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RELATIONSHIP BETWEEN PHOSPHOLIPID COMPOSITIONS AND TRANSPORT ACTIVITIES OF AMINO ACIDS IN *ESCHERICHIA COLI* MEMBRANE VESICLES

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Summary

Cells of *Escherichia coli* were incubated in broth medium in the presence of 5 mM of hydroxylamine which completely inhibited growth but did not affect viabilities. Hydroxylamine is known to inhibit phosphatidylserine decarboxylase. A large amount of phosphatidylserine (up to 20% of total phospholipids), which did not occur in normal cells, accumulated accompanied with a decrease in phosphatidylethanolamine. Higher uptake activities of serine and glutamate were observed with the hydroxylamine-treated cells than control cells. When membrane vesicles from hydroxylamine-treated cells were prepared, they also displayed higher uptake activities of serine, proline, glutamate, and threonine than those of normal membranes. When hydroxylamine-treated cells were incubated with chloramphenicol, at concentrations which almost completely inhibited protein synthesis, the composition of phosphatidylserine decreased with a concomitant increase in that of phosphatidylethanolamine. The phospholipid composition of these cells incubated for 5 h with chloramphenicol became almost normal. Membranes vesicles prepared from such cells displayed reduced uptake activities, which were close to those of normal vesicles. These results were interpreted as indicating the altered transport activities due to the altered phospholipid composition.

Introduction

Phospholipids are one of the integral components of biological membranes. They contribute not only to the structure of these organelles but also to their functions. The major species of phospholipids found in *Escherichia coli* are phosphatidylethanolamine, phosphatidylglycerol and cardiolipin which comprise 70—80, 5—15 and 5—15%, respectively [1]. Phosphatidylserine, phos-

phatidic acid and CDP-diglyceride are also present as minor components of *E. coli* lipids and they are rapidly metabolized [1,2]. Recently, Hawrot and Kennedy [3] suggested that phosphatidylethanolamine was an essential phospholipid in *E coli.*

In bacterial membranes, phospholipids have been demonstrated to be required for the functions of membrane-located enzymes, for example, those related to oxidative phosphorylation [4,5], active transport [6,7] and lipopolysaccharide synthesis [8]. Several workers have investigated the relationship between unsaturated fatty acids and the activities of sugar and amino acid transport systems of *E. coli* [9—14]. Tsukagoshi and Fox [13] reported that an abortive induction of lactose transport system was due to a non-fluid and immobile state of membrane lipids. Milner and Kaback [6] reported that phospholipase D inhibited the vectorial phosphorylation of α-methylglucoside in bacterial membrane preparation, but to a less extent the uptake of proline. Kundig and Roseman [7] showed that phosphatidylglycerol was an essential constituent of membrane-bound enzyme II of the phosphotransferase system.

There are, however, few investigations concerning the physiological function of phospholipid species on active transport systems. For this purpose, two approaches could be considered, namely (a) the isolation of mutants defective in a single step of the biogenesis of membrane lipids and (b) the treatment of cells with an inhibitor of phospholipid metabolism resulting in an alteration of phospholipid composition. Many mutants defective in enzymes of phospholipid synthesis of *E. coli* have been isolated. Although many mutants defective in the early steps of the synthesis of phosphatidic acid have been reported, only a few mutants defective in the later steps have been isolated [3,15—17]. For the first approach described above, the later mutants are useful. The distortion of the phospholipid composition in bacteria can be achieved in many ways, for example, the treatment with various drugs or altered growth conditions. Most of the drugs, which induce the distortion, give rise to the accumulation of cardiolipin [18—21]. The accumulation of cardiolipin has been observed as a consequence of either the death of cells or the ceasing of cell growth. It has been reported that serine hydroxamate [22] and hydroxylamine [1,23,24] are the inhibitors of phosphatidylserine decarboxylase and bring about the accumulation of phosphatidylserine. Therefore, these drugs might be useful for the second approach mentioned above.

To our knowledge, no investigation has been reported on the effect of the accumulation of phosphatidylserine on bacterial transport systems. In this paper, the relationship between phospholipid compositions and transport activities is described, using hydroxylamine as the inhibitor of phosphatidylserine decarboxylase.

Materials and Methods

Bacterial strain, medium and growth conditions. E. coli W2252 was used throughout the experiment. The cells were grown at 30° C with shaking in a nutrient broth (pH 7.0), containing 1% Polypeptone (Daigo Eiyo Kagaku Co., Ltd., Osaka), 1% meat extract (Kyokuto Seiyaku Co., Ltd., Tokyo) and 0.5% NaCl. Bacterial growth was measured by absorbance (A) at 660 nm using a

Hitachi photoelectric colorimeter (FPW-4).

Extraction and characterization of phospholipids. The cells were grown in 5 ml of the nutrient broth containing 10 μCi/ml of [^{32}P]orthophosphate ($^{32}P_i$ culture). After indicated manipulations a portion (1 ml) of the culture was withdrawn and phospholipids were extracted by the method of Bligh and Dyer [25]. Phospholipids were separated by thin-layer chromatography on a silica gel plate (E. Merck, Darmstadt; Art. 5724) with a solvent system of chloroform/methanol/acetic acid (65 : 25 : 10, by vol.). Radioactive spots on the thin-layer plate were detected by autoradiography, and each spot was scraped off from the plate and placed in a glass counting vial. Radioactivity was counted by a liquid scintillation spectrometer (Packard, Tri-Carb Model 3385) using PPO-toluene scintillation fluid. Each phospholipid was characterized from the R_F value and the composition of phospholipids was determined from the ratio of radioactivities.

When the alteration of phospholipid composition during the preparation of membrane vesicles was examined, a 20 ml portion of the $^{32}P_i$ culture at *A* 0.8 was withdrawn and membrane vesicles were prepared as described below. An aliquot in each step of preparation was subjected to the phospholipid extraction as the case of whole cells. Phospholipids were analyzed as above.

Uptake of amino acid by whole cells. The cells were grown in the nutrient broth to *A* 0.8, harvested in the cold and washed twice with cold 0.1 M potassium phosphate buffer (pH 7.2). The washed cells were resuspended in this buffer at *A* 1.5. For studies of amino acid uptake by whole cells, 50-μl aliquots of the cell suspensions were diluted to a final volume of 100 μl containing, in final concentrations, 50 μg/ml of chloramphenicol and radioactive amino acid. The samples in small test tubes were preincubated at 25°C for 5 min and the reaction was started by the addition of labeled amino acid. After incubation at 25°C for 1 min, each sample was rapidly diluted with 2 ml of ice-chilled 10 mM Tris · HCl (pH 7.3) containing 0.5 mM $MgCl_2$ and 0.15 M NaCl (washing buffer) to terminate the reaction according to the method of Anraku [26]. The diluted sample was immediately filtered through a Millipore HAWP membrane filter (25 mm diameter) and washed once with 2 ml of the washing buffer. The dilution, filtration and washing procedure were performed within 20 s. The filter was immediately removed from a suction apparatus to a glass counting vial. The vial was incubated overnight at 37°C after addition of 1.5 ml of 1.67% sodium dodecyl sulfate solution. Radioactivity was counted by the liquid scintillation spectrometer using toluene-Nonione scintillation fluid [27]. Following concentrations of amino acids in μM were used for the uptake studies; L-serine, 15.4; L-glutamic acid, 10.0; L-lysine, 3.9; L-alanine, 12.6; L-phenylalanine, 5.4.

Preparation of membrane vesicles and uptake studies. Membrane vesicles were prepared according to the method described by Kaback [28]. The cells grown to *A* 0.8 were harvested in the cold and washed twice with cold 10 mM Tris · HCl (pH 8.0). Washed cells were resuspended (80 ml per g wet weight) in 30 mM Tris · HCl (pH 8.0) containing 20% sucrose and incubated at 30°C with stirring in the presence of 10 mM potassium EDTA and 0.5 mg/ml of lysozyme. After about 30 min of incubation, almost all cells were converted to spheroplasts. The spheroplasts were centrifuged in the cold and lysed

osmotically at 37°C. The spheroplast lysate was centrifuged and the pellet was resuspended at 0°C with homogenizing in 0.1 M potassium phosphate buffer (pH 6.6) containing 10 mM EDTA. Whole cells and cellular debris were removed by centrifugation at 500 × *g* for 15 min. Then, the supernatant fluid (crude membranes) was carefully decanted and subjected to the centrifugation at 40 000 × *g* for 30 min. The low and high speed centrifugations were repeated until the supernatant from the low speed centrifugation became free from whole cells as examined by a phase contrast microscope. The high speed pellet was washed five times by resuspension and vigorous homogenization in 0.1 M potassium phosphate buffer (pH 6.6) containing 10 mM EDTA. After the last wash, the membrane vesicles were resuspended by homogenization in 0.1 M potassium phosphate buffer (pH 6.6) at a concentration of 4—5 mg protein per ml, frozen in small aliquots in solid CO_2/ethanol and stored in a deep freezer at −80°C.

For studies of amino acid uptake by membrane vesicles, 50-μl aliquots of the membrane vesicles were diluted to a final volume of 100 μl containing, in final concentrations, 50 mM potassium phosphate buffer (pH 6.6), 10 mM $MgSO_4$, 20 mM ascorbate plus 0.1 mM phenazine methosulfate as energy source and labeled amino acid. The samples in small test tubes were preincubated at 25°C for 15 min. The reaction was started by the addition of labeled amino acid. Ascorbate plus phenazine methosulfate were added just before the addition of substrate. After incubation at 25°C for 1 min, each sample was diluted with 2 ml of 0.5 M LiCl to terminate the reaction and was immediately filtered through a Millipore HAWP membrane filter (25 mm diameter). The filters were washed once with 2 ml of 0.5 M LiCl. The dilution, filtration, and washing procedures were performed within 20 s. The other procedures were same as the uptake studies by whole cells. Following concentrations of amino acids in μM were used for the uptake studies, L-serine, 15.4; L-proline, 8.4; L-glutamic acid, 10.0; L-threonine, 12.1; L-alanine, 12.6; L-lysine, 3.9; glycine, 23.0; L-phenylalanine, 5.4.

The amounts of protein in membrane vesicles and whole cells were determined by the method of Lowry et al. [29] with bovine serum albumin as a standard.

Labeled compounds. All labeled compounds were purchased from the Japan Radioisotope Association (Tokyo). [U-^{14}C]Alanine(171 Ci/mol), [2-^{14}C]-glycine(114 Ci/mol), L-[U-^{14}C]lysine(342 Ci/mol), L-[U-^{14}C]serine(174 Ci/mol), L-[G-^{3}H]glutamic acid(34 Ci/mmol) and L-[G-^{3}H]proline(677 Ci/mol) were the products of the Radiochemical Centre (Amersham). L-[3-^{3}H(N)]-Phenylalanine(16.1 Ci/mmol) and L-[G-^{3}H]threonine(1.8 Ci/mmol) were the products of New England Nuclear (Boston). Carrier-free [^{32}P]orthophosphate was the product of Commissariat à l'Energie Atomique (Gif-sur-Yvette).

Results

Effect of hydroxylamine on growth of E. coli

The cells were grown in the nutrient broth medium and at *A* 0.8, various amounts of hydroxylamine were added and cultures were further continued. As shown in Fig. 1, hydroxylamine over 5 mM inhibited cell growth almost

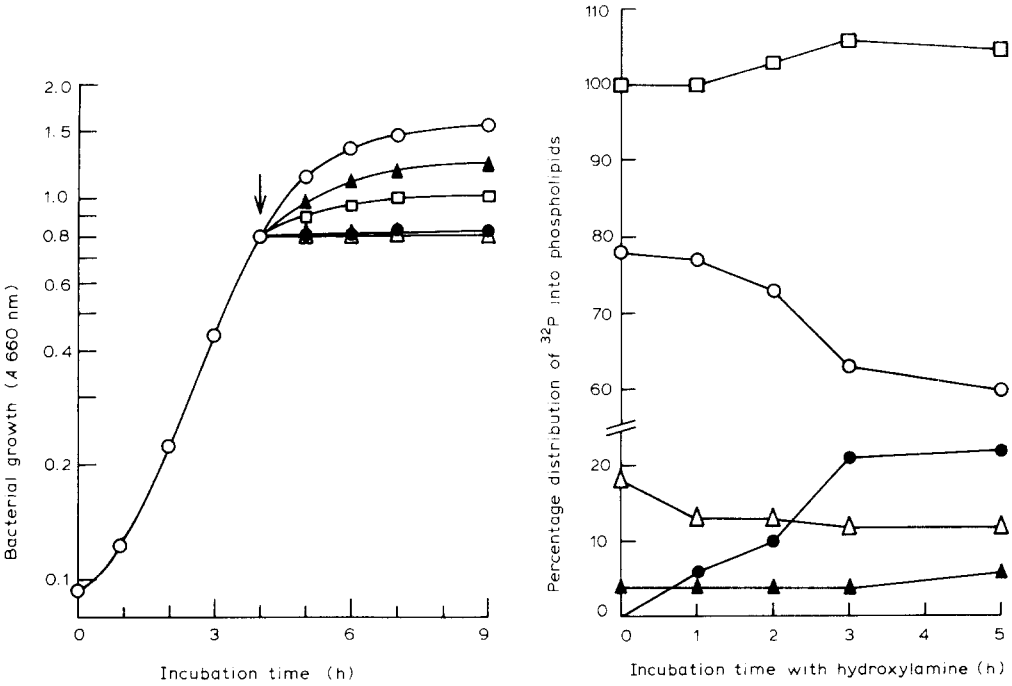

Fig. 1. Effect of hydroxylamine on growth. The cells were incubated in the nutrient broth. Hydroxylamine was added on the culture at the time indicated by an arrow (*A* 0.8) and incubation was continued. Final concentrations of hydroxylamine added were: 0 (○), 1 (▲), 2.5 (□), 5 (●) and 10 mM (△).

Fig. 2. Effect of hydroxylamine on phospholipid composition. To the $^{32}P_i$ culture at *A* 0.8, 5 mM of hydroxylamine was added and incubations was continued. The ratio in percentage of the amount of total phospholipid at indicated incubation time to that of zero time was plotted against incubation time with hydroxylamine. The phospholipid composition at indicated time was expressed by percentage distribution of ^{32}P. □, total phospholipids; ○, phosphatidylethanolamine; ●, phosphatidylserine; △, phosphatidylglycerol; and ▲, cardiolipin.

completely. However, hydroxylamine at 5 mM did not affect viabilities (data not shown).

Effect of hydroxylamine on phospholipid compositions

The percentage compositions of phosphatidylethanolamine, phosphatidylglycerol and cardiolipin in this organism were approx. 76—80, 16—19 and 3—5 on phosphorus basis, respectively, and phosphatidylserine was not detected in the cells grown in the nutrient broth. When hydroxylamine was added, however, the amount of phosphatidylserine increased with the increasing concentrations of this reagent up to 5 mM. Usually phosphatidylserine occupied about 20% of the total phospholipids after incubation for 3 h with 5 mM of hydroxylamine. The percentage composition of each phospholipid during incubation with hydroxylamine is shown in Fig. 2. There was a slight increase in the level of total phospholipids. However, a significant decrease in phosphatidylethanolamine occurred with a concomitant increase in phosphatidylserine. The level of cardiolipin or phosphatidylglycerol tended to increase or decrease, respectively. However, these changes occurred to small extents as

TABLE I

THE RATE OF PHOSPHOLIPID IN THE PRESENCE OF HYDROXYLAMINE

To the culture at *A* 0.8, 10 μCi of $^{32}P_i$ per ml was added simultaneously with hydroxylamine (5 mM) and incubation was continued. At indicated period, phospholipids were extracted and chromatographed and the radioactivity of each phospholipid fraction was counted. Inhibition of each phospholipid synthesis was calculated from the percent distribution of each phospholipid of the cells grown under ordinary conditions. n.d., not detected; —, stimulation.

Phospholipid	Percent distribution under ordinary conditions	Incubation time with hydroxylamine and $^{32}P_i$ (h)								
		1			3			5		
		Incorporation of $^{32}P_i$		Inhibition (%)	Incorporation of $^{32}P_i$		Inhibition (%)	Incorporation of $^{32}P_i$		Inhibition (%)
		cpm	%		cpm	%		cpm	%	
Phosphatidylserine	n.d.	7 370	73	—∞	26 987	64	—∞	33 675	41	—∞
Phosphatidylethanolamine	78	339	3	96	3 371	8	90	23 746	29	63
Phosphatidylglycerol	18	2 089	21	—17	10 064	24	—33	20 384	25	—39
Cardiolipin	4	314	3	25	1 584	4	0	3 971	5	—25
Total phospholipids	100	10 112	100		42 006	100		81 776	100	

compared with the large changes in phosphatidylethanolamine and phosphatidylserine.

In order to know the rate of synthesis of each phospholipid, cells were grown to *A* 0.8 and 10 μCi of $^{32}P_i$ per ml of culture was added simultaneously with hydroxylamine and the culture was further incubated. As shown in Table I, an almost complete inhibition of phosphatidylethanolamine synthesis and a remarkable accumulation of phosphatidylserine were observed with cells incubated for 1 or 3 h with hydroxylamine. Synthesis of phosphatidylglycerol and cardiolipin was not significantly affected. For these reasons, the following experiments were performed with the cells incubated for 3 h with 5 mM of hydroxylamine.

TABLE II

UPTAKE OF AMINO ACID BY WHOLE CELLS

Substrate	Uptake of amino acid (nmol/mg protein per min)	
	Control cells	Hydroxylamine-treated cells
L-Serine	1.14	2.04
L-Glutamic acid	0.13	0.80
L-Lysine	0.15	0.06
L-Alanine	0.46	0.48
L-Phenylalanine	0.13	0.16

TABLE III

ALTERATION OF PHOSPHOLIPID COMPOSITION DURING THE PREPARATION OF MEMBRANE VESICLES

Step of preparation	Phospholipid compositions (%)						
	Normal			Hydroxylamine treated			
	Phosphatidyl-ethanolamine	Phosphatidyl-glycerol	Cardiolipin	Phosphatidyl-serine	Phosphatidyl-ethanolamine	Phosphatidyl-glycerol	Cardiolipin
Whole cells	77	18	5	21	65	10	4
Washed cells	76	20	4	—	—	—	—
Spheroplasts	82	14	4	9	84	5	2
Spheroplast lysate	78	10	12	12	75	5	8
Crude membranes	79	10	11	—	—	—	—
Membrane vesicles	78	11	11	12	74	5	9

Effect of hydroxylamine treatment on the transport activity of whole cells

The effect of hydroxylamine treatment on the transport of amino acids by whole cells was examined. As shown in Table II, the treatment gave rise to the stimulated transport activities of serine and glutamic acid, while that of lysine was inhibited. No significant difference in the activities toward alanine and phenylalanine was observed. It is possible to consider that the accumulation of phosphatidylserine and/or the decrease in phosphatidylethanolamine in the hydroxylamine-treated cells are responsible for the altered transport activities. In order to ensure this hypothesis, the transport activities of membrane vesicles were examined.

Alteration of phospholipid composition during the preparation of membrane vesicles

Little information is available concerning the phospholipid composition of membrane vesicles of *E. coli* with regard to the alteration of the composition during the preparation of membrane vesicles. Table III shows the changes in phospholipid composition during the preparation of normal membrane vesicles. Although no significant change in the composition was noted between intact and washed cells, significant increase in phosphatidylethanolamine and decrease in phosphatidylglycerol compositions were observed at the spheroplast formation. When the spheroplasts were lysed osmotically, the resulted lysate contained less phosphatidylethanolamine and more cardiolipin as compared to the spheroplasts. No significant change in phospholipid composition occurred after this procedure. Therefore, the phospholipid composition of normal membranes was relatively rich in cardiolipin and poor in phosphatidylglycerol as compared to that of whole cells.

During the preparation of membrane vesicles from hydroxylamine-treated cells (hydroxylamine-treated membranes), a similar profile was observed as normal membranes, with the exception of the presence of phosphatidylserine as shown in Table III. When spheroplasts were formed, there was a remarkable decrease in phosphatidylserine and phosphatidylglycerol. Significant decrease in phosphatidylethanolamine and increase in cardiolipin were observed at the osmotic shock process.

TABLE IV

UPTAKE OF AMINO ACIDS BY NORMAL AND HYDROXYLAMINE-TREATED MEMBRANES

Substrate	Uptake of amino acid (pmol/mg protein per min)		Ratio B/A
	Normal membranes (A)	Hydroxylamine-treated membranes (B)	
L-Serine	62	431	7.0
L-Proline	105	348	3.3
L-Glutamic acid	29	70	2.4
L-Threonine	30	70	2.3
L-Alanine	35	35	1.0
L-Lysine	28	27	0.96
Glycine	63	53	0.84
L-Phenylalanine	11	9	0.82

TABLE V

INHIBITORY EFFECT OF HYDROXYLAMINE ON UPTAKE OF AMINO ACID BY NORMAL MEMBRANES

Substrate	Uptake of amino acid (pmol/mg protein per min)		Inhibition (%)
	Without hydroxylamine	With hydroxylamine *	
L-Serine	62	33	47
L-Glutamic acid	34	24	29
L-Alanine	35	22	37
L-Lysine	30	22	27

* Hydroxylamine · HCl was added to the reaction mixture at final concentration of 5 mM.

Transport activity of hydroxylamine-treated membranes

As shown in Table IV, uptakes of serine, proline, glutamic acid and threonine by hydroxylamine-treated membranes were 2—7 times as high as those by normal membranes. On the other hand, no significant difference in the uptake activities of alanine, lysine, glycine and phenylalanine was found between normal and hydroxylamine-treated membranes. No relationship was found between the chemical nature of amino acids and the elevated transport activities.

The observed stimulation of uptakes in hydroxylamine-treated membranes is not due to the direct action of hydroxylamine. As shown in Table V about 30—50% inhibition of serine, glutamic acid, alanine and lysine uptakes was observed when hydroxylamine at 5 mM was added to normal membranes.

Effect of incubation on phospholipid composition of hydroxylamine-treated cells

To the $^{32}P_i$ culture at A 0.8, 5 mM of hydroxylamine was added and incubation was continued for 3 h. The cells were harvested by centrifugation in the cold, washed twice with cold 10 mM Tris · HCl (pH 8.0) containing 50 μg/ml of chloramphenicol and resuspended in the same buffer of the original volume containing chloramphenicol (50 μg/ml). They were further incubated with gentle stirring at 30° C. Chloramphenicol at this concentration was sufficient to inhibit protein synthesis almost completely as measured by the incorporation of [^{3}H]phenylalanine into trichloroacetic acid-insoluble materials.

As shown in Fig. 3, the phospholipid composition of the hydroxylamine-treated cells changed during incubation in the presence of chloramphenicol. The composition of phosphatidylserine decreased from 20% to about 5% within 4 h, with a concomitant increase in phosphatidylethanolamine. An increase in cardiolipin and a decrease in phosphatidylglycerol were observed, but these shifts were not so dramatic as the case of phosphatidylethanolamine and phosphatidylserine. The results indicated that the inhibition of phosphatidylserine decarboxylase by hydroxylamine is reversible. Phosphatidylserine occupied 3% of total phospholipids even after 5 h incubation and did not disappear by the prolonged incubation for 10 h. Therefore, an almost complete recovery in the phospholipid composition seems to be achieved by

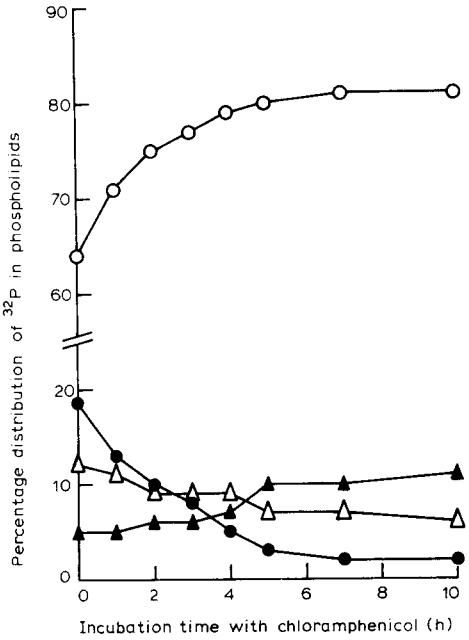

Fig. 3. The phospholipid compositions during incubation with chloramphenicol after hydroxylamine treatment. Experimental procedure were described in the text. At time intervals a portion of the culture was withdrawn and the phospholipid compositions were analyzed by percentage distribution of ^{32}P. ○, phosphatidylethanolamine; ●, phosphatidylserine; △, phosphaditylglycerol; and ▲, cardiolipin.

incubation for 5 h in the presence of chloramphenicol.

Membrane vesicles were prepared from cells which were treated with hydroxylamine and then incubated for 5 h in the presence of chloramphenicol. These membrane vesicles were designated as hydroxylamine-chloramphenicol-treated membranes. As shown in Table VI, the phospholipid composition of

TABLE VI

ALTERATION OF PHOSPHOLIPID COMPOSITION DURING THE PREPARATION OF MEMBRANE VESICLES FROM CELLS INCUBATED WITH CHLORAMPHENICOL AFTER HYDROXYLAMINE TREATMENT

Step of preparation	Phospholipid composition (%)			
	Phosphatidyl-serine	Phosphatidyl-ethanolamine	Phosphatidyl-glycerol	Cardiolipin
Hydroxylamine-treated cells *	18	64	12	6
Chloramphenicol-treated cells **	2	79	8	11
Spheroplasts	3	88	6	3
Spheroplast lysate	3	75	8	14
Membrane vesicles	3	76	7	14

* Cells which were incubated with hydroxylamine (5 mM) for 3 h.

** Cells which were incubated with chloramphenicol for 5 h after hydroxylamine treatment.

TABLE VII

UPTAKE OF AMINO ACIDS BY VARIOUS MEMBRANE PREPARATIONS

Substrate	Uptake of amino acid (pmol/mg protein per min)			
	Normal membranes	Hydroxylamine-treated membranes	Hydroxylamine-chloramphenicol-treated membranes	Chloramphenicol-treated membranes
L-Serine	62	431	143	109
L-Proline	105	348	141	213
L-Threonine	30	70	45	41
L-Glutamic acid	29	70	60	40

the hydroxylamine-chloramphenicol-treated membranes was essentially identical to that of cells incubated with chloramphenicol after the hydroxylamine treatment. When the phospholipid composition of hydroxylamine-chloramphenicol-treated membranes was compared to that of normal membranes, three points could be noted; (a) phosphatidylserine was present only in hydroxylamine-chloramphenicol-treated membranes as a minor component, (b) the composition of cardiolipin of hydroxylamine-chloramphenicol-treated membranes was higher and phosphatidylglycerol was lower and (c) the ratio of phosphatidylethanolamine plus phosphatidylserine to phosphatidylglycerol plus cardiolipin was similar (cf. Tables III and VI).

Amino acid uptake activities of hydroxylamine-chloramphenicol-treated membranes

In order to know incubation with chloramphenicol alone has some effect on transport activities, the cells grown to A 0.8 were treated with chloramphenicol for 5 h. Membrane vesicles from the chloramphenicol-treated cells were designated as chloramphenicol-treated membranes. The activities of amino acid uptakes of normal, hydroxylamine-treated, hydroxylamine-chloramphenicol-treated and chloramphenicol-treated membranes were examined. As shown in Table VII, hydroxylamine-treated membranes showed higher activities than normal membranes, while hydroxylamine-chloramphenicol-treated membranes had much less activities than hydroxylamine-treated membranes except the uptake of glutamate. It is evident that incubation of hydroxylamine-treated cells in the presence of chloramphenicol, which resulted in the normal phospholipid composition, also brought about the normal levels of uptake activities. The uptake activities of chloramphenicol-treated membranes were generally higher than the normal membranes. These results indicate that the stimulated activities of hydroxylamine-treated membranes are due to the accumulation of phosphatidylserine and/or the decrease in phosphatidylethanolamine, but not to the treatment of chloramphenicol.

Discussion

In this report, we used hydroxylamine as an inhibitor of phosphatidylserine decarboxylase and observed the accumulation of phosphatidylserine which was

not detected in *E. coli* cells grown under ordinary conditions. The extent of phosphatidylserine accumulation in the cells treated with this drug was almost similar to that in the temperature-sensitive mutants of phosphatidylserine decarboxylase at elevated temperature as reported by Hawrot and Kennedy [3]. They discussed that the cessation of growth in those mutants could result from the lack of sufficient amounts of phosphatidylethanolamine to participate in some vital membrane functions for which other phospholipids could not substitute, and that the accumulation of phosphatidylserine might itself adversely affected membrane functions and thereby resulted in cell death. In the present study, however, the viability was not affected by the treatment with 5 mM hydroxylamine. Transport activities of certain amino acids were stimulated both in hydroxylamine-treated cells and in hydroxylamine-treated membranes. Moreover, the elevated activities became almost normal in the hydroxylamine-chloramphenicol-treated membranes. Since the hydroxylamine-chloramphenicol-treated cells were prepared in the presence of chloramphenicol, these results suggest that the shifts in transport activities were induced by the changes in phospholipid composition and not by the changes involving protein sysnthesis.

The effect of hydroxylamine on transport activities of amino acids was heterogeneous in that some activities were stimulated but other activities were almost normal or inhibited. Possibly, the functional proteins related to each transport system might be activated or suppressed because of their conformational alterations due to the changes in the composition of surrounding phospholipids. In this connection, Beebe [30] reported the decreased transport activities of amino acids, carbohydrates and nucleosides in a phosphatidylethanolamine-deficient mutant of *Bacillus subtilis* and suggested that the deletion of phosphatidylethanolamine resulted in a loss of structural integrity of the membranes and an aberration in functional transport activities. Also, Holden et al. [31] have reported the heterogeneous alteration of amino acid transport activities in a pantothenate-deficient *Lactobacillus plantarum* and suggested that a reduction in membrane lipid content heterogeneously affected the operation and/or synthesis of amino acid transport systems and that at least some transport activities were affected by the surrounding membrane lipid components.

During the preparation of membrane vesicles, the alteration of phospholipid composition especially the increase in cardiolipin and the decrease in phosphatidylglycerol were observed. The alteration was most significant when the spheroplasts were lysed by the osmotic shock. The cardiolipin content of normal membrane vesicles almost doubled that of intact cells. Since the accumulation of cardiolipin was induced by either the death of cells or the ceasing of cell growth, the accumulation of cardiolipin might be considered to be related to the distortion of the membrane structure. So far, every attempt to avoid this change in phospholipid composition during the preparation of membrane vesicles was unsuccessful.

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