point observed in Fig. 3 may denote the beginning of a lateral phase separation.

The dependence of membrane enzyme activity on the fluidity of the membrane is a problem which is of great interest. The purification of lipid-dependent membrane enzymes such as the *staphylococcus aureus* C-55 isoprenoid alcohol phosphokinase (23) provides a system for the examination of such lipid–protein interaction. Studies performed in this laboratory indicate that the activity of this enzyme is strongly dependent on the fluidity of the lipid cofactor. Thus, homeoviscous adaptation may also play some role in regulating membrane-bound enzyme activity, particularly in maintaining appropriate reaction rates when cells are grown at different temperatures.

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