THE EFFECT OF MEMBRANE LIPID UNSATURATION ON GLYCOSIDE TRANSPORT

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The effect of temperature upon the rates of β-galactoside and β-glucoside transport has been studied in an unsaturated fatty acid auxotroph of *E. coli* K12 grown on oleic acid or linoleic acid supplemented media. Arrhenius plots of the data for both transport systems are biphasic with changes in slope extrapolating to approximately 13° and 7°C for cells grown with oleate and linoleate supplementation respectively. The slopes of the curves are, however, similar for both fatty acids above and below the transition points. These data suggest that the membrane may exist in two physical states, one above and the other below a transition point determined by the composition of the lipid phase.

The availability of unsaturated fatty acid auxotrophs of *E. coli* K12 (1) permits a direct study of the contribution of lipid composition to transport processes. The unsaturated fatty acids incorporated into lipid by these mutants are derived solely from fatty acid supplements provided in the culture medium. In this report we describe the effects of degree of fatty acid unsaturation and temperature upon the rate of transport mediated by the independent β-galactoside and β-glucoside transport systems.

METHODS. Strain 30⁻ is an unsaturated fatty acid auxotroph derived by nitrosoguanidine mutagenesis and penicillin selection from a β-glucoside fermenting derivative of *E. coli* K12 strain M0 which is F⁻ and *str*. Genetic studies indicate that 30⁻ is not defective in β-hydroxydecanoyl thioester dehydrase (2). Strain 30⁻ was grown at 37° on medium A (3) containing 1% Difco casamino acids as a carbon source and 0.25% Tween 40; fatty acid supplements (Hormel Institute, Austin, Minn.) were added at a concentration of 0.02%. The β-galactoside transport system was induced

with 0.5 mM IPTG[1] for a 30 minute period, and the β-glucoside transport system, with 0.1 mM TPG for 3-5 generations of growth. Cultures were harvested at a cell density of 1 x 10^9 per ml and washed once with the above medium supplemented with 40 μg per ml of chloramphenicol, but lacking inducer and casamino acids. The cells were suspended in wash medium for assay of transport.

Transport was assayed by previously described techniques which measure the release of nitrophenol from ONP-galactoside and PNP-glucoside, except that the fatty acid used for growth was included in the assay mixture at 50 μg per ml and reactions were terminated with Na_2CO_3 instead of K_2CO_3 (4,5). For the β-galactoside transport system, transport of ONP-galactoside is rate limiting for intracellular hydrolysis by β-galactosidase, which is present in considerable excess (6). In the case of the β-glucoside system (4), hydrolysis of PNP-glucoside occurs subsequent to phosphorylation by the phosphotransferase described by Kundig, *et al.* (7). The phosphorylated glucoside is cleaved by specific phosphoglucosidases (figure 1). Phosphoglucosidase activity measured in extracts with PNP-glucoside-6-phosphate as substrate is 5- to 10-fold greater than the rate of hydrolysis of PNP-glucoside by intact cells indicating that formation of the glycoside phosphate is rate limiting for its hydrolysis. The ratio of the specific activities of PNP-glucoside hydrolysis by intact cells to phosphorylation by the β-glucoside specific enzyme II, in an *in vitro* system where enzyme II is rate limiting, remains constant throughout the course of induction. This suggests that the activity of enzyme II, rather than that of the P-HPr generating system, is rate limiting for glucoside hydrolysis by intact cells since the P-HPr generating system is present at the same level throughout the course of induction.

RESULTS. The effect of temperature on the rates of β-glucoside and β-galactoside transport are given in figures 2 and 3. These data yield

[1]Abbreviations used are: IPTG, isopropyl-1-thio-β-galactoside; TMG, methyl-1-thio-β-galactoside; ONP-galactoside, *o*-nitrophenyl-β-galactoside; TPG, phenyl-1-thio-β-glucoside; PNP-glucoside, *p*-nitrophenyl-β-glucoside.

phosphoenolpyruvate + HPr $\xrightarrow[Mg^{++}]{\text{Enzyme I}}$ P-HPr + pyruvate

P-HPr + PNP-glucoside $\xrightarrow[Mg^{++}]{\text{Enzyme II}}$ HPr + PNP-glucoside-6-P

PNP-glucoside-6-P $\xrightarrow{\text{Phosphoglucosidase}}$ glucose-6-P + *p*-nitrophenol

Figure 1. Scheme for generation of *p*-nitrophenol from PNP-glucoside by *E. coli* K12. Here, phosphate is transferred from phosphoenolpyruvate to HPr, a protein cofactor, in a reaction catalyzed by enzyme I. The phosphate is then transferred from P-HPr to the 6-OH position of the aryl-β-glucoside which is subsequently cleaved by a phospho-β-glucosidase.

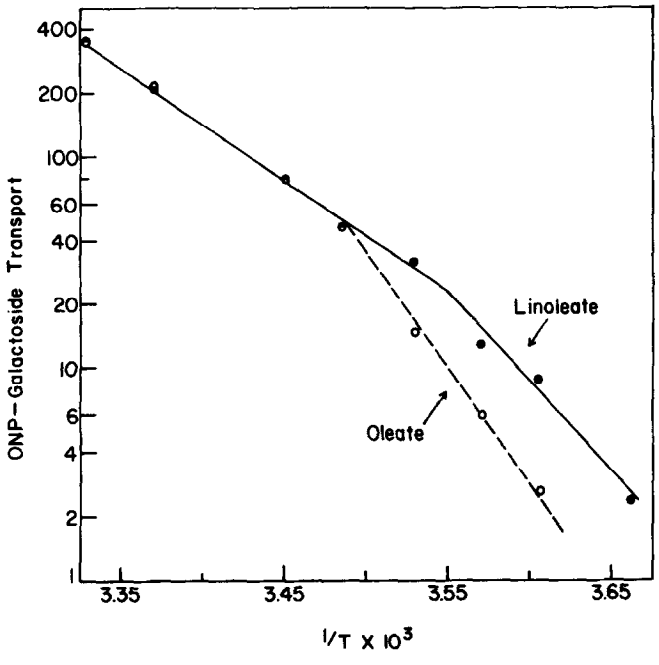

Figure 2. Temperature dependence of ONP-galactoside transport. Details are described in the text. Units are nmoles of *o*-nitrophenol released from ONP-galactoside per hour by 2 x 10^9 cells.

Arrhenius plots with transition points in slope at 13-14° for cells grown on oleate supplemented medium and 7-8° for cells grown on linoleate supplemented medium. No significant differences in the rate of growth (generation time of 86 minutes) or extent of induction were observed for

cultures grown with these fatty acid supplements. Two aspects of these curves are striking. First, the slopes of the plots above and below the transition points are similar for each transport system. Second, the transition points for both transport systems are identical within the range of experimental error.

A number of lines of evidence indicate that the phosphorylation by an enzyme II in intact cells is coupled to penetration of the membrane barrier (8-10). The enzyme II dependent phosphorylation of TPG was studied as a function of temperature utilizing membrane fragments prepared by sonic oscillation from cells grown with oleate or linoleate supplementation (table 1).

TABLE 1

Fatty Acid Present During Growth	Enzyme II Activity (Sonic Extract) 28°	20°	15°	10°	5°
$\Delta^{9,12}$	129	69	33	14	7
Δ^{9}	117	57	31	15	6
ratio $\Delta^{9,12}/\Delta^{9}$	1.1	1.2	1.1	0.9	1.1

Table 1: Activity of the β-glucoside specific enzyme II in sonic membrane fragments prepared from the cells used for the study shown in figure 3. Assays were performed in a previously described system in which enzyme II is rate limiting for TPG phosphorylation (4). The results are expressed in arbitrary units.

In the *in vitro* system, the rate of phosphorylation as a function of temperature is independent of membrane lipid composition. The other activities of the phosphotransferase system in extracts were studied as a function of temperature (data not shown) but yielded no discontinuities over the temperature range from 5° to 30°. A major difference between the phosphotransferase reaction systems in the intact cell (figure 3) and in disrupted

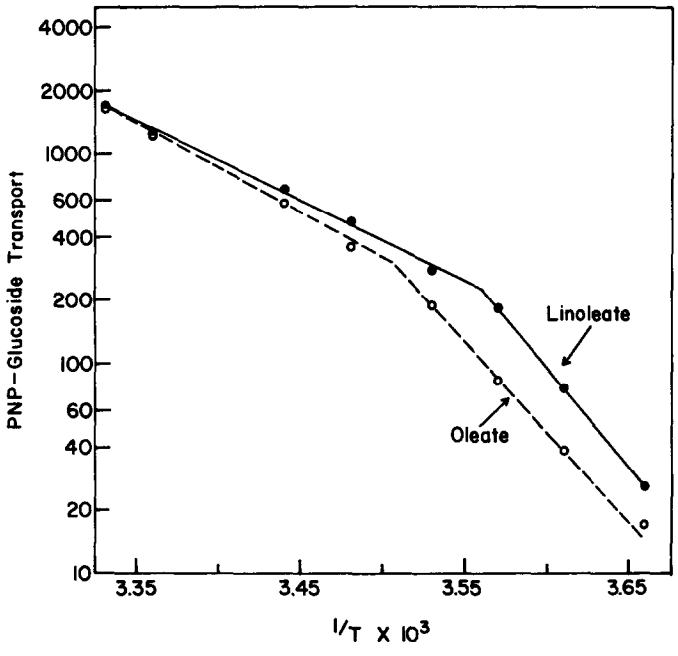

Figure 3. Temperature dependence of PNP-glucoside transport. Details are described in the text. Units are nmoles of *p*-nitrophenol released from PNP-glucoside per hour by 2 x 10^9 cells.

cell preparation (table 1) is the lack of a membrane barrier in the latter. The fact that lipid composition affects the phosphotransferase system in the intact cell but not in extracts suggests that penetration of the membrane by the substrate for phosphorylation occurs only in intact cells, and that the process affected by lipid composition is membrane penetration.

DISCUSSION. The transition points in the Arrhenius plots for β-glucoside and β-galactoside transport shown in figures 2 and 3 are compatible with the hypothesis that "carrier" proteins of transport systems are part of an ordered lipoprotein structure which undergoes a phase transition at a temperature which is a function of the fatty acid composition of the lipid components. This hypothesis is supported by the striking similarities in the transition temperatures in Arrhenius plots for two transport systems which are genetically and mechanistically

independent. Schairer and Overath have recently studied methyl-1-thio-β-galactoside (TMG) accumulation mediated by the β-galactoside transport system over the temperature range between 15° and 40° in an unsaturated fatty acid auxotroph grown in media supplemented with a variety of fatty acids including trans-Δ^9-octadecenoic (elaidic), oleic and linoleic acids (11). The Arrhenius constants calculated from their data in the 15-25° decade for oleate and linoleate grown cells are approximately one-third of those determined by our method of following β-galactoside transport. These differences probably arise from the fact that ONP-galactoside hydrolysis by intact cells measures a unidirectional flux, whereas TMG accumulation measures a balance between influx and efflux, the latter process having a much higher temperature coefficient than the former (12). These authors observed a pronounced difference in the slopes of the Arrhenius plots only in the case of elaidic acid. They concluded that elaidic acid grown cells were unique from the standpoint of exhibiting a higher temperature coefficient for transport, but noted an apparent transition point in the plot for elaidic acid grown cells at approximately 25°. Our study indicates that elaidic acid is not unique, but merely gives rise to a higher transition temperature. The fact that transition points are observed in decreasing order from elaidic to oleic to linoleic acids is compatible with the conclusion that the membrane exists in two physical states, one above and the other below a transition temperature determined by the liquidity of the lipid components.

Fox has published data compatible with the idea that transport proteins synthesized *de novo* associate preferentially with lipids synthesized simultaneously with the formation of the transport protein (13). The unique transition temperatures noted for transport in unsaturated fatty acid auxotrophs grown in media supplemented with different fatty acids provides an independent means to test this hypothesis.

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