# Regulation of Lipid Composition in *Acholeplasma laidlawii* and *Escherichia coli* Membranes: NMR Studies of Lipid Lateral Diffusion at Different Growth Temperatures†

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ABSTRACT: Lipid lateral diffusion coefficients have been directly determined by pulsed field gradient NMR spectroscopy on macroscopically aligned, fully hydrated lamellar phases containing dimyristoylphosphatidylcholine and total lipid extracts from *Acholeplasma laidlawii* and *Escherichia coli*. The temperature dependence of the diffusion coefficient was of the Arrhenius type in the temperature interval studied. The sharp increase in the diffusion coefficient at the growth temperature of *E. coli* obtained by FRAP measurements, using a fluorescent probe molecule (Jin, A. J., Edidin, M., Nossal, R., and Gershfeld, N. L. (1999) *Biochemistry 38*, 13275−13278), was not observed. Thus, we conclude that the lipid structural properties (i.e., those affecting the lipid phase behavior), rather than the lipid dynamics, are involved in the adjustment of the membrane lipid composition. Further support for this conclusion is given by the finding that lipid extracts from *A. laidlawii* grown at different temperatures have about the same diffusion coefficients. Finally, the lipid lateral diffusion in bilayers of phospholipids was found to be much faster than that in bilayers of mainly glucolipids, which can be understood in terms of a free volume theory for the diffusion process.

The lipid bilayer has long been used as a model for biological membranes. The awareness of the formation of nonlamellar structures, occurring for a number of membrane lipids in aqueous solution, has gradually changed the view on the functional role played by membrane lipids in cell processes; surprisingly, this knowledge still cannot be found in general biochemistry textbooks. There is a great deal of experimental evidence from organisms in several kingdoms showing that lipids actively participate in many important functions of the cell (*1*−*11*). It is well documented by now that the physicochemical properties of the lipid matrix can trigger a direct feed-back signal on the activity of lipid synthesizing enzymes. The activity of such enzymes can be modulated by the fractions of anionic and/or nonlamellar-forming lipids present in the membrane. Numerous investigations also show that nonlamellar-forming lipids are important for membrane structures and membrane-associated processes (*12*).

It has been shown that many, if not all, organisms adapt their membrane lipid composition to the prevailing environmental and physiological conditions (*5*, *13*−*17*). The regulation of the membrane lipid composition by the cell wall-less bacterium *Acholeplasma laidlawii* strain A has been extensively studied in our laboratory (*16*, *18*−*35*). The organism can be grown under conditions, where the regulatory changes occur predominantly in the structure of the polar headgroups. We have also studied the metabolic regulation and the phase equilibria of the membrane lipids from wild-type cells of the Gram-negative bacterium *Escherichia coli* (*36*). This bacterium has only three main membrane phospholipids, which occur frequently in prokaryotic as well as eukaryotic organisms, and the regulation of the lipid composition in wild-type cells is brought about by changes in the acyl chain structure (*36*, *37*). This is a very common response to changes in the environmental temperature among a variety of organisms (*13*, *14*). We have conclusively shown that for the two prokaryotic organisms *A. laidlawii* and *E. coli*, the cells strive to maintain a certain balance between the lipids constituting a bilayer and those forming reversed nonlamellar structures. Hence, this balance is achieved by adjusting the composition of the polar headgroups (*A. laidlawii*) or the acyl chains (*E. coli*) to the prevailing growth conditions. Accordingly, the adjustments performed by the cells are able to keep the membrane lipids in a "window" between a gel crystalline phase and reversed nonlamellar phases (Figure 1) (*16*, *18*, *25*, *26*, *30*, *32*, *33*, *36*).

Recently, an anomalous jump in the lipid translational diffusion ($D_L$)[1] of a fluorescent probe was observed at the growth temperature by fluorescence recovery after photobleaching (FRAP) for a lamellar phase of total lipids from *E. coli* (*38*). It was suggested that this dramatic change in $D_L$ is involved in the adjustment of the membrane lipid composition.

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[1] Abbreviations: PC's, phosphatidylcholines; DMPC, dimyristoylphosphatidylcholine; MGlcDAG, monoglucosyldiacylglycerol; DGlcDAG, diglucosyldiacylglycerol; $L_\alpha$, lamellar liquid crystalline; $T_m$, gel-to-liquid crystalline transition temperature; pfg, pulsed field gradient; NMR, nuclear magnetic resonance; $D_L$, lipid translational diffusion constant; FRAP, fluorescence recovery after photobleaching.

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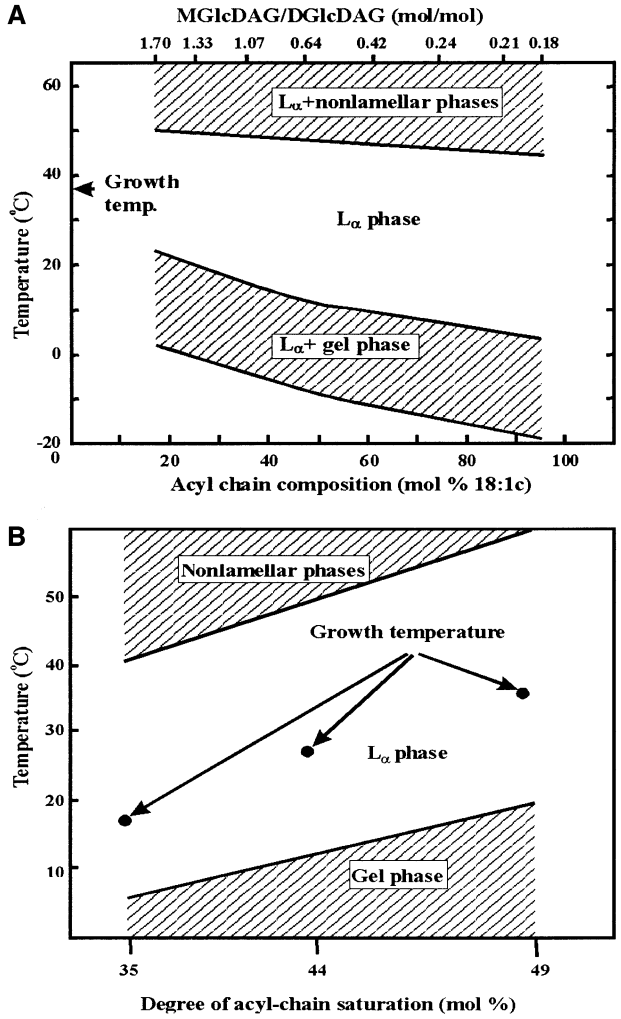


FIGURE 1: (A) Phase equilibria of total lipid extracts, containing different fractions of palmitoyl and oleoyl chains (lower *x*-axis), from membranes of *A. laidlawii* strain A grown at 37 °C. Water contents were 20 wt %. The upper *x*-axis shows the metabolically obtained MGlcDAG/DGlcDAG ratios. The lower hatched area denotes the gel-to-liquid crystalline phase transition interval, and the upper hatched area denotes the appearance of $H_{II}$ and/or reversed cubic phases. Adapted from refs *15* and *25*. (B) Phase equilibria of total lipid extracts from *E. coli* cells grown at 17, 27, and 37 °C. The water content was 20 wt %. The *x*-axis shows the fraction of saturated acyl chains in the lipids. The line of the lower hatched area denotes the temperatures at which a gel phase appears, and the line of the upper hatched area denotes the temperatures at which an $H_{II}$ phase appears. Data from ref *36*.

As emphasized above, our model, which is wholly based on the phase behavior of the lipids, implies that a balance between lipids forming lamellar and nonlamellar phases plays a critical role in the regulation of the lipids in the membrane. Thus, the dynamics of the lipids play only a secondary role as long as the lipids are in the liquid crystalline state. Therefore, the work by Jin et al. (*38*) prompted us to measure $D_L$ for total lipid extracts from *E. coli* and *A. laidlawii* membranes by diffusion NMR with pulsed field gradients (pfg's), with which the lipid lateral diffusion coefficient can be directly determined and monitored as a function of temperature.

## MATERIALS AND METHODS

*E. coli* wild-type strain K12 and *A. laidlawii* strain A-EF22 were grown in the media described in previous work (*16*, *36*). The *E. coli* cells were grown at 27 °C, and the *A. laidlawii* cells were grown at 30 and 37 °C in a medium supplemented with 75 $\mu$M palmitic acid and 75 $\mu$M oleic acid. Dimyristoylphosphatidylcholine (DMPC) was purchased from Sigma and used without further purification.

The oriented samples for the pfg-NMR diffusion measurements were prepared by a method similar to that described by Kurtze et al. (*39*). Thirty-five glass plates (2.5 × 14 × 0.08 mm; Marienfeldt Lab. Glassware, Bad Mergentheim, Germany), each of them covered by a thin film of 225 $\mu$g lipid, were stacked on top of each other and placed in the center of a 68 mm long glass tube with square cross section. The sample was put in an $^2H_2O$ vapor (Larodan Fine Chemicals) atmosphere, and $^2H_2O$ was finally added to give a water content of 70% w/w. Plastic spacers were placed on each side of the stack of glass plates in order to keep them at the center of the glass tube, which was finally sealed by wax and Parafilm. The sample was then allowed to equilibrate at 40 °C (DMPC) or 25 °C (*E. coli* and *A. laidlawii* lipids) for 1 week before the measurements. During this time the DMPC sample was brought under the gel−liquid crystalline phase transition temperature several times in order to anneal the membranes. After the equilibration period, the samples looked partially dark in crossed polarizers, indicating a partial orientation of the lipid bilayers parallel to the glass plates.

The oriented sample was placed horizontally into a Varian/Chemagnetics pfg-diffusion $^1$H/BB NMR probe capable of producing pulsed magnetic field gradients in the *z*-direction (defined to be in the direction of the main magnetic field) of up to 10 T/m with submillisecond switching times. A goniometer stage permitted the sample to be rotated along its long axis in order to orient the bilayers into the magic angle where the pfg-diffusion experiments could be performed (*40*). A heated air stream passing over the sample controlled the temperature to within ±0.2 °C. $^{31}$P NMR spectra were recorded at 162.13 MHz using the Hahn echo sequence with WALTZ-decoupling of the protons. The pfg-NMR diffusion experiments were performed at a proton frequency of 400.51 MHz with the stimulated spin−echo technique (SSE) (*41*). The pulse sequence timing is (90-$\tau$-90-$T$-90-$\tau$-acquire) with the gradient pulses, of length $\delta$ and strength $g$, following immediately after the first and the third 90° rf pulses. This sequence is specifically suitable for measurements in samples with short $T_2$ relaxation times (*40*). In a diffusion experiment, typically 20−40 spectra were recorded, in which $g$ was varied, keeping all the other parameters constant. The diffusion coefficient, $D$, was then determined from a nonlinear fit of the signal attenuation of the lipid peaks to the Stejskal−Tanner equation (*42*)

$$\ln\left(\frac{S}{S_0}\right) = -\gamma^2 g^2 \delta^2 D\left(\Delta - \frac{\delta}{3}\right) \qquad (1)$$

where $S$ and $S_0$ are the signal amplitudes with and without gradient pulses, $\gamma$ is the gyromagnetic ratio, and $\Delta$ is the separation between the two gradient pulses. The experiment was repeated at least three times for which $\Delta$ was varied between 10 and 500 ms. No variation of the diffusion coefficient on $\Delta$ was observed, indicating that the diffusion occurred unobstructed on this time scale (*43*−*49*). Finally, the diffusion coefficients obtained were multiplied by a factor

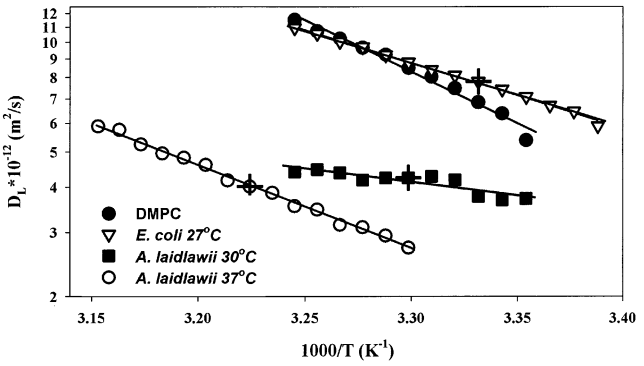


FIGURE 2: Lipid lateral diffusion coefficients of $L_\alpha$ phases with the following contents: DMPC/$^2H_2O$, *E. coli* lipids/$^2H_2O$, and *A. laidlawii* lipids/$^2H_2O$, as a function of temperature. $D_L$ is a mean value of eight measurements with the diffusion time varying between 15 and 500 ms. The crosses mark the growth temperatures of *E. coli* and *A. laidlawii*.

of 1.5, since the translation diffusion is measured along the *z*-axis, while the lipid motion actually occurs along the bilayers oriented with their normal at 54.7° with respect to this axis (*40*).

## RESULTS AND DISCUSSION

According to $^{31}P$ NMR spectroscopy, the lamellar gel to lamellar liquid crystalline ($L_\alpha$) phase transition temperature ($T_m$) for oriented samples with DMPC was 24 ± 0.5 °C. The samples with *E. coli* and *A. laidlawii* lipids were in the liquid crystalline state at all temperatures from 20 to 35 °C. The melting temperature for these samples is expected to be between 10 and 20 °C (Figure 1) (*25*, *36*).

The $^{31}P$ NMR line shape was a superposition of a powder pattern and a narrow peak (not shown). The chemical shift of the narrow peak varied between the outer edges of the powder pattern as the sample was rotated in the magnetic field, according to the relation

$$\nu = \frac{\mathrm{csa}}{3}(3\cos^2\theta - 1) \qquad (2)$$

where $\theta$ is the angle between the normal to the glass plates and the main magnetic field and csa is the motion-averaged chemical shift anisotropy of the phosphate group (*50*). This confirms that this signal originates from lipid bilayers oriented parallel to the glass plates. The remaining powder pattern signal derives from unaligned liquid crystal material seeping out from the edges of the glass plate stack. This part of the signal was unaffected by sample rotation as expected for a random distribution of orientations.

The $^1H$ NMR signal from the lipids was very broad except at the magic angle orientation, where the static dipole interactions become zero, thereby causing a narrowing of the lines (*40*). The orientation of the macroscopically aligned sample was adjusted to give the largest signal from the lipids in a spin−echo experiment, and the diffusion experiments were then performed at this setting.

Figure 2 shows $D_L$ obtained for $L_\alpha$ phases of DMPC/$^2H_2O$, of *E. coli* lipids/$^2H_2O$, and of *A. laidlawii* lipids/$^2H_2O$ as a function of temperature. For all the lipids, $D_L$ increases monotonically over the entire temperature region, and Arrhenius plots give a calculated energy of activation for the diffusion process of 50.8 kJ/mol for DMPC, 34.5 kJ/mol for the *E. coli* lipids, 14.3 kJ/mol for the *A. laidlawii* lipids grown at 30 °C, and 44.1 kJ/mol for those grown at 37 °C. The activation energy for DMPC compares favorably with earlier values obtained on similar systems (*51*). The unexpectedly low activation energy obtained for the *A. laidlawii* lipids grown at 30 °C needs further studies before a firm interpretation of this finding can be given.

From Figure 2 it can be inferred that there is a striking difference in $D_L$ for the *A. laidlawii* lipids, which consist of 70−80 mol % glucolipids (*32*, *52*, *53*), and for the DMPC and *E. coli* lipids, which consist of only phospholipids (*36*); the diffusion motion of the latter is much more rapid. Moreover, in contradiction to the reported FRAP experiment by Jin et al. (*38*), no pointed increase in the lateral diffusion coefficient is observed around the growth temperature of the bacteria or at the so-called critical temperature, *T**, for DMPC introduced by Gershfeld (*54*). With the existing data, it is difficult to explain this discrepancy. In both the NMR and FRAP experiments, the lipid bilayers are fully hydrated; i.e., they are in equilibrium with excess water. However, the NMR diffusion is performed on a single-phase system, namely, a lamellar phase, while the FRAP experiment is carried out on a fluorescent probe molecule in a lamellar phase in contact with an excess water reservoir (i.e., a two-phase system). Maybe, this difference in experimental setup could be the source for the observed discrepancy.

The faster diffusion motion of the phospholipids is probably caused by the "looser" packing of the lipids in the bilayer compared to lamellae composed of glucolipids. Surface balance studies on monolayers (*20*) and X-ray scattering on lamellar liquid crystalline phases (*21*, *55*−*57*) have shown that the area per lipid molecule at the water/lipid interface is smaller for the glucolipids (MGlcDAG and DGlcDAG) than for DMPC or phosphatidylcholines (PC's) with unsaturated acyl chains. The slower diffusion of the glucolipids can be understood by using a free volume theory (*43*): bilayers with closely packed glucolipids leave a smaller free area for lipid diffusion, resulting in a smaller diffusion coefficient than PC's with a much larger interfacial area in bilayers, allowing them to diffuse faster. Furthermore, since the chain entanglements are less in lipids with saturated chains (e.g., DMPC), they should diffuse more rapidly than phospholipids with unsaturated acyl chains (e.g., *E. coli* lipids), provided that the comparison is made at the same temperature relative to $T_m$ for the different systems. This is also observed experimentally (Figure 2). We have reported such effects previously in connection with studies of the effect of cholesterol on $D_L$ in PC and sphingomyelin bilayers (*58*). Thus, according to the free volume theory, the finding that the lateral diffusion is the same for bilayers obtained from *A. laidlawii* grown at different temperatures implies that the packing of the lipids in the membrane is similar at the two growth temperatures. Again, this is exactly what one should expect to see according to our model of the regulation of the lipid composition in the membrane. A balance between lamellar and nonlamellar-forming lipids is maintained, which in turn depends on the lipid molecular shape, which is anticipated to have a strong influence on the bilayer packing.

## FINAL REMARKS

The lateral diffusion coefficients of the lipids from membranes of both *A. laidlawii* grown at 30 and 37 °C and

*E. coli* grown at 37 °C increase with temperature, and no anomaly is observed at the growth temperatures. Therefore, we conclude that the lipid dynamics is not a significant issue in the adjustment of the membrane lipid composition. Thus, our model involving lipid structural properties (i.e. molecular shape) still is most appropriate for a description of this lipid regulation. Finally, it should be clearly emphasized that our model based on structural aspects and lipid-phase behavior has also been shown to have a very good predictability. Most noticeably, the existence of a new lipid, not previously observed, in the membranes of *A. laidlawii* could be predicted (*16*).

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