A COMPARISON OF CHARACTERISTIC TEMPERATURES FOR TRANSPORT IN TWO UNSATURATED FATTY ACID AUXOTROPHS OF ESCHERICHIA COLI

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The rate of sugar transport as a function of temperature has been compared in two unsaturated fatty acid auxotrophs. One of these, the parent strain 30E, can β-oxidize the unsaturated fatty acid supplements, whereas the β-oxidation defective progeny strain 30Eβox$^-$ cannot. In a previous study, Arrhenius plots for transport of β-glucosides and β-galactosides by strain 30Eβox$^-$ revealed striking departures from linearity at both a lower and an upper characteristic temperatures. By electron spin resonance (esr) these temperatures were shown to correlate with the temperatures where the membrane lipids undergo a transition from a totally solid state to a solid–liquid equilibrium and from a solid–liquid equilibrium to a totally liquid state, respectively (1). In the present study with strain 30E we have made the following observations:

1. Arrhenius plots for transport rate are usually more complex, often revealing three characteristic temperatures. Two of these correlate with the upper and lower characteristic temperatures observed in strain 30Eβox$^-$. The third characteristic temperature falls between the previously described upper and lower ones.

2. In cells supplemented during growth with elaidate, the third characteristic temperature was identical within experimental limits for both β-glucoside and β-galactoside transport, indicating that it is likely to arise from some interaction in the bulk lipid phase. This conclusion is supported by the fact that the boundary of a change in physical state is also observed at this temperature by electron spin resonance.

3. In cells supplemented during growth with oleate, two or three characteristic temperatures were observed depending upon the transport system studied. Although glucoside and galactoside transport had the same lower characteristic temperature, these systems had no common upper characteristic temperature.

4. In cells supplemented during growth with the lipid density label, bromostearic acid, three characteristic temperatures were observed for β-glucoside transport in both strains 30E and 30Eβox$^-$.

INTRODUCTION

Arrhenius plots for transport rate are characterized by discontinuities in slope or have slope intercepts at approximately those temperatures where alterations in the physical state of membrane lipids are detected (1–5). A one component phospholipid bilayer system undergoes a relatively sharp thermal transition from a liquid to a para-crystalline or solid state at a characteristic temperature that reflects the degree of saturation of the fatty acids. In two component phospholipid bilayer model systems, two charactertistic temperatures have been observed. These mark the beginning and end of transitions from a totally liquid state to a liquid–solid equilibrium and a liquid–solid equilibrium to a totally solid state (1, 6). These characteristic temperatures have been denoted t_h and t_l, respectively, and the course of the liquid–solid equilibrium over the temperature range limited by t_h and t_l is governed by a phase separation process that requires lateral mobility of the bilayer lipids (6). Upper and lower characteristic temperatures have also been observed in membranes of biological origin and in lipids derived from these membranes, not only where the lipids approximate a two component system, but also where the lipid composition is more complex (7).

The use of unsaturated fatty acid auxotrophs has enabled investigators to manipulate the fatty acid composition of membrane phospholipids in *Escherichia coli* and other organisms, facilitating study of the effects of membrane lipid physical state on membrane function (8–18). In a previous study we used an *E. coli* auxotroph defective in the β-oxidation of the unsaturated fatty acid supplement supplied for growth. With this bacterial strain we observed at least two slope intercepts and/or discontinuities in Arrhenius plots for transport at approximately the characteristic temperatures (t_h and t_l) detected by electron paramagnetic resonance using the spin labels, TEMPO (1), or 5N10 and 6N11 (7).

Prior to isolation of the β-oxidation defective unsaturated fatty acid auxotroph (strain 30Eβox$^-$), the parent strain (30E) had been used extensively in characterizing relationships between membrane fatty acid composition and the temperature dependence of membrane function (13–18). In these earlier studies we did not examine transport rate over a sufficiently broad temperature range to determine if events occurring at the higher characteristic temperature affect membrane functions such as transport. In the present paper, we report a reinvestigation of the temperature dependence of transport rate in strain 30E and correlate these data with those obtained with the β-oxidation defective progeny strain 30Eβox$^-$.

METHODS

Growth and Media

Strain 30E, an unsaturated fatty acid auxotroph of *E. coli* K12, was used in the studies reported here. Its properties have been described in detail (16, 19). Cells were grown in medium A (20) supplemented with 1% Difco casamino acids, 5 μg/ml of thiamine·HCl, 0.5% of the nonionic detergent Triton X–100 (Rohm and Haas), and 0.02% of the essential fatty acid. Elaidic (*trans*-9-octadecenoic) and oleic (*cis*-9-octadecenoic) acids were purchased from the Hormel Institute, Austin, Minn. Bromostearate (9, 10-monobromostearic acid) was prepared by a published procedure (16). Cultures of 500 ml were grown with vigorous rotary agitation at 37°C in 2 liter

flasks. Cultures supplemented with bromostearate were grown at 40°C. Cells were grown with the indicated essential fatty acid for at least 4 doublings of cell mass, except for cells supplemented with bromostearate, which were grown for only 3 doublings of cell mass. Growth was followed turbidimetrically.

Induction and Assay of Transport

For induction of β-glucoside transport, salicin (Aldrich Chemical Co.) was included in the medium at a concentration of 0.1% for at least 4 generations. For induction of β-galactoside transport, isopropyl-l-thio-β-galactopyranoside was included in the medium at 0.2 mM during the final hour of growth. The transport assay procedures are described elsewhere (14).

Fatty Acid Analysis

Strains 30E and 30Eβox$^-$ (1) were grown in 50 ml cultures supplemented with bromostearate. The cells were harvested in log phase and washed with medium A containing 0.5% Triton X–100 to remove adsorbed fatty acids, and then twice with medium A to remove remaining Triton. The washed cell pellets were incubated with 2.2 ml of methanol at 65°C for 10 min, after which time 4.4 ml of chloroform was added and incubation continued at room temperature for 2 hr. Cell debris was removed by filtering the chloroform–methanol extract through a plug of glass wool, and phospholipids were isolated by chromatography on silicic acid (Bio-Sil HA, Bio-Rad Laboratories, Richmond, Ca.). Fatty acid methyl esters were obtained by transesterification of the phospholipids with BF_3-methanol (Applied Science Laboratories), and the methyl esters were separated by gas–liquid chromatography on a 6 ft by 1/8 in cholumn of 3% SE 30 on 100/120 Gas Chrom Q.

RESULTS

Arrhenius plots for β-glucoside and β-galactoside transport in cells of strain 30E grown with elaidate or oleate supplements are described in Figs. 1 and 2. The plots obtained with elaidate-grown cells show three departures from linearity for both glucoside and galactoside transport. Two of these are clear discontinuities at approximately 39° and 35°C, and at both we observed increased transport rate with a decrease in assay temperature. The third departure from linear slope is at approximately 30°C, and at this apparent discontinuity there is no increase in rate with decreasing temperature. W. Kleemann of Stanford University has performed spin label analysis with an *E. coli* cytoplasmic membrane fraction derived from elaidate-grown cells of strain 30E using the spin label probe TEMPO as described previously (1,6). These data (not shown) also indicate three departures from linearity in Arrhenius plots of the TEMPO spectral parameter vs. 1/°K, and the characteristic temperatures obtained by esr correlate with those shown in Figs. 1A and 2A. It is therefore likely that the 3 characteristic temperatures observed in elaidate-grown cells of strain 30E correspond to a like number of phase boundaries (i.e., discrete beginning or end points of phase separation processes).

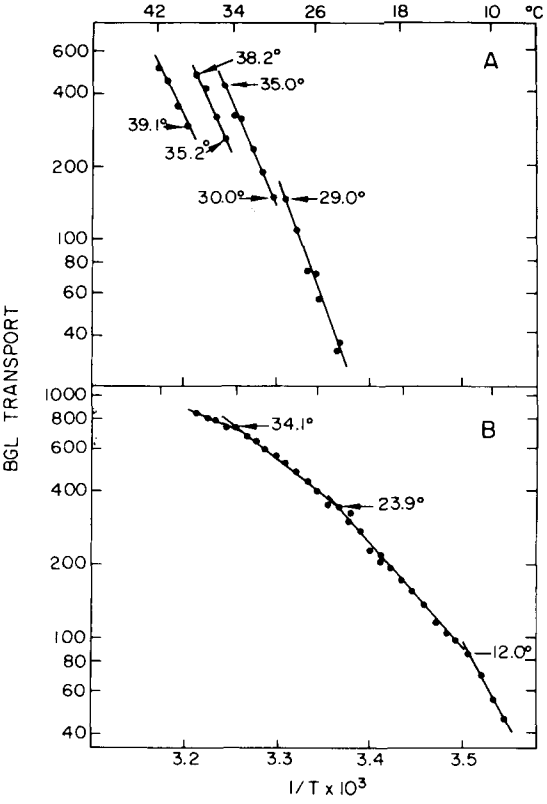

Fig. 1. Arrhenius plots for β-glucoside transport by cells of strain 30E grown at 37°C in medium supplemented with elaidate (A) or oleate (B). Units are nmoles *p*-nitrophenyl-β-glucoside transported in 20 min by 2 × 10^9 cells.

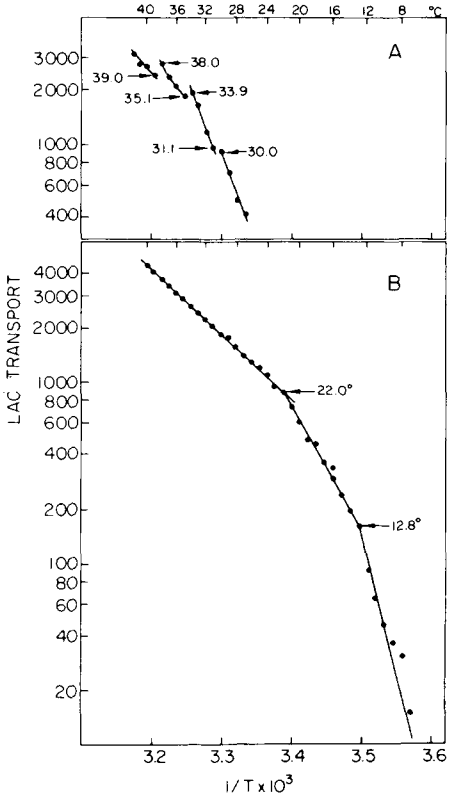

Fig. 2. Arrhenius plots for β-galactoside transport by cells of strain 30E grown at 37°C in medium supplemented with elaidate (A) or oleate (B). Units are nmoles of *o*-nitrophenyl-β-galactoside transported in 20 min by 10^9 cells.

Arrhenius plots of β-glucoside or β-galactoside transport in oleate-grown cells of strain 30E are biphasic (Fig. 2B) or triphasic (Fig. 2A). There is excellent agreement for the lowest slope intercept (12–13°) and for the one above it (22–24°). A third slope intercept was detected for β-glucoside transport, but not for β-galactoside transport.

The characteristic temperatures for transport by cells of strain 30E and the progeny strain 30Eβox$^-$ grown with oleate or elaidate are compared in Table I. For cells grown with oleate or elaidate there is excellent agreement for the lower characteristic temperatures (t^*_l). Values for t^*_h correlate well for elaidate-grown but not for oleate-grown cells. Characteristic temperatures for glucoside transport by cells of both strains grown with bromostearate have also been compared (Table I and Fig. 3). As in the case of elaidate-grown cells of strain 30E, three characteristic temperatures are observed. At the highest of these there is an increase in rate with decreasing temperature. The lowest characteristic temperature is indicated by an upward rather than a downward change in slope.

TABLE I. Summary of Characteristic Temperatures for Transport in Two Related Unsaturated Fatty Acid Auxotrophs of *E. Coli*

Fatty acid supplement for growth	30Eβox⁻ (progeny)				(30E (parent)			
	β-glucoside		β-galactoside		β-glucoside		β-galactoside	
	t^*_h	t^*_l	t^*_h	t^*_l	t^*_h	t^*_l	t^*_h	t^*_l
Oleate	26.0[a] & 21.8	14.4[a]	–	–	34.1 & 23.9	12.0	22.0	12.8
Elaidate	38.6[a]– 38.8	32.1[a]	38.0[a]– 38.9	32.3[a]	38.2– 39.1 & 35.0– 35.2	29.0– 30.0	38.0– 39.0 & 33.9– 35.1	30.0– 31.1
Bromostearate	37.9– 38.9 & 34.0	22.0[b]	–	–	37.1– 38.0 & 33.0	21.0[b]	–	–

[a]From Linden et al. (1).

[b]The observed t^*_l values lie between 22° and 23° in strain 30Eβox⁻ and 21° and 22° in strain 30E (Fig. 3). The upper and lower characteristic temperatures detected in Arrhenius plots of transport vs. 1/T are denoted t^*_h and t^*_l, respectively. The temperature ranges are shown where discontinuities are observed in the Arrhenius plots. Actual slope intercepts are denoted by a single temperature.

Excellent correlation was observed between all the characteristic temperatures for transport in strains 30E and 30Eβox$^-$ grown with bromostearate in spite of the fact that the fatty acid composition of the cellular phospholipids differed considerably (Table II). The lowest of the characteristic temperatures detected by transport correlates with the characteristic temperature detected by electron spin resonance with hydrocarbon probes (7). Figure 4 describes the results obtained with the spin label 5N10 and inner membranes from strain 30Eβox$^-$ grown with bromostearate. The experiment described was conducted exactly as were the experiments described previously by Linden et al. (7). This procedure usually detects only the lower characteristic temperature detected by TEMPO binding (7), i.e., the temperature at which all the membrane lipids appear to be in a solid state. Figure 4B describes an additional experiment in which the membranes were first incubated with spin label at high temperature (44°C) prior to the first esr scan. The slope intercept detected upon lowering of the temperature is identical to that obtained by methods that detect t_l. When the temperature program is reversed, however, there is an apparent hysteresis. We have no explanation for this phenomenon.

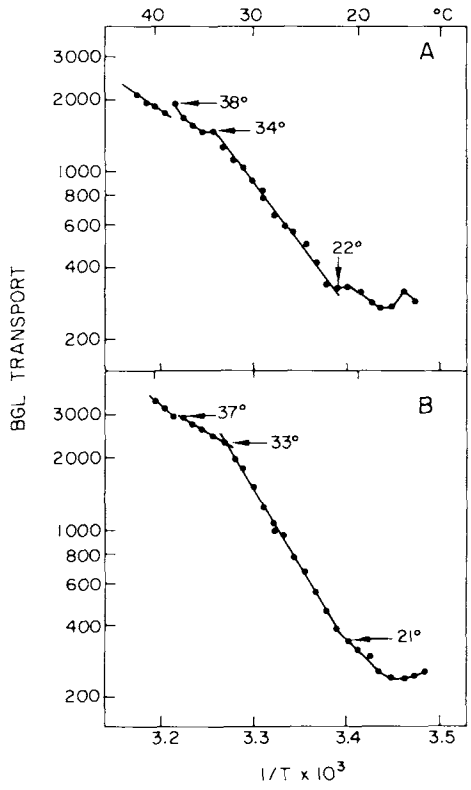

Fig. 3. Arrhenius plots for β-glucoside transport by cells of strain 30Eβox$^-$ (A) and 30E (B) grown at 39°C in medium supplemented with bromostearate. Units are nmoles of *p*-nitrophenyl-β-glucoside transported per hr by 2 X 10^9 cells.

TABLE II. Fatty Acid Composition of Phospholipids Derived from Cells Grown in Medium Supplemented with Bromostearic Acid

Fatty acid	30E	30Eβox⁻
Myristate (14:0)	13.8	20.2
Palmitate (16:0)	40.4	37.9
Bromomyristate (14:Br)	4.0	–
Oleate (18:1)	2.0	5.2
Bromopalmitate (16:Br)	25.9	–
Bromostearate (18:Br)	12.4	35.3
Other	1.5	1.5
%Brominated fatty acids	42.3	35.3

Fatty acid methyl esters were obtained and analyzed as described in Methods. Values are expressed as percent of the total fatty acid content.

DISCUSSION

In our initially published reports that correlated lipid structure and the kinetic behavior of membrane transport systems, we described only a single "transition temperature" (13, 14, 16). For cells grown with any one of a variety of essential fatty acids, we observed the same transition temperature for two transport systems known to have no common protein component. We therefore concluded that the physiological transition in transport rate arose from a change in physical state in the bulk lipid phase. A similar conclusion was drawn by Overath and his colleagues who correlated transitions in transport rate and other physiological phenomena with the temperature-dependent behavior of extracted cellular phospholipids at the air–water interface (2). It was not until recently, however, that a clear physical rationale for the physiological transition could be made (1).

It has long been known that lipid transitions in membranes have a broad temperature range. It is obvious from the studies of Chapman, Steim, Engelman, and their collaborators that the order ⇋ disorder transition in membrane lipids occurs over a 10–20°C range of temperature (21–23). This is in contrast to the behavior of a pure lipid species, e.g., dipalmitoylphosphatidylethanolamine, for which only a sharp, major transition (melting temperature) is observed. Since only a single physiological transition was observed in the early structure–function studies on transport, it was not entirely clear if this single transition correlated with (a) the point where all the membrane lipids become frozen (t_l), (b) the point where all lipids become liquid (t_h), or (c) some point in between. This situation was clarified in collaborative studies between Harden McConnell's laboratory and ours (1). A careful analysis of the effects of temperature on the phase behavior of lipids, either in the membrane or in the extracted state, and of the effects of temperature on transport kinetics revealed that two physiological transitions can be observed in transport, and that these correlate with t_h and t_l.

In this collaborative study with Wright and McConnell we employed the auxotrophic strain 30Eβox$^-$, which is incapable of fatty acid β-oxidation. The results obtained with this strain caused us to engage in the study reported here. We had previously used strain 30E (the parent strain of 30Eβox$^-$) in a number of studies on membrane structure–function relationships and assembly (13–18).

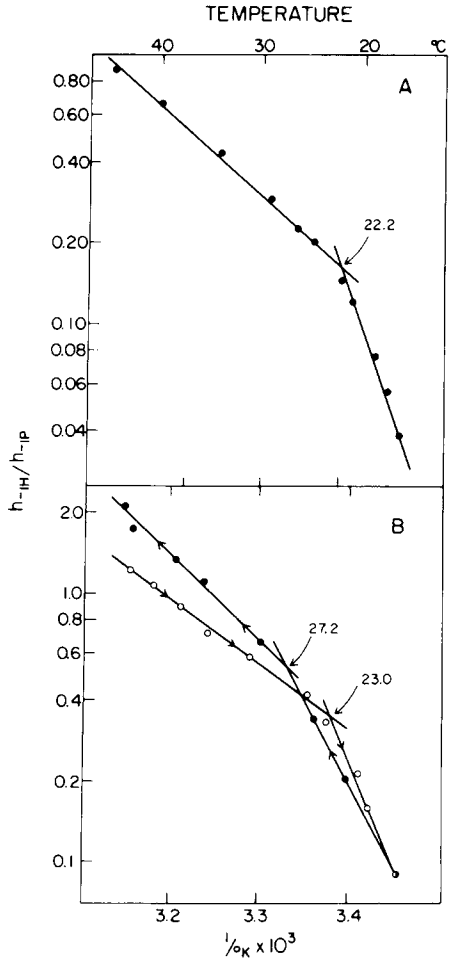

Fig. 4. Response of a spin labeled hydrocarbon (5N10) (7) in bromostearate membranes to temperature. The ratios of the two high field lines of the first derivative spectra are plotted as a function of the reciprocal of absolute temperature. For details of spin labeling methodology, see the companion paper by Linden, Keith, and Fox (7). (A) Spectra were determined on a 10 μl sample containing 5×10^{-5} M spin label and bromostearate inner membranes suspended at a concentration of 7.5 mg of protein per ml. Inner membranes were prepared from strain 30Eβox$^-$ by a previously described procedure (1, 16). The membranes were first incubated with spin label for 90 min at ice bath temperature. Spectra were then taken at various temperatures in ascending order. Samples were held for 10 min after each increase in temperature before initiating the esr scan. (B) Conditions were as described in (A) except that the concentration of the spin label was 2.5×10^{-5} M, and the membranes were initially incubated with spin label for 10 min at the highest temperature (44°C) prior to taking the first spectrum. Spectra were then taken in descending order until the lowest temperature was reached after which point the spectra were taken in ascending order.

It was therefore imperative to determine the origin of the physiological transitions observed with strain 30E. It is clear from the data reported here, most of which are summarized in Table I, that all the physiological transitions reported earlier with strain 30E for cells grown with elaidate, oleate, or bromostearate supplements correspond to t_l, the critical temperature where the membrane lipids pass from a totally solid state to a liquid–solid equilibrium.

Our studies reported here amplify one important feature observed earlier and reveal an entirely new one. In elaidate-grown cells of strain 30E, we observed increased transport rate with decreased transport assay temperature at t_h, indicating that this phenomenon is not unique for elaidate-grown cells of strain 30Eβox$^-$ (Figs. 1A and 2A). Furthermore, this same type of behavior is observed at the apparent t_h (38–39°C) in bromostearate-grown cells of both strains (Fig. 3). In addition, we have observed a third characteristic temperature in elaidate-grown cells of strain 30E and in bromostearate-grown cells of 30E and 30Eβox$^-$. Though the origin of this transition intermediate between t_l and t_h is not clear, it seems to be a property of the bulk lipid phase in elaidate-grown cells since its effects are observed in the properties of both transport systems and also in spin labeling experiments using isolated membranes and the nitroxide probe, TEMPO. This third characteristic temperature might arise from some type of nonequilibrium phase behavior, e.g., the existence of two imiscible liquid phases, each having its own onset of freezing (t_h).

The precise nature of the effect of lipid physical state on transport is still not known. We previously concluded that the striking increase in rate of transport sometimes observed at t_h is an indication that some step during transport is enhanced by an increase in the lateral compressibility of the membrane lipids (1). This, however, is compatible with a number of plausible models for the mechanism of transport, e.g., it cannot distinguish between carrier- and channel former-mediated transport. The nature of the influence of lipid structure on transport at t_l is equally obscure. There are two radical changes in the organization and dynamics of the *E. coli* cytoplasmic membrane that occur at t_l. First, the bulk lipids freeze and lateral diffusion ceases (1, 6). Second, the integral membrane proteins detected by freeze-fracture electron microscopy on the inner face of the cytoplasmic membrane separate laterally so that large patches are formed that are denuded of particles, and the particles (presumably the integral membrane proteins) become concentrated in dense patches (Kleeman, W. and McConnell, H. M., to be published). As the membrane lipids freeze, the proteins that mediate transport might separate laterally, remaining preferentially in the liquid lipid milieu. Thus transport might be influenced at t_l by any or all of the following: (a) lateral compressibi reaching a minimum below t_l, (b) a cessation of lateral diffusion of membrane lipids and proteins, (c) a severe restriction in the rotational diffusion of proteins, and (d) maximal protein–protein interaction (patching). The third alternative is the easiest to test. This is in our own minds one of the more interesting uncertainties, since restriction of protein rotational motion by solidification of the bulk membrane lipid might be expected to have a far more pronounced effect upon carrier-mediated than channel former-mediated transport. The effects of

lipid physical state on the rotational mobility of membrane proteins could probably be examined in the retinal outer rod system using existing technology (24).

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