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CONTROL OF FATTY ACID COMPOSITION OF *ACHOLEPLASMA LAIDLAWII* MEMBRANES

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Summary

The temperature-dependent pattern of incorporation of palmitate and oleate from the growth medium into *Acholeplasma laidlawii* membrane lipids correlates with the physical state of the membrane defined by calorimetry. Both the pattern and the state can be changed at will by changing the fatty acid composition of the membrane lipids. The ratio of palmitate to oleate incorporated is independent of temperature when the membrane bilayer is below its transition and fully ordered, but becomes temperature dependent upon the onset of the transition and continues to be temperature dependent when the membrane is above its transition and fully fluid. This behavior is mimicked by the physical binding of palmitate and oleate to bilayers of extracted membrane lipids and to bilayers of lecithin. Selective binding by membranes may provide a means for controlling lipid fatty acid composition without invoking an enzymatic mechanism.

Introduction

A characteristic of nearly all organisms is their ability to accomodate the fatty acid composition of their lipids to growth temperature [1]. Most commonly, the principal biochemical adjustment is an increase in the fraction of unsaturated fatty acids in lipids synthesized at lower temperatures. The obvious physical effect of such alterations is a depression of lipid melting points. These biochemical changes at low growth temperatures result in an increase in membrane fluidity and a decrease in membrane transition temperatures [2,3]. Some adjustment of bilayer fluidity may itself be physiologically important, even in membranes which may have no transition or, as in the case of *Micrococcus lysodeikticus*, have a transition well below growth temperature [4]. But in organisms whose membrane transition exists at or just below growth temperature, as in the case of *Escherichia coli* [2,5], the shift in the fatty acid spectrum at lower growth temperatures is absolutely essential to maintain a functional fluid state. As membranes pass through a transition from high to low temperature they become progressively more crystalline. This increased order can give rise to aberrant behavior, including changes in enzyme kinetics, cell leakage, cessation of cell division, and even cell lysis. If these and other calamitous effects are to be avoided the increase in unsaturation or the inclusion of other fatty acids of low melting point becomes a physiological necessity.

The detailed mechanisms responsible for modulating fatty acid composition are unknown, but evidence gleaned from *Escherichia coli* suggests that two separate processes may operate [6]. The first occurs at the level of fatty acid synthesis, and can be rationalized in terms of the temperature response of one or more enzymes in the synthetase system. The second is at the level of phosphatidic acid synthesis in membranes, where membrane-bound transacylase enzymes appear to be "programmed" to select increasingly lower proportions of saturated fatty acids as temperature is decreased [7]. In cells whose fatty acids are endogenously synthesized both processes may play a role. However, insight into the selective process in vivo at the membrane level is best obtained from organisms growing largely upon exogenous sources of fatty acids. *A. laidlawii* is such an organism, since it requires unsaturated fatty acids and preferes to use exogenous saturated acids rather than endogenously synthesized ones. Furthermore, extensive studies of the physical and physiological effects of modifying the composition of membrane lipids in *A. laidlawii* by growing cells in fatty acid-enriched media [8—10] have shown that membrane transition temperatures can be shifted considerably, from about —20°C to the physiological temperature range. By examining the pattern of incorporation of labeled palmitate and oleate as representative fatty acids into the membrane lipids of *A. laidlawii* grown in various fatty acid supplements, we have been able to define the nature of the temperature-dependent selection program at the membrane level and to show that the pattern of incorporation correlates with the membrane transition determined calorimetrically. In addition, the pattern of temperature-dependent binding of fatty acids to extracted membrane lipids and to model lipid bilayers is quite similar to that of incorporation into membrane lipids [2].

Materials and Methods

Incorporation of fatty acids into membrane lipids

A. laidlawii was grown at 37°C to late log phase in tryptose [11], either unmodified or supplemented with oleate or palmitate and lipid-depleted bovine serum albumin (Calbiochem). In initial experiments, mixed [^{3}H]oleate and [^{14}C]palmitate (New England Nuclear) was added to cells at room temperature in growth medium, then 0.5 ml aliquots of the cells were dispensed into test tubes inserted into an aluminium block heated at one end and cooled at the other to provide a linear temperature gradient. The time lapse between adding label and dispensing the last aliquot was about 45 s, samples within the block reached thermal equilibrium within 30 s, and uptake took place for 7.5 min (about 0.1 generation). To avoid incubation with label at room temperature, the protocol was later modified by adding 0.1 ml aliquots of cells in growth medium to 0.4 ml of labeled fatty acids in growth medium pre-incubated in tubes in the gradient block to attain thermal equilibrium. Results obtained by both protocols were essentially the same.

The reaction was quenched and lipids were simultaneously extracted by adding to each tube 1.76 ml chloroform/methanol (1 : 2, v/v) containing unlabeled *A. laidlawii* lipids as a carrier. The extracted lipids were the washed by adding 0.625 ml chloroform, followed by 0.625 ml water and thorough agitation on a vortex mixer [12]. After evaporation of the solvent, the lipids were separated from free fatty acids on silica gel G plates with petroleum/ether/acetic acid (25 : 25 : 1, v/v). The origin and immediate surroundings, containing the phospholipids and glycolipids, were scraped into a glass scintillation vial and incubated overnight in 1.0 ml 1.0% sodium dodecyl sulfate at 37°C. After addition of 10 ml of scintillation fluid (1ℓ Triton X-100, 2ℓ toluene, 8 g PPO, 0.2 g POPOP), the samples were counted in a dual-channel scintillation spectrometer. Appropriate controls assured separation of free fatty acids from polar lipids, elution of label from the silica gel, no uptake by heat-killed cells, and negligible incorporation of label into non-lipid components.

Chloramphenicol (80 µg/ml) added to cell suspensions to block protein synthesis before adding label or placing aliquots in the gradient block had no effect upon the results.

Binding of fatty acids by lipid bilayers

Unlabeled *A. laidlawii* lipids were extracted and washed by the method of Bligh and Dyer [12]. L-α-Dipalmitoyl phosphatidylcholine was obtained commercially, and egg phosphatidylcholine was prepared by column chromatography on silica gel.

For binding studies, pieces of Whatman 3MM filter (approximately 1 cm^2) paper were impregnated in a vacuum with a 10% solution of lipid in chloroform and dried in a vacuum dessicator. Approximately 10 mg of lipid was deposited in each piece. Mounted on nichrome wires, the impregnated papers were hydrated by incubating for 1 h in 0.1 M Tris · HCl buffer at pH 8.5. For all lipid preparations, calorimeter scans of thermal transitions were unaffected by entrapment within the pores of the paper. The papers were incubated for 4 h in the aluminium temperature gradient block in test tubes containing 4.0 ml of a suspension of mixed [^{3}H]oleate and [^{14}C]palmitate in Tris buffer, then removed from the tubes, blotted to remove excess buffer, and counted in the scintillation spectrometer using the sodium dodecyl sulfate/Triton X-100 solvent. Washing the papers in buffer to remove unbound fatty acids was unnecessary, since the label retained in the buffer wetting the papers was at least an order of magnitude less than the counts bound by the trapped lipids.

Calorimetry

Thermograms were obtained with a Perkin-Elmer DSC-2 scanning calorimeter, modified in this laboratory for improved stability and signal/noise ratio [13]. Lipid samples of 1—3 mg mass were encapsulated in sealed sample pans with water or Tris buffer (10—12 µl) and incubated for a short time above their transition temperatures, then scanned in both ascending and descending modes at rates of 5 and 10 degree/min on the 0.2 mcal/s range. Mixed lipids were prepared by dissolving the components together in chloroform and evaporating the solvent before exposure to buffer.

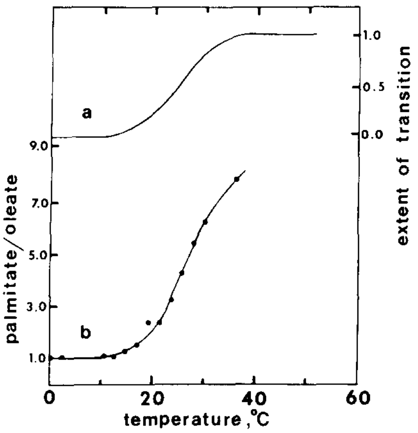
Membranes were prepared by hypotonic lysis of cells in Tris buffer at pH 7.4, then washed in the centrifuge in the same buffer. The washed membranes were pelleted in the centrifuge at high speed or concentrated by vacuum dialysis at 4°C, transferred to a sample pan, then sealed and scanned under the same conditions used for lipids. Sample sizes were usually 10—15 mg net weight, and contained 10—20% dry matter.

Results

The temperature "program" for selection of exogenous fatty acids is revealed by plotting the ratio of [^{14}C]palmitate to [^{3}H]oleate (palmitate/oleate ratio) simultaneously incorporated into the membrane lipids [7] while the bilayer transition within the membrane is described by the calorimeter. Comparison of palmitate/oleate with calorimeter scans shows the temperature program to correlate with the physical state of the bilayer, which in turn depends upon the fatty acid composition of the membrane lipids. As a consequence, the program and resultant palmitate/oleate plots can be changed at will by changing the fatty acid composition. The greatest alteration in the physical state of a membrane of fixed fatty acid composition occurs during a transition, where the bilayer passes from an ordered state at low temperature to a disordered state at higher temperatures. Below the transition lateral diffusion is inhibited and the lipids are fractionally crystallized within the plane of the membrane, while above it free diffusion and mixing takes place [2]. The progression from one state to the other can be sudden or quite gradual, depending upon the fatty acid heterogeneity of the lipids. Similarly, the greatest changes in the palmitate/oleate ratio of incorporated fatty acids occur at the beginning of the transition, where fatty acid selection becomes strongly temperature dependent. At temperatures below the transition, the ratio of palmitate to oleate incorporated is nearly independent of temperature, while during the transition it is strongly temperature dependent. The palmitate/oleate ratio thus shows a pronounced change in slope at the low-temperature end of the transition.

These phenomena are illustrated in the first two figures. Fig. 1 is a plot of the palmitate/oleate ratio of incorporated fatty acids and the extent of membrane melt determined calorimetrically in *A. laidlawii* grown in unsupplemented tryptose. There is a clear correlation between the two. Below the transition, which begins gradually at approx. 15°C, fatty acid selection is unaffected by temperature. During the transition, however, selectivity becomes strongly dependent upon temperature, and the palmitate/oleate plot roughly parallels the fraction melted. The increase of palmitate with respect to oleate is approximately eight-times greater at 40°C, toward the end of the transition, than at its beginning.

The effects of shifting the membrane transition temperature are shown in Fig. 2, plots of the palmitate/oleate ratio and extent of transition in *A. laidlawii* grown in oleate-enriched and plamitate-enriched media. The transition in oleate cells is lowered compared to tryptose-grown cells, beginning at about 0°C and ending at about 20°C. From 20°C to 40°C the membrane bilayer is completely fluid. Over the entire range, from 0 to 40°C, the temperature program has a positive slope and selectivity is strongly temperature dependent.



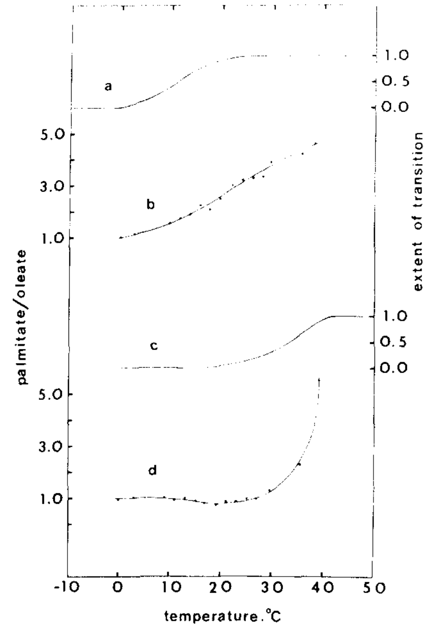


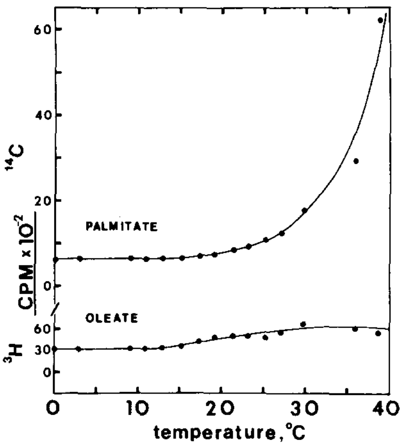
Fig. 1. Correlation of the temperature dependence of fatty acid incorporation into membrane lipids with the state of the membrane bilayer in *A. laidlawii* cells grown in unsupplemented tryptose medium. Extent of transition (curve a) is obtained from the integrated calorimeter scan of the transition in membranes, and progresses from 0.0 for a fully ordered bilayer to 1.0 for a fully fluid bilayer. Palmitate/oleate (curve b) is the ratio of [^{14}C]palmitate to [^{3}H]oleate activities incorporated into the lipids of the same cells, and is normalized to 1.0 at the starting temperature.

Fig. 2. The effect of altering the state of the membrane bilayer upon the incorporation of fatty acids into membrane lipids. For cells grown in an oleate-enriched medium, the extent of transition (curve a) shows the membrane to be undergoing a transition below 20°C and fully fluid thereafter. The accompanying ratio of [^{14}C]palmitate to [^{3}H]oleate incorporation (curve b) is strongly temperature dependent both during and after the transition. In palmitate-enriched cells, the extent of transition (curve c) shows the membrane bilayer to be below its transition and ordered over the entire physiological temperature range. The accompanying palmitate/oleate incorporation ratio (curve d) is nearly independent of temperature.

Thus the increasing preference for palmitate over oleate with increasing temperature appears to be characteristic of fluid membranes. It vanishes when the membrane solidifies. The temperature-independence of selectivity by membranes below their transition temperature is clearly emphasized in Fig. 2d, a plot of the palmitate/oleate ratio for palmitate-enriched cells with a high transition temperature.

Figs. 1 and 2, taken together, define the relation between the thermal response of fatty acid incorporation and the state of the lipids in the membrane. When the membrane is crystalline, temperature dependence is weak or absent. As the membrane enters a transition, and passes from the ordered state to the fluid state, selectivity becomes temperature dependent. When the membrane of oleate-enriched cells is fully fluid, above its transition, selectively continues to be temperature dependent.

In Figs. 1 and 2, the palmitate/oleate ratios have been normalized to 1.0 at 0°C for convenience in presentation. The origin of the temperature dependence of the ratios is revealed by the absolute counts of palmitate and oleate. Incorporation by cells grown in palmitate-enriched medium is shown in Fig. 3. At temperatures below the transition few counts of palmitate are incorporated, and the incorporation is independent of temperature. Upon the onset of the transition a large increase in the rate of incorporation of plamitate begins, accompanied by a relatively much smaller proportional increase in oleate incorporation. By 37°C, palmitate incorporation has increased by a factor of ten, while oleate incorporation has little more than doubled. The shapes of the two curves differ, with the oleate curve flattening at higher temperatures. In cells grown in unsupplemented tryptose medium (Fig. 4), the proportional increase of palmitate incorporation is again more strongly temperature dependent than oleate, except that the entire curve is shifted to lower temperatures to again coincide with the transition. Oleate incorporation again increases only slightly with temperature, and even appears to decrease again at higher temperatures to a rate near that observed at 0° C. The rate of palmitate incorporation becomes constant at the highest temperature and may even decrease. This effect is not seen in oleate-grown cells (Fig. 5), and might be explained by endogenous synthesis of saturated fatty acids. Nevertheless, it does obscure the correlation between the calorimetrically observed transition and the shift in uptake curves. The same pattern of a strong temperature response in palmitate uptake and a much weaker relative response in oleate uptake is repeated by oleate-grown cells. The rate of palmitate uptake in no longer temperature-independent at lower temperatures as it is in palmitate-enriched cells and cells grown in unsupplemented tryptose medium, since even at 0° C the membrane transition has already begun. At temperatures above the transition, where the membrane is completely fluid, the rate of palmitate uptake continues to be strongly temperature dependent. The rate of oleate uptake again seems to plateau at higher temperatures.



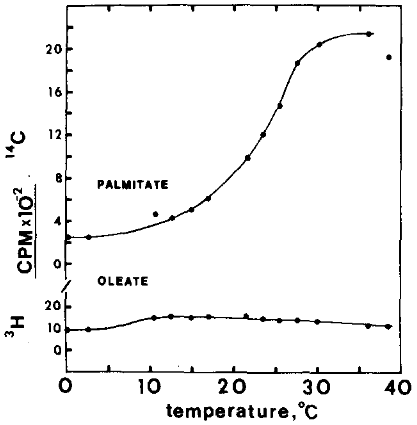


Fig. 3. The simultaneous incorporation of [^{14}C]palmitate and [^{3}H]oleate into the lipids of palmitate-enriched *A. laidlawii* cells. The ratios platted in Figs. 1 and 2 were obtained from the data presented in this and Figs. 4 and 5. The ordinate scales are chosen so that relative increases in cpm of plamitate and oleate are comparable (i.e. doubling of both palmitate and oleate produce identical increments in the ordinate).

Fig. 4. The simultaneous incorporation of [^{14}C]palmitate and [^{3}H]oleate into the lipids of cells grown in unsupplemented tryptose medium.

The possibility of artefacts must be considered, since the state of the labeled fatty acids in the incubation medium is ill-defined. Some may be in solution, some bound to other components in the medium, and others could exists as droplets or micelles. All could experience physical changes with temperature which might affect their availability to the cells. However, the plots correlate well with calorimeter scans of the membranes and can be changed drastically without changing the composition of the incubation medium used during incorporation. Thus, the plots apparently reflect events associated with the cells and not with unexpected changes in the medium. Rate-limiting steps occurring in the medium or exhaustion of the supply of labeled fatty acids are also not implicated, since the palmitate/oleate ratio of incorporated fatty acids did not change with increased incubation time (24 min, 4 times the usual procedure) at constant cell concentration or with cell concentration (increased by a factor of 4) at constant incubation time of 6 min. Ordinarily, approx. 5% of the total labeled fatty acids added to the medium were incorporated during incubation. Endogenous synthesis of saturated fatty acids in *A. laidlawii* is low compared to uptake from exogenous sources. It can be discounted as a perturbing factor, since it would be expected to flatten the curves to some extent but could not account for the observed differences between curves.

The correlation between the state of the membrane bilayer and the pattern of incorporation of palmitate and oleate into membrane lipids appears to be mimicked by the physical binding of fatty acids by lipid bilayers. The palmitate/oleate ratio of labeled fatty acids incorporated into the membrane lipids of

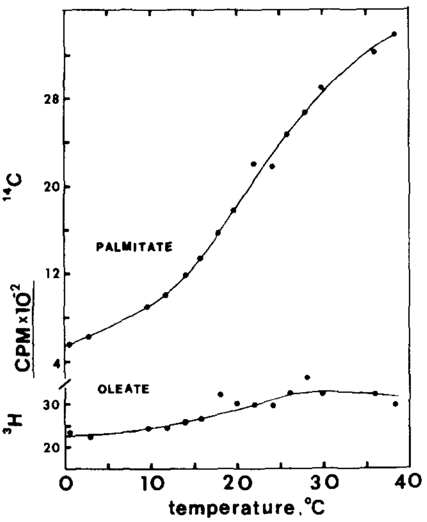


Fig. 5. The simultaneous incorporation of [^{14}C]palmitate and [^{3}H]oleate into the lipids of oleate-enriched cells.

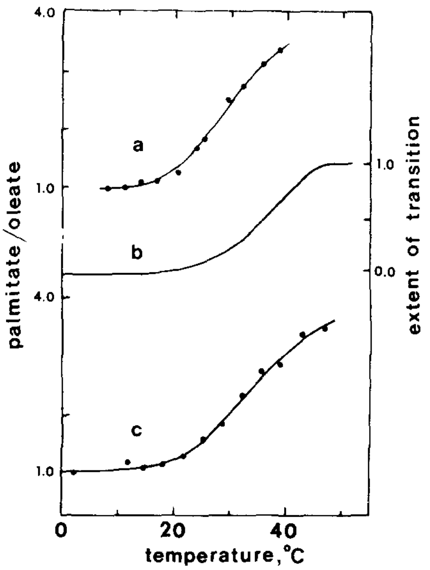


Fig. 6. Correlations between the palmitate/oleate ratio incorporated into cellular membrane lipids (curve a), the extent of transition in membranes (curve b), and the palmitate/oleate ratio of fatty acids physically bound to bilayers of extracted membrane lipids (curve c). Both incorporation and binding curves refflect the state of the bilayer, and are identical within experimental error. The cells were grown in unsupplemented tryptose medium.

cells grown in unsupplemented tryptose medium is shown in Fig. 6, together with the extent of the membrane transition seen by calorimetry and the palmitate/oleate ratio of fatty acids physically bound by lipids extracted from the membrane. All data come from the same culture of *A. laidlawii* grown on unsupplemented tryptose medium. As seen in Fig. 1, incorporation again parallels the transition. However, selective binding of fatty acids by bilayers of extracted lipids, which undergo the same transition as bilayers within membranes, behaves in the same way. Within the limits of experimental error, incorporation and binding curves are identical. It is not known whether selective binding is kinetically or thermodynamically determined, but preliminary data suggests a thermodynamic mechanism since the same behavior is observed for experiments of 1—4 h binding. Absolute counts of fatty acids bound by extrated lipids are plotted in Fig. 7. The pattern is quite similar to that seen in incorporation by live cells. Palmitate binding is not strongly dependent upon temperature below the transition, but at the onset of the transition becomes strongly temperature dependent. Oleate binding, on the other hand, is proportionally less responsive to temperature.

Transition-dependent selective binding of fatty acids is not confined to bilayers of *A. laidlawii* lipids. The same phenomenon occurs with mixed phosphatidylcholine. Fig. 8a, b displays calorimetric and binding data for bilayers

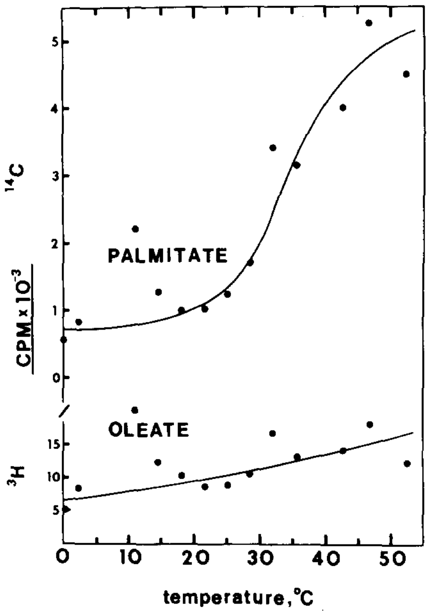


Fig. 7. The simultaneous binding of [^{14}C]palmitate and [^{3}H]oleate by bilayers of lipids extracted from the membranes of *A. laidlawii* grown in unsupplemented tryptose medium. Scatter is greater than in studies of incorporation by cellular lipids because of variations in the amount of lipid deposited in filter paper strips, but the scatter is eliminated in ratio plots of binding (Fig. 6b).

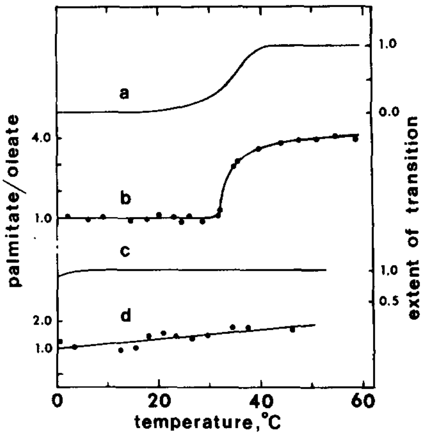


Fig. 8. Selective binding of fatty acids by phosphatidylcholine bilayers. A mixture of 25% egg phosphatidylcholine and 75% dipalmitoyl phosphatidylcholine gives the transition shown in curve a and the [^{14}C]-palmitate/[^{3}H]oleate binding ratio in curve b. Pure egg phosphatidylcholine has the transition seen in curve c and the binding ratio in curve d. Since the transition in egg phosphatidylcholine occurs almost entirely below 0° C, the bilayers are fluid over nearly the entire temperature range shown.

containing 75% dipalmitoyl phosphatidylcholine and 25% egg phosphatidylcholine, which, because of its saturation, broadens the dipalmitoyl phosphatidylcholine transition and lowers it below its customary 41°C. Below the transition selective binding is again indepnednt of temperature, as it is in binding by *A. laidlawii* lipids and in incorporation into lipids by cells. The binding curve again changes with the onset of the transition, and becomes strongly temperature dependent during it. Above the transition, the completely fluid bilayers bind proportionally far more plamitate than below, and the palmitate/oleate plot continues to show a decreased but still positive slope. Although the shape of the palmitate/oleate plot does not appear to correspond as closely to the extent of the transition as it does in the case of incorporation by cells or binding by extracted membrane lipids, the phenomenon of transition-dependent selectivity is displayed by all three systems.

The temperature-dependent selective binding shown by fluid lamellar phosphatidylcholine in Fig. 8b (i.e. a positive slope from 40 to 55°C) is also characteristic of pure egg phosphatidylcholine, which is far more unsaturated than the egg phosphatidylcholine/dipalmitoyl phosphatidylcholine mixture. The egg phosphatidylcholine transition, shown in Fig. 8c, is 95% complete at 0°C, so that the bilayers are essentially fluid over the entire 0—50°C range. The binding curve is featureless and linear, with a positive slope. Although their slopes differ, the behavior of palmitate/oleate plots of fatty acid binding by egg phosphatidylcholine and fluid bilayers in oleate-enriched *A. laidlawii* (Fig. 2a, b) are analogous even though the lipid of *A. laidlawii* contain no phosphatidylcholine [14].

Discussion

The pattern of incorporation of palmitate and oleate into *A. laidlawii* lipids is consistent with the thermal response of most other organisms, whose lipids become progressively enriched in unsaturated fatty acids as growth temperature decreases. However, although the palmitate/oleate ratio of incorporated fatty acids increases rapidly with increasing temperature, such behavior does not occur until the onset of the membrane transition.

The palmitate/oleate ratios of incorporated fatty acids are nearly independent of temperature at temperatures below the membrane transition, but at the beginning of the transition begin to increase, and during the transition they continue increasing in a roughly exponential manner. In the fully fluid membrane of oleate-enriched cells (Fig. 2), the palmitate/oleate ratio continues to remain strongly temperature dependent at temperatures well above the completion of the transition. Absolute incorporations reveal that the large increases in palmitate/oleate ratios result mostly from large relative increases in palmitate rather than in oleate incorporation. Compared to palmitate, the relative changes in oleate are only weakly temperature dependent. In at least one case, that of cells grown in ordinary tryptose medium (Fig. 4), the rate of incorporation of oleate at higher temperatures decreases with increasing temperature. Similar decreases may occur at higher temperatures in both palmitate-enriched (Fig. 3) and oleate-enriched cells (Fig. 5).

These results can be explained in terms of temperature-dependent enzyme activities. One possibility is a transacylase whose temperature dependence is far more pronounced for straight-chain saturated fatty acids such as oleate. Alternatively, two enzymes might be active in *A. laidlawii*, one of which, specific for straight-chain saturates, is more temperature dependent than the others. Similarly, temperature-dependent selectivity might be explained by the thermal response of acyl CoA synthetase. If such speculations are correct, the absence of temperature dependence in the palmitate/oleate ratios and in the absolute incorporations at temperatures below the membrane transition may simply reflect the fact that growth does not occur below the transition [10]. Cells which are not growing would not be expected to be synthesizing new lipids at an appreciable rate. Alternatively, one could argue that the transacylase enzyme or enzymes become inactivated in an ordered bilayer matrix.

Although these or other mechanisms which explain temperature-dependent selectivity in terms of the thermal response of fatty acid specific enzymes are consistent with the incorporation studies, the physical binding of palmitate and oleate by extracted lipids and model phosphatidylcholine bilayers suggest a viable alternative. The remarkable similarities shared by fatty acid incorporation into membrane lipids in live cells and fatty acid binding by extracted membrane lipids suggest that the pattern of uptake from the growth medium may be determined by the physical properties of the membrane lipid bilayer. The palmitate/oleate plots of both incorporation and binding resemble each other closely (Fig. 6), and show the same pattern of absolute counts. Palmitate incorporation and binding are both strongly temperature dependent, while oleate incorporation and binding are not (Fig. 7). Furthermore, both incorporation and binding are less temperature-dependent at temperatures below the transition than above.

Temperature-dependent selective binding of fatty acids by bilayers apparently is not limited to the lipids of *A. laidlawii*. It is also a property of phosphatidylcholine, which does not occur in *A. laidlawii* or bacteria but is a major phospholipid of animal and plant membranes. *A. laidlawii* contains only glycolipids and acidic phospholipids [14] which bear a negative charge, whole phosphatidylcholine is zwitterionic. However, in spite of very different surface charges, the effect of temperature upon fatty acid binding is essentially the same in both cases. Evidently selectivity depends largely upon the changing apolar hydrocarbon core of the bilayer rather than the polar interface. Since transition-mediated selective binding seems to be a general property of bilayers, the control of membrane fluidity and transition temperatures by this mechanism may be common in Nature. The temperature program of the acylase enzymes in membranes of *E. coli* K12 [7] correlates very well with calorimetric determinations of the inner membrane transition in that organism [2].

The mechanism for selective binding is unknown. It could be thermodynamically or kinetically controlled. If it is an equilibrium phenomenon, as preliminary evidence suggests, it could be explained in terms of the temperature dependence of the partition of fatty acids between the apolar interior of the bilayer and the exterior aqueous medium. The relative affinity for long-chain saturated acids compared to unsaturated acids is considerably greater for a fluid bilayer than for the same bilayer in an ordered state. Thus this affinity continuously increases as the bilayer progresses through its order-disorder transition and the fraction of fluidity increases. Once the bilayer is above its transition, at least in oleate-enriched cells, selectivity continues to be temperature dependent. The thermal response in this fully fluid region may depend upon both the lipid classes in the membrane and their fatty acid composition. Lateral phase separation [15] of membrane lipids may affect the changes in incorporation and binding which occur during the transition by changing the lipid composition of the fluid portions of the bilayer, since, as the membrane solidifies, the shrinking areas of fluidity become progressively enriched in unsaturated lipids. If the esterifying enzymes are free to diffuse within the membrane they may be excluded from solidified portions of the bilayer and remain with the changing fluid regions [8] where they would have access to fatty acids or fatty acid derivatives. Although incorporation and binding studies have been carried out only with palmitate and oleate, we regard oleate as a model compound for other fatty acids of low melting point. Any structural modification of a long-chain saturated fatty acid would affect both solubility and melting point.

If the pattern of fatty acid incorporation can be explained in terms of the physical properties of the bilayer, it is unnecessary to invoke an enzymatic mechanism for the selection of fatty acids to control transition temperatures and maintain fluidity in membranes of *A. laidlawii*. Suppose the acylase protein or proteins are imbedded in the membrane bilayer, and are responsible solely for catalyzing the esterification of membrane lipids. That is, they have little or no innate ability to distinguish between various fatty acids or to change their selectivity for fatty acids with changing temperature. They accept and use fatty acids or fatty acid derivatives supplied to them by the bilayer. The selective temperature program is a property of the bilayer itself. In a sense, the acylase enzyme is a lipoprotein, comprising a protein imbedded in a bilayer. Catalytic activity resides in the protein, but the bilayer provides temperature-programmed selectivity.

Such selectivity in fatty acid incorporation would tend to maintain bilayer fluidity constant as growth temperature changes. Furthermore, since the slopes of palmitate/oleate plots change abruptly toward the low temperature and of the transition, selective binding could provide a homeostatic mechanism, operating at the membrane level, to sense the thermodynamic state of the membrane and make appropriate adjustments to maintain the transition below or largely below growth temperature. In this sense, lipid bilayers would be capable of controlling their own physical state. In cells growing largely upon exogenous fatty acids, selective binding could be the major means of controlling membrane transitions. It is not the sole means, however. In *E. coli*, which carries out appreciable endogenous synthesis, the fatty acids synthesized by the synthetase system differ from those incorporated into membrane lipids. In such organisms, it seems likely that selective binding plays a role in modulating the fatty acid spectrum before incorporation.

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