# A BIOPHYSICAL STUDY OF PROTEIN-LIPID INTERACTIONS IN MEMBRANES OF *ESCHERICHIA COLI*

## FLUOROMYRISTIC ACID AS A PROBE

MARTIN P. N. GENT, PATRICIA F. COTTAM, AND CHIEN HO, *Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213 U.S.A.*

ABSTRACT Fluorine-19 nuclear magentic resonance spectroscopy and transport assays have been used to investigate and compare the membrane properties of unsaturated fatty acid auxotrophs of two strains of *Escherichia coli*, K1060B5 and ML 308-225-*UFA*-8. A fluorinated analog of myristic acid, 8, 8-difluoromyristic acid, can be incorporated into the membrane phospholipids by substitution for oleate in the growth medium. Growth for one generation on 8, 8-difluoromyristate results in a 20% content of fluorinated fatty acid in the membranes, changes in the protein to lipid ratio, and altered transport of methyl β-D-thiogalactopyranoside. The differences in membrane composition and transport behavior seen in oleate supplemented *E. coli* K1060B5 relative to ML 308-225-*UFA*-8 are enhanced by the incorporation of 8, 8-difluoromyristate. The phase transition behavior becomes distinctly different and some differences in lipid organization persist above the transition temperature. Concomitantly, the rate and extent of concentration of methyl β-D-thiogalactopyranoside are reduced two-fold more in *E. coli* K1060B5 compared to ML 308-225-*UFA*-8. Such behavior suggests that these fluorinated fatty acid supplemented strains of *E. coli* are useful to study subtle differences in protein–lipid interactions and their effects on the function of membrane-bound enzymes.

### INTRODUCTION

Active transport, the transport of substrate from a low external concentration to a higher internal level, is an important function of cytoplasmic membranes. This function is affected by the number of transport proteins, by their interactions with the surrounding lipids, and by their interactions with other proteins responsible for coupling transport to metabolic energy. The kinetics and energy requirements of this process have been investigated extensively (Harris, 1972; Hatifi and Djavadi-Ohaniance, 1976). In bacteria, genetic manipulation has provided important information concerning the nature and regulation of the transport of β-galactosides across the cytoplasmic membrane of *Escherichia coli* (Horecker et al., 1960; Fox et al., 1967; Rotman et al., 1968; Jones and Kennedy, 1969). However, the nature of the membrane architecture surrounding the site of transport of these substances is not fully understood. Since the lactose carrier protein (or the so-called "permease") has not been

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Dr. Gent's present address is the Connecticut Agricultural Experiment Station, New Haven, Conn. 06504.

isolated in a purified catalytically active form, reconstitution experiments have not been possible. It appears that the structural requirements for transport of these substances must, by necessity, be examined *in situ* (Lancaster and Hinkle, 1977 *a* and *b;* Teather et al., 1977). This can be accomplished by adding exogenous membrane components, such as lipids, to a cell preparation and observing their effect on the transport activity (Yariv et al., 1969; Sullivan et al., 1974), or it can be done by comparative studies of bacterial strains similar in their expression of the lactose gene products, but with different membrane compositions or properties (Ohta et al., 1977; Therisod et al., 1977).

To understand the nature of the structual requirements for transport, it is necessary to know more than the composition of the membrane. Specifically, the nature of the protein-lipid interactions and the alterations due to changes in composition must be determined. There are several methods for studying the architecture of membranes (Singer and Nicolson, 1972; Paton et al., 1978). Nuclear magnetic resonance (NMR)[1] spectroscopy is a powerful method for studying the motion of lipids and the perturbations in lipid motion due to interactions with proteins (Seelig and Niederberger, 1974; Gent and Prestegard 1977; Peterson and Chan, 1977). We have developed a $^{19}$F-NMR technique that is a sensitive measure of lipid–lipid and lipid–protein interactions (Gent et al., 1978, Gent and Ho, 1978). The technique is informative because of the sensitivity of magnetic resonance to molecular motions. The utility for biological applications lies in the fact that no other fluorine resonances exist except for the added fluorinated fatty acid. Thus, the resonance is not overlapped or obscured and the maximum possible information is obtained, even from systems of complex chemical composition. For instance, complex resonance shapes can be deconvoluted into narrow (fluid lipid) and broad (gelled lipid) components of defined intensity. The resonance can be measured, or fitted, by comparison to theoretical line shapes predicted from considerations of the lipid motion (Gent and Ho, 1978). Three parameters are involved: the intensity, proportional to the amount of lipid; the dipolar width, related to the order parameter used in discussions of nitroxide spin labels; and the width of the central spike, which has no simple physical explanation, but which appears to be sensitive to protein–lipid interactions (Sheetz and Chan, 1972; Gent and Ho, 1978). Using this technique we have examined two strains of *E. coli,* K1060B5 and ML 308-225-*UFA*-8, both of which are unsaturated fatty acid auxotrophs, constitutive in their expression of the *lac y* gene and able to grow for more than one generation on 8, 8-difluoromyristate. The membranes of the two organisms have rather different compositions and $^{19}$F-NMR studies show different protein–lipid interactions. We discuss these results, of rather subtle differences in lipid behavior seen in conjunction with large changes in transport ability, with respect to the membrane organization necessary for efficient transport.

## MATERIALS AND METHODS

### *Materials*

The fluorinated fatty acid, 8, 8-difluoromyristic acid, was synthesized as described previously (Gent et al., 1978). Oleic acid and palmitoleic acid were purchased from Nu Check Prep. *N*-Methyl-*N'*-

[1] *Abbreviations used in this paper:* NMR, nuclear magnetic resonance; NMG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; TMG, methyl β-D-thiogalactopyranoside; TLC, thin layer chromatography; PE, phosphatidylethanolamine; GC, gas chromatography; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; EPR, electron paramagnetic resonance.

nitro-*N*-nitrosoguanidine (NMG) was purchased from K & K Laboratories. Uniformly $^{14}$C-labeled methyl β-D-thiogalactopyranoside (TMG) was purchased from New England Nuclear, Boston, Mass. Unlabeled TMG and chloramphenicol were purchased from Calbiochem-Behring Corp., American Hoescht Corp., San Diego, Calif., and Brij 58 from Sigma Chemical Co., St. Louis, Mo. Other chemicals were reagent grade and were used without further purification.

### *Media, Bacterial Strains, and Growth of Cells*

Cell cultures for preparation of membranes, membrane vesicles, or NMR studies were grown with vigorous aeration at 37°C in minimal broth medium of the following composition: 60 mM potassium phosphate buffer, pH 7.0; 0.1% $(NH_4)_2SO_4$; 0.01% $MgSO_4 \cdot 7H_2O$; and 0.46% sodium succinate. Thiamine and oleate, when required, were used at a final concentration of 100 μg/ml. Brij 58 was added to 250 μg/ml. To examine cells grown in the presence of 8, 8-difluoromyristate, cultures were first grown in minimal succinate medium containing oleate to log phase, centrifuged, washed with saline, and resuspended at the desired density in minimal succinate medium supplemented with 25 μg/ml 8, 8-difluoromyristate. Growth was followed turbidimetrically with a Fisher Electrophotometer (Fisher Scientific Co., Pittsburgh, Pa.).

*E. coli* ML 308-225 ($i^-z^-y^+a^+$) (Winkler and Wilson, 1966) was the gift of Dr. H. R. Kaback. *E. coli* K1060B5 ($i^-z^+y^+a^+$ *Fab*B, *Fad*E, *Thi*$^-$) was supplied by Dr. D. F. Silbert (Silbert et al., 1973). ML 308-225-*UFA*-8 was derived from ML 308-225 by mutagenesis with nitrosoguanidine. Cells were grown to mid-log phase in L broth (Lennox, 1955) at 37°C, centrifuged at 4°C and resuspended in L broth to a concentration of $5 \times 10^{10}$ cells/ml. 100 μg/ml of NMG was added and the culture incubated at 37°C for 30 min without aeration. NMG was then removed by centrifugation, the cells were washed with minimal succinate, and finally resuspended in minimal succinate containing 100 U/ml penicillin. After five generations of growth in the presence of penicillin, fatty acid auxotrophs were selected by plating appropriate dilutions on minimal succinate-oleate plates and replicating onto minimal succinate plates and succinate plates supplemented with oleate. The unsaturated fatty acid biosynthetic deficiency was confirmed by growth experiments with oleate or palmitoleate as an unsaturated fatty acid supplement.

### *Analysis of Membrane Proteins and Lipids*

Protein and lipid determinations were made on membrane vesicles prepared according to Kaback (1971) as well as on the particulate fraction recovered from cells sonicated for 10 min with a sonifier cell disrupter (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) at 4°C. Membrane particles and membrane vesicle preparations were determined to be free of whole cells by phase contrast microscopy.

Lipids were extracted by the method of Bligh and Dyer (1959) and resolved into individual species by thin layer chromatography (TLC) on silica gel (Type 0, New England Nuclear) using chloroform:methanol:water at 65:25:4 vol/vol/vol), and visualized with iodine vapor. Fatty acids were released from the phosphatidylethanolamine (PE) fraction by the method of Silbert et al. (1973) and determined as their methyl esters by gas chromatography (GC) (Perkin-Elmer Corp., Instrument Div., Norwalk, Conn., Sigma 3). Total phospholipid was determined either from the GC analysis of the fatty acids (assuming 80% of the phospholipids are PE) or from phosphate analysis of TLC spots digested in concentrated perchloric acid for 2 h at >200°C (Yang et al., 1967).

Membrane proteins were determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

### *Measurement of Active Transport*

Measurements of rate and extent of accumulation of $^{14}$C-TMG were made by the rapid millipore filtration technique. Determinations were made on log phase cells, centrifuged, washed with saline, and resuspended in minimal succinate medium containing the fatty acid supplement. Growth was stopped at the appropriate time by the addition of 25 μg/ml chloramphenicol and the cultures were placed in an ice bath and kept cold until the onset of the transport experiment, usually 10 min. After equilibration of the cells at 37°C, the experiment was started by adding a measured amount of cell culture to prewarmed

$^{14}$C-TMG. 1-ml samples were removed, filtered, and washed. The filters were dried and counted in a liquid scintillation counter (Packard Model 3800, Packard Instrument Co., Inc., Downers Grove, Ill.) with a toluene-based scintillator.

### *NMR Experiments*

$^{19}$F-NMR experiments were done on membrane vesicles prepared according to Kaback (1971) at a concentration of 25 mg protein/ml or membrane lipids at 25–30 mg lipid/ml. The buffer was a 2:1 mixture of $H_2O$:$D_2O$ containing 0.01 M potassium phosphate at pH 6.6 and 50 mg/ml chloramphenicol. $^{19}$F-NMR spectra at 84.7 MHz were taken on a Bruker HFX-90 spectrometer modified for wide band (Bruker Instruments, Inc., Billerca, Mass.), Fourier transform operation with quadrature phase sensitive detection (Nicolet Model TT1025, Nicolet Instrument Corp., Madison, Wisc.). A 50-kHz sweep width was used, 20 kHz of which was analyzed via line fitting. Each observed resonance was fitted with a simulated spectrum derived from the theory of magnetic resonance line shape in the presence of restricted motion (Gent and Ho, 1978). Three parameters are used to fit the resonance:[2] the intensity under the resonance; the dipolar width, $\upsilon_{DD}$ (proportional to the order parameter, $Sz$); and the width of the central spike, $\upsilon_0$. The quality of the spectra and the meaning of these parameters are described in our earlier work (Gent and Ho, 1978). If the resonance was not satisfactorily fit with a single simulated resonance, it was assumed that a broad resonance due to frozen lipid and a narrow one due to fluid lipid were present and the observed resonance was fit by a summation of a broad and a narrow line shape.

### *TEMPO Experiments*

2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) was added to a membrane vesicle preparation containing 25–30 mg/ml of membrane protein to a final concentration of $1 \times 10^{-4}$ M. The sample was put into a 50-$\mu$l capillary sample tube, sealed, and placed in an electron paramagnetic resonance (EPR) spectrometer (Bruker Model ER-418S) operating at the x-band frequency. The temperature was calibrated with samples of dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine containing TEMPO. Typically, a temperature scan took 2 h with the temperature increased gradually during this period. All samples were subjected to the same rate of increase, which was reproducible to $\pm 1$°C. The results are presented as the ratio (not corrected for the $^{13}$C-satellite intensity) of the heights of the components of the high-field line. A detailed description of the technique was given by Schimshick and McConnell (1973).

## RESULTS

### *Composition Analysis*

To carry out a structural examination of the membranes from two different strains of *E. coli*, we have incorporated $^{19}$F-labeled fatty acids into the phospholipids of the two unsaturated fatty acid auxotrophs described above. Growing the cells for one generation in the presence of 8,8-difluoromyristate but in the absence of oleate results in ~27 mol % content of fluorinated fatty acid in the membrane phospholipids (Table I). During the growth period the amount of oleate decreases as 8,8-difluoromyristate is incorporated. We have found that more fluorinated fatty acid can be incorporated when the cells are grown for two or more generations (Table I). However, the growth rate decreases and the K1060B5 strain forms elongated

[2]The following $^{19}$F-resonance parameters are used in this paper: $\upsilon_{DD}$ is the line width due to the dipolar interactions between the two geminal fluorine atoms and between the fluorine and nearby hydrogen atoms; $\upsilon_0$ is the line width of the central spike and is due to dipolar interactions that are averaged to zero in $<10^{-5}$ s in fluid lipid regions; and $S_z$ is the order parameter which can be used to describe the motion of the phospholipids. For a detailed discussion on $^{19}$F-NMR as applied to membrane systems, refer to Gent and Ho (1978).

structures (Gent et al., 1978). To eliminate the effect of gross structural defects on the transport assays, the cells in the following experiments were supplemented with 8,8-difluoromyristate for only one generation.

The lipid composition of the two strains of *E. coli* is similar. TLC analysis of the phospholipids followed by staining with iodine vapor shows essentially the same distribution of lipid species. Fatty acid analysis of the methyl esters derived from the phosphatidylethanolamine spot also shows that the ratio of saturated to unsaturated plus fluorinated fatty acids is the same (Table I). There are two main differences. First, when grown on 8,8-difluoromyristate, the K1060B5 strain has a greater amount of myristate, 14:0, relative to palmitate, 16:0. Second, although both strains have the same total amount of unsaturated fatty acids, the ML 308-225-*UFA*-8 strain has a much larger amount of palmitoleate, 16:1, probably because it is not prevented from degrading the oleate supplement to this species. The similarity of the phospholipid composition of these two strains is reflected in the phase transition in the phospholipid extract, which has the same transition width and is shifted to a higher temperature by only 2°C in ML 308-225-*UFA*-8 (see below).

Nevertheless, there is some significant difference in the total lipid composition of these two strains. Such a difference is necessary to explain the very different line widths seen for the total lipid extracts (see below). This difference disappears after an acetone precipitation step to purify the K1060B5 phospholipids. A membrane component that may have a fluidizing effect on phospholipid membranes is ubiquinone. We looked for concentration differences of this component by UV spectroscopy at 280–290 nm. The two strains have approximately the same amount of this component, as a percentage of the total weight of membrane, regardless of the fatty acid supplement. We suspect that the dramatic narrowing of the $^{19}$F-NMR resonance of the lipid extract of K1060B5 may be due to some other lipid soluble component, perhaps a lipoprotein. Ames (1974) has shown that major outer membrane proteins of ML 308-225 and K12 strains of *E. coli* differ from each other and from *Salmonella typhimurium*. In *Salmonella* these proteins (33,000–36,000 daltons) are dependent on the nature of the lipopolysaccharides. (It should be noted that in our present studies, inner and outer membranes have not been separated).

TABLE I
FATTY ACID COMPOSITION OF *E. COLI* MEMBRANES*

| Strain | No. of generations growth | Mole % of fatty acids‡ | | | | |
|---|---|---|---|---|---|---|
| | | 14:0 | 16:0 | 16:1 | 18:1 | 8,8-$F_2$ |
| K1060B5 | | | | | | |
| Oleate (*18:1*) | 1 | 2.6 | 47.9 | 4.1 | 40.0 | — |
| 8,8-$F_2$ (*14:0*) | 1 | 12.8 | 37.0 | 2.0 | 17.8 | 27.5 |
| | 2 | 14.0 | 35.0 | 1.0 | 7.0 | 37.0 |
| ML 308-225-*UFA*-8 | | | | | | |
| Oleate (*18:1*) | 1 | 3.6 | 47.3 | 14.5 | 29.1 | — |
| 8,8-$F_2$ (*14:0*) | 1 | 9.4 | 45.0 | 7.8 | 10.3 | 26.1 |
| | 2 | 8.6 | 41.0 | 3.7 | 6.7 | 36.0 |

*Cells grown on oleate at 37°C, then transferred to new fatty acid supplement medium and growth continued.
‡The numbers do not add up to 100% because not all fatty acid species are reported.

The most significant compositional difference between these two strains of *E. coli* is in the ratio of protein to lipid (Table II). In both cases these two components together make up 90–95% of the total weight of the membrane. However, when the cultures are supplemented with 8,8-difluoromyristate, the phospholipid component of K1060B5 is 26.5% by weight of the membrane, whereas in the ML 308-225-*UFA*-8 strain, the phospholipid is only 12.8%. It can be seen in Table II that this supplement increases the lipid composition in K1060B5 and decreases it in ML 308-225-*UFA*-8, relative to the oleate supplemented cells. The compositional differences can largely explain both the phase transition behavior, as seen by $^{19}$F-NMR, and the properties of the membrane above the phase transition temperature, as we show below.

*Transport Studies*

Incorporation of 8,8-difluoromyristate into either the ML 308-225-*UFA*-8 or K1060B5 strain of *E. coli* changes the behavior of the lactose transport system. Fig. 1 shows the effect on transport of $^{14}$C-TMG. It is apparent that in both strains there is a decreased level of accumulation at the steady state which occurs with a change in the lipid composition. Table III, which contains values for the steady-state level and the initial velocity of influx at 30 s (averaged over several experiments), clearly demonstrates this point. The ratio of the steady state level over the initial velocity remains the same in each case despite a tenfold change in the rate of uptake. This suggests that the effect is on the rate of uptake of TMG rather than an enhancement of the rate of efflux. The presence of 8,8-difluoromyristate has a greater effect on the transport of TMG through the K1060B5 membrane than that through ML 308-225-*UFA*-8 (see Fig. 1 and Table III). ML 308-225-*UFA*-8 shows a 75% decrease while K1060B5 shows a 90% decrease for the steady-state ratio of the substrate concentration on the inside vs. the outside of the cells.

These results are not due to the membrane phase transition. Other authors have shown (Therisod et al., 1977) using strains ML 308-225 and K1059 of *E. coli* that there is a temperature-dependent decrease in the number of binding sites of the lactose permease for dansylgalactoside. The effect of decreasing temperature overlaps the temperature range of the conformational order-disorder transition of membrane lipids. The present measurements at 37°C will not register temperature-dependent effects of the lipid phase transition, which

TABLE II
PROTEIN AND PHOSPHOLIPID COMPOSITION OF THE MEMBRANE OF *E. COLI*

| Strain | Supplement | Weight | |
|---|---|---|---|
| | | Protein | Lipid |
| | | *(%)* | *(%)* |
| K1060B5 | Oleate (>3 generations) | 68 | 21 |
| K1060B5 | 8,8-difluoromyristate (1 generation) | 68.5 | 26.5 |
| ML 308-225-*UFA*-8 | Oleate (>3 generations) | 75 | 16 |
| ML 308-225-*UFA*-8 | 8,8-difluoromyristate (1 generation) | 83 | 12.8 |

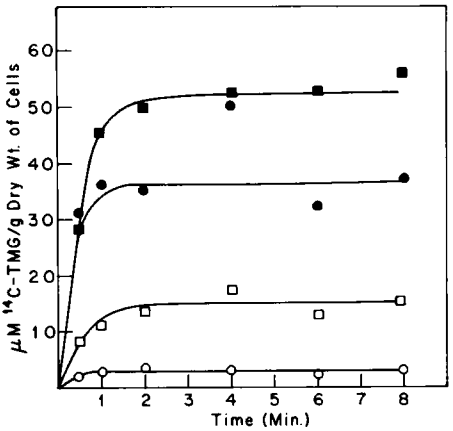


FIGURE 1 Uptake of $^{14}$C-labeled methyl β-D-thiogalactopyranoside (TMG) by two strains of unsaturated fatty acid auxotrophs of *E. coli* at 37°C: (■) ML 308-225-*UFA*-8 in oleic acid; (□) ML 308-225-*UFA*-8 in 8,8-difluoromyristic acid; (●) K1060B5 in oleic acid; (○) K1060B5 in 8,8-difluoromyristic acid.

occurs in the range of 20°–35°C for the fluorinated membrane of K1060B5 and 0°–20°C for the oleate enriched membrane.

### *$^{19}$F-NMR Studies of Lipid Fluidity and the Phase Transition*

One possible explanation for the different active transport behavior of the ML 308-225-*UFA*-8 strain relative to K1060B5 is that the organization of the cytoplasmic membrane matrix is different. This should be detectable by $^{19}$F-NMR studies. The results of a $^{19}$F-NMR investigation of intact membranes, isolated lipids, and purified phospholipids have been presented previously for the K1060B5 strain (Gent and Ho, 1978). We have used the same technique to examine the ML 308-225-*UFA*-8 strain. It should be mentioned that the $^{19}$F-NMR spectra of whole cells and membrane vesicles are the same. We have found distinct differences between the two strains, both in the high temperature region above the phase transition and in the progress of the lipids from the fluid to the frozen state. The temperature dependence of the line widths, derived from a computer simulation of the $^{19}$F resonance, is plotted in Figs. 2 and 3. If the temperature dependence of the phospholipid motion is ignored for the moment, and the results obtained at 37°C and higher are compared, we can say the following. The dipolar line width parameter, $\upsilon_{DD}$, is the same in the two intact membranes,

TABLE III
INFLUX AND ACCUMULATION OF $^{14}$C-METHYL β-D-THIOGALACTOPYRANOSIDE BY LOG PHASE UNSATURATED FATTY ACID AUXOTROPHS OF *E. COLI* AT 37°C

| Strain | Supplement | $V_{in}$ μM/min/g dry wt | Concentration gradient at steady state |
|---|---|---|---|
| K1060B5 | 18:1 | 46 | 45 |
| K1060B5 | 8,8-$F_2$ 14:0 | 6 | 4 |
| ML 308-225-*UFA*-8 | 18:1 | 77 | 63 |
| ML 308-225-*UFA*-8 | 8,8-$F_2$ 14:0 | 17 | 16 |

~3,100 Hz. $\upsilon_{DD}$ is also the same in the purified phospholipids of the membranes, ~2,000 Hz, for K1060B5 and ML 308-225-*UFA*-8. However, the total lipid extract of K1060B5 has a very narrow $\upsilon_{DD}$ = 200 Hz. In contrast, total lipids from ML 308-225-*UFA*-8 have the same line width as the purified phospholipids. Thus, there is a membrane component that exerts a disordering effect on the K1060B5 lipid that is not seen in the ML 308-225-*UFA*-8 strain. This effect disappears on purification of the phospholipids. It is also not evident in the intact membrane. In K1060B5 the proteins must somehow counteract the fluidizing effect that is apparent in the total lipid extract.

The line width of the central spike of the $^{19}F$ resonance, $\upsilon_0$, appears to be more sensitive to the relative proportion of interacting lipid and protein than $\upsilon_{DD}$ (Gent and Ho, 1978). This parameter is the same in all the lipid extracts, $\upsilon_0$ = 40 Hz at 37°C. It is slightly different in the two intact membranes. K1060B5 has a value of 80 Hz and ML 308-225-*UFA*-8 a value of 100 Hz at 37°C. This difference implies that K1060B5 has less interaction of lipid with protein than ML 308-225-*UFA*-8.

The progress of the lipid phase transition in the two cytoplasmic membranes shows a more fundamental difference than any other properties mentioned so far. As we reported previously, K1060B5 membranes have a phase transition that involves clustering of the membrane protein (Gent and Ho, 1978). Above the phase transition the protein interacts uniformly with all the lipid. Below the transition most of the protein interacts with a small proportion of lipid which remains fluid. The $^{19}F$ resonance of the lipids reflects this phenomenon in that only one resonance is seen above 35°C while narrow and broad components are superimposed at temperatures below this. The data in Figs. 2 and 3 actually correspond to the line width temperature dependence of the narrow resonance. The broad resonance is assumed to have $\upsilon_{DD}$ = 7,500 Hz and $\upsilon_0$ = 500 Hz, although this is hard to determine accurately in the presence of a sharp component. In the ML 308-225-*UFA*-8 strain, the temperature dependence of the

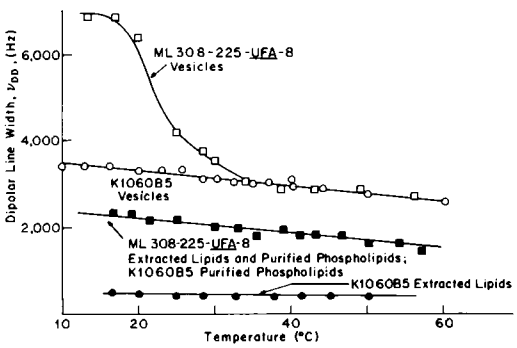


FIGURE 2

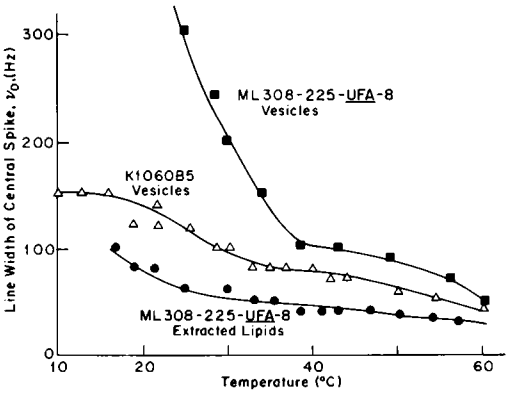


FIGURE 3

FIGURE 2 $^{19}F$-resonance line width (dipolar line width, $\upsilon_{DD}$) of *E. coli* membrane vesicles, extracted lipids, and purified phospholipids as a function of temperature. *E. coli* cells were grown for one generation on 8,8-difluoromyristate at 37°C.

FIGURE 3 $^{19}F$-resonance line width (line width of the central spike, $\upsilon_0$) of *E. coli* membrane vesicles and extracted lipids as a function of temperature. *E. coli* cells were grown for one generation on 8,8-difluoromyristate at 37°C.

resonance shape is best approximated by a single resonance that broadens greatly as the phase transition range is traversed, eventually giving rise to a resonance with a width of $\upsilon_{DD}$ = 7,500 Hz and $\upsilon_0 \simeq 500$ Hz. It appears that no protein clustering is induced by the transition and the lipid continues to interact with all the protein. Further evidence for this is seen in the temperature dependence of the sharp resonance intensity. The lipids in the intact ML 308-225-*UFA*-8 membrane tend to melt earlier than the isolated lipids, as would be expected of a more heterogenous system (Fig. 4), whereas the bulk of the lipids in the K1060B5 membrane melt at a higher temperature than the isolated lipids (Fig. 9 in Gent and Ho, 1978). The latter is explained by the preference of the protein clusters for the more unsaturated, lower melting lipids. Therefore, the protein-lipid interactions in these two strains of *E. coli* are sufficiently different that the protein clustering is induced by the lipid phase transition in K1060B5 but not in ML 308-225-*UFA*-8.

### *TEMPO Studies of the Membrane*

Other techniques also report on the physical state of the phospholipid component of membranes. One of the simplest experiments to perform is TEMPO partitioning in which the ratio of the nitroxide spin label in lipid vs. aqueous phases is measured (Linden et al., 1973; Shimshick and McConnell, 1973; Kleeman and McConnell, 1974). This ratio decreases when the lipid component freezes into the gel phase. The temperature dependence of TEMPO partitioning is shown in Fig. 5. The purified phospholipids show a proportionally larger partition ratio because there is a greater concentration of lipid in this sample (~25 vs. ~10 mg/ml). However, differences in the lipid concentration between the membranes of ML 308-225-*UFA*-8 and K1060B5 are not evident in the TEMPO partitioning. Breaks in the temperature coefficient of the partition function appear at 20° and 37°C, in the extracted

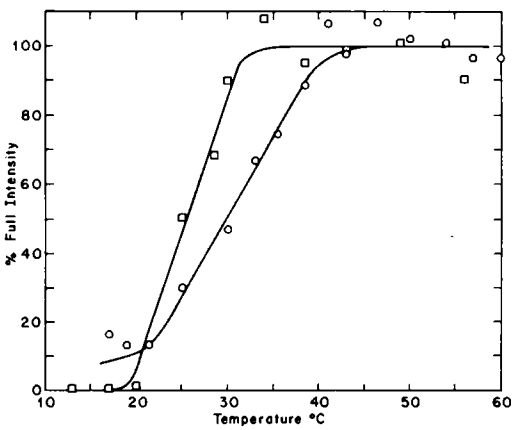


FIGURE 4

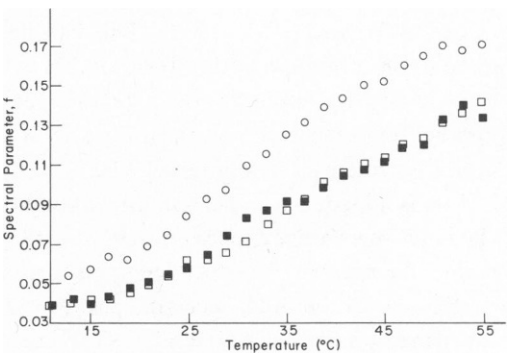


FIGURE 5

FIGURE 4 Intensity due to fluid lipids of the $^{19}F$ resonance of 8,8-difluoromyristate of *E. coli* ML 308-225-*UFA*-8: (□) isolated membrane vesicles; and (○) extracted lipids.

FIGURE 5 TEMPO partitioning in *E. coli* membrane vesicles and phospholipids as a function of temperature: (○) K1060B5 phospholipids at 25 mg/ml: (□) ML 308-225-*UFA*-8 membrane vesicles at 44 mg/ml; and (■) K1060B5 membrane vesicles at 43 mg/ml. *E. coli* cells were grown for one generation on 8,8-difluoromyristate at 37°C.

lipids corresponding to the solidus and fluidus boundaries of the phase transition. These breaks are not discernable in the intact membranes. The most interesting point to note is that there is little difference in the TEMPO partition ratio for the two strains except in the center of the transition. In this particular temperature zone the TEMPO partitioning shows K1060B5 to have more fluid lipid than ML 308-225-*UFA*-8. As with the $^{19}$F-NMR results, the most significant difference in the physical properties of the lipids of these two strains is seen within the phase transition, not at a higher temperature.

## DISCUSSION

The observed differences in transport behavior between K1060B5 and ML 308-225-*UFA*-8 strains of *E. coli* could be due to either a different lactose permease (carrier protein) or a different membrane architecture and behavior. The β-galactoside transport system can be altered genetically to a form in which it is decoupled from metabolic energy and acts like a facilitated diffusion mechanism for lactose transport (Wilson and Kusch, 1972; West and Wilson, 1973; Fried, 1977). Other mutants have been isolated in which many active transport systems are unable to accumulate a variety of compounds against a concentration gradient, indicating a general perturbation of the coupling of membrane related respiration to active transport (Hong and Kaback, 1972; Hong, 1977). Since the isolation procedures for the K1060B5 and ML 308-225-*UFA*-8 mutants did not select for these altered lactose transport genotypes, and since differences in membrane composition and physical behavior are clearly evident, we hypothesize that the transport effects seen are due to the general membrane architecture alone. An altered lipid composition can lead to altered membrane-related activities (Baldassare et al., 1976; Vanderwinkel et al., 1976).

One means by which a membrane compositional change could result in an altered transport activity is through perturbation of the normal protein-protein and protein-lipid interactions. There appears to be a boundary layer of lipid around integral membrane proteins that is physically distinguishable from the bulk lipid at least on the EPR time scale of $10^{-7}$ sec (Jost et al., 1970; Jost et al., 1973). This can be viewed as solubilization lipid necessary for the dissolution of protein within the membrane. The affinity for this interaction, with respect to the affinity for protein-protein binding or association, will determine both the extent of aggregation of protein within the plane of the membrane and the rate of any process that requires protein-protein interactions.

Lipids added as a sonicated vesicle solution to *E. coli* cells quickly move from the outer to the inner membrane (Jones and Osborn, 1977 *b*), illustrating that the lipid does not provide a stable framework in which proteins are embedded. Although lipids migrate from one membrane to the other, certain specific enzyme activities are restricted to the outer or the inner membrane (Overath et al., 1975; Smyth et al., 1978). Thus, the organization of proteins provides the orientation for vectorial biochemical activities.

We observe changes, in both the ratio of protein to lipid and the strength of interaction of the two components, between K1060B5 and ML 308-225-*UFA*-8. Either or both of these alterations could affect active transport. Small increases in the amount of membrane lipid caused by fusion of pure lipid to cell membranes will not necessarily cause biochemical alterations. However, addition of lipid in quantities greater >20 or 30% of the original leads to

decreases in membrane-related respiration and other toxic effects (Baldassare et al., 1977; Jones and Osborn, 1977 *a*). The relative difference in lipid content is ~50% in the two strains of *E. coli* under discussion (Table II). Therefore, either the increased lipid content or the different composition of the total extract could cause the impaired active transport of K1060B5 relative to ML 308-225-*UFA*-8.

The change in protein-lipid interactions, indirectly implying a change in protein solubilization and protein-protein interactions, is evident in the phase transition behavior of the two strains of *E. coli*. The lipid phase transition phenomenon has been intensively studied in pure lipid dispersions and in mixtures of lipids and purified proteins (Chapman, 1975; Lee, 1977). It is apparent that the presence of proteins strongly changes the melting temperature and physical characteristics of the boundary lipid (Jost et al., 1970; Chapman, 1975; Overath et al., 1975). The lipid phase transition likewise affects the activity and solubility in the plane of the membrane of integral proteins in *E. coli* (Van Heerikhuizen et al., 1975; Bayer et al., 1977; Letellier et al., 1977). The qualitative differences in the phase transition between K1060B5 and ML 308-225-*UFA*-8 lipids suggest altered protein-lipid and protein-protein interactions. In K1060B5 the protein-lipid interaction is sufficiently weak, or conversely protein-protein interactions are stronger, so that the lipid segregates as it freezes. In ML 308-225-*UFA*-8 this does not happen and the protein and lipid are presumed to keep a random distribution. This difference in protein-lipid interactions is only faintly seen above the phase transition, particularly in the $\upsilon_0$ line width parameter of the $^{19}F$ resonance. This parameter seems to be sensitive to the ratio of protein interacting with lipid (Gent and Ho, 1978). If the different transport behavior in these two strains of *E. coli* is due to the different membrane compositions observed, it is not the lipid order parameter that is the crucial factor but some more difficult to define aspect related to either protein-protein or protein-lipid interactions.

We have presented evidence to suggest differences in protein-lipid interactions between the two strains of *E. coli*. These differences are not black and white, with thorough mixing of lipid and protein in one situation and complete segregation in the other. The protein-protein interactions necessary for active transport are likely to be sensitive to slight changes in affinity with respect to the lipid solubilization. Thus, either a change in the protein to lipid ratio or a change in lipid composition could affect membrane behavior. This would change the probability of protein-protein interactions but not eliminate them.

In conclusion, $^{19}F$-NMR spectroscopy indicates clearly that there are altered protein-lipid interactions in two strains of *E. coli*, K1060B5 and ML 308-225-*UFA*-8. It appears that 8,8-difluoromyristic acid acts as an unsaturated fatty acid for these two unsaturated fatty acid auxotrophs of *E. coli* and is incorporated into their lipids. We are constructing additional fatty acid auxotrophs of *E. coli* and *S. typhimurium* with which we plan to extend these investigations and to assess critically the fluorine-19 probe in biological membranes.

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