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EFFECT OF CHANGES IN FATTY ACID COMPOSITION OF PHOSPHOLIPID SPECIES ON THE β-GALACTOSIDE TRANSPORT SYSTEM OF *ESCHERICHIA COLI* K-12

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SUMMARY

On lowering the growth temperature of *Escherichia coli* K-12 from 37 to 17 °C, the cells resumed growth after a lag period of 40 min. During the lag period, the transition points in Arrhenius plots of the preinduced β-galactoside transport system were not changed while the saturated/unsaturated fatty acids ratio decreased gradually in phosphatidylethanolamine, rapidly in phosphatidylglycerol and little in cardiolipin.

INTRODUCTION

It has been demonstrated that the permeability of model membranes increases by the introduction of double bonds into the paraffin chains of the membrane phospholipids[1]. Induction of the β-galactoside transport system of *Escherichia coli* requires phospholipids[2] containing unsaturated fatty acids[3] for fully functional structure. The temperature profile of β-galactoside transport characterized for instance by the transition temperatures obtained from Arrhenius plots is changed by the degree of unsaturation in the membrane phospholipids in the unsaturated fatty acid auxotroph[4–7].

It was thought to be of interest to see if the function of the transport system requires either the bulk phospholipid environment or the localized specific phospholipid species in the wild-type cells. In a preceding paper[8], *cis*-vaccenic acid content was demonstrated to increase in phospholipids after a downward shift of the growth temperature of *E. coli* B. With this background, the present paper deals with the relationship between the degree of unsaturation of the individual phospholipids and the transition points in Arrhenius plots of β-galactoside transport activity of *E. coli* K-12.

EXPERIMENTAL

Bacterial growth

E. coli K-12 was grown at 37 °C in a modified M9 medium[9] supplemented with 1% casamino acids as carbon source. Growth was followed turbidimetrically at 660 nm.

Assay of preinduced β-galactoside transport activity after lowering the growth temperature

Isopropyl-β-D-thiogalactopyranoside was added to the culture medium to a final concentration of 0.5 mM during the early exponential growth phase. During the middle exponential phase (A_{660} 1.0), cells were collected by centrifugation at $9000 \times g$ for 5 min at 25–30 °C, washed, resuspended in the same volume of fresh medium and grown for an additional 15 min (A_{660} 1.25) at 37 °C. The flasks were rapidly cooled to 17 °C, and incubation was continued. A part of the cells, removed at 0, 15, 30 and 45 min, was used for the assay of transport, the remainder was immediately lyophilized for phospholipid analyses after being washed with cold 0.05 M KCl. For the assay of transport, cell samples were chilled immediately upon removal from the culture, and chloramphenicol was added to a final concentration of 50 μg/ml. Within 3 min the cells were collected on a Millipore filter HA, washed with cold modified M9 medium supplemented with 0.2% glucose and chloramphenicol (50 μg/ml), and resuspended in the same solution. *In vivo* *o*-nitrophenyl-β-D-galactopyranoside hydrolysis (transport activity) was measured by incubating 0.5 ml of a cell suspension (0.5 mg dry wt of cells) with 2 mM *o*-nitrophenyl-β-D-galactopyranoside for 1, 3, 5 and 10 min at various temperature. The reaction was terminated by the addition of 1 ml of cold 1 M Na_2CO_3. After centrifugation, the color was determined at 420 nm. Control experiments were carried out with 10 mM formaldehyde, an inhibitor of the permease[10,11].

Preparation and analysis of phospholipids

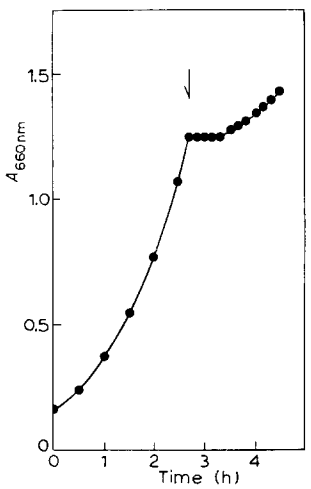
Phospholipids were extracted[12] from the lyophilized cells prepared as mentioned above and separated by two-dimensional thin-layer chromatography as described previously[13].

Analysis of fatty acids

The fatty acids in the phospholipids were determined as their methyl esters by gas–liquid chromatography as described previously[13].

RESULTS

On lowering the growth temperature from 37 to 17 °C during the exponential growth phase, the cells resumed growth after a lag period of 40 min (Fig. 1). During the lag period, the changes in fatty acid composition of the total phospholipids, phosphatidylethanolamine, phosphatidylglycerol and cardiolipin, after the shift in temperature are shown in Tables I, II, III and IV. *cis*-Vaccenic acid was the only fatty acid which increased in all phospholipid species with a corresponding decrease in palmitic acid during the lag period, while palmitoleic acid comprised a large portion of the unsaturated fatty acids, unlike the distribution in *E. coli* B[13]. A dramatic decrease in the saturated/unsaturated fatty acids ratio was found in phosphatidylglycerol 15 min after lowering the growth temperature, while the ratio decreased linearly in phosphatidylethanolamine for 45 min, and virtually no change was observed in cardiolipin (Fig. 2). The change in the saturated/unsaturated fatty acids ratio of the total phospholipids resembled that of phosphatidylethanolamine as expected.



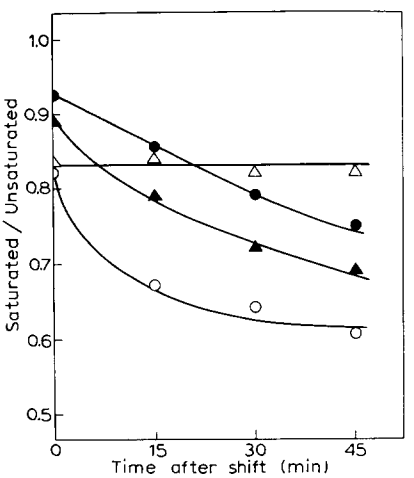


Fig. 1. Growth curve of *E. coli* K-12 after lowering the growth temperature from 37 to 17 °C. When a culture had progressed to middle exponential growth phase at 37 °C, the temperature was decreased to 17 °C at the point indicated by the vertical arrow.

Fig. 2. Changes in the saturated/unsaturated fatty acids ratio in the phospholipids during the lag period after lowering the growth temperature. Values in Tables I, II, III and IV were used for calculation. Saturated/unsaturated fatty acids ratio of total phospholipids (▲), phosphatidylethanolamine (●), phosphatidylglycerol (○) and cardiolipin (△).

TABLE I

CHANGES IN FATTY ACID COMPOSITION IN TOTAL PHOSPHOLIPIDS DURING THE LAG PERIOD AFTER LOWERING THE GROWTH TEMPERATURE

Values are expressed as weight percentage of total fatty acids in the total phospholipid preparation before separation by thin-layer chromatography. Each value may vary ±1% between two separate runs of the same sample on the gas–liquid chromatograph. Trace amounts of stearic acid and lactobacillic acid were detected.

	Period of growth at 17 °C			
Fatty acid	*0 min*	*15 min*	*30 min*	*45 min*
Myristic acid	5	4	4	4
Palmitic acid	40	38	36	35
Palmitoleic acid	32	33	33	33
Methylene-hexadecanoic acid	3	3	3	2
cis-Vaccenic acid	18	20	22	24
Unidentified*	2	2	2	2
Saturated	47	44	42	41
Unsaturated**	53	56	58	59

* The retention time of unidentified fatty acids relative to that of palmitic acid was 2.50.
** Methylenehexadecanoic acid was regarded as an unsaturated fatty acid.

TABLE II

CHANGES IN FATTY ACID COMPOSITION IN PHOSPHATIDYLETHANOLAMINE DURING THE LAG PERIOD AFTER LOWERING THE GROWTH TEMPERATURE

Conditions used are defined in Table I.

	Period of growth at 17 °C			
Fatty acid	*0 min*	*15 min*	*30 min*	*45 min*
Myristic acid	5	5	5	5
Palmitic acid	41	39	38	37
Palmitoleic acid	31	32	32	32
Methylene-hexadecanoic acid	4	3	3	4
cis-Vaccenic acid	17	19	21	21
Unidentified	2	2	1	1
Saturated	48	46	44	43
Unsaturated	52	54	56	57

TABLE III

CHANGES IN FATTY ACID COMPOSITION IN PHOSPHATIDYLGLYCEROL DURING THE LAG PERIOD AFTER LOWERING THE GROWTH TEMPERATURE

Conditions used are defined in Table I.

	Period of growth at 17 °C			
Fatty acid	*0 min*	*15 min*	*30 min*	*45 min*
Myristic acid	3	2	2	2
Palmitic acid	41	37	36	35
Palmitoleic acid	28	28	28	27
Methylene-hexadecanoic acid	1	1	1	1
cis-Vaccenic acid	26	31	32	34
Unidentified	1	1	1	1
Saturated	45	40	39	38
Unsaturated	55	60	61	62

Transition points in Arrhenius plots of transport activity have been found to be dependent upon the fatty acid composition of the membrane phospholipids[5-7]. On this basis the transition points of the transport system which had been preinduced at 37 °C were determined at short intervals during the lag period after lowering the growth temperature. If the changes in unsaturation of the total phospholipids from zero time to 45 min reflect the transition point–fatty acid diagram by Overath *et al.*[7], two straight lines at 45 min should intersect below the point measured at 20 °C. However, the points were 21.3 ± 0.5, 21.1 ± 0.4, 21.4 ± 0.4 and 21.5 ± 0.5 °C at 0, 15, 30 and 45 min, respectively (Fig. 3).

Thus, no change was observed in transition points while the degree of unsaturation of phosphatidylethanolamine and phosphatidylglycerol increased.

TABLE IV

CHANGES IN FATTY ACID COMPOSITION IN CARDIOLIPIN DURING THE LAG PERIOD AFTER LOWERING THE GROWTH TEMPERATURE

Conditions used are defined in Table I.

	Period of growth at 17 °C			
Fatty acid	*0 min*	*15 min*	*30 min*	*45 min*
Myristic acid	3	3	3	3
Palmitic acid	40	41	40	41
Palmitoleic acid	30	28	28	28
Methylene-hexadecanoid acid	2	2	2	2
cis-Vaccenic acid	23	24	25	25
Unidentified	2	2	2	1
Saturated	45	46	45	45
Unsaturated	55	54	55	55

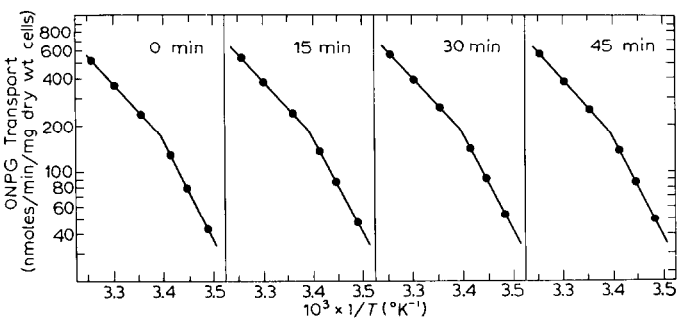


Fig. 3. Changes in transition points in Arrhenius plots of the preinduced transport system during the lag period after lowering the growth temperature. Transport activity was assayed at 14, 17, 20, 25, 30 and 34 °C under the conditions described in Experimental. The period of growth at 17 °C is shown at the top of the figure. ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

DISCUSSION

The relationship between the fatty acid composition of the environmental bulk phospholipids and the transition point of the transport system has been investigated by the use of cells of the unsaturated fatty acid auxotroph growing on media supplemented with oleate[6,7], linoleate[6] and palmitelaidate[7] which are not commonly found in *E. coli*. However, the chemical nature of the phospholipids has been described to determine the properties of the permeability barrier[14], and the differences in the content of unsaturated fatty acids, especially *cis*-vaccenic acid, among phosphatidylethanolamine, phosphatidylglycerol and cardiolipin[13] may support the different functions of the three phospholipids. The results obtained from the transition point experiments with cells of *E. coli* K-12 with a lag period at 17 °C suggest that the phospholipids responsible for the transition point of the transport system may include high proportions of cardiolipin whose fatty acid composition was not changed during the lag period or that the phospholipids localized at the site of transport can not be renewed readily during the lag period, in contrast with the environmental bulk phospholipids.

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