Short Communication

STIMULATIVE EFFECT OF BOVINE SERUM ALBUMIN ON THE TRANSPORT ACTIVITY OF SERINE IN MEMBRANE VESICLES FROM *ESCHERICHIA COLI*

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In the preceding papers (1, 2), we have shown that lipid A, fatty acids, and their derivatives inhibit the accumulation of succinate or amino acids by membrane vesicles from *Escherichia coli* W2252, which couples to the oxidation of electron donors. We found that the inhibition of transport activities by lipid A and fatty acids was due to the action of these compounds as proton conductors on membrane vesicles and that this inhibition was partly overcome by the addition of bovine serum albumin which could remove these inhibitors from membrane vesicles (3). We also noticed that bovine serum albumin stimulated amino acid transport activities (serine, alanine, glycine, glutamate, leucine, and phenylalanine) of membrane vesicles (3), which was in agreement with the observation of MIZUSHIMA (4). We report here a further study on the stimulative effect of bovine serum albumin on the transport activity of serine in membrane vesicles from *E. coli* W2252.

The effect of bovine serum albumin on the removal of lipid materials from membrane vesicles was studied. ¹⁴C-acetate-labeled membrane vesicles (0.4 mg protein, and the labeling procedure is given in the legend to Table 1) were diluted to 200 μ l final volume containing in final concentrations, 0.05 M potassium phosphate buffer (pH 6.6), 0.01 M MgSO₄, and 1.8% bovine serum albumin (Sanko Pure Chemicals Co., Tokyo). The mixture was incubated at 25° for 15 min and diluted with 0.5 ml of 0.05 M potassium phosphate buffer (pH 6.6). It was filtered through a Millipore HAWP membrane filter. The filter was washed twice with 0.5 ml of the same buffer. The radioactivities of the filter and filtrate were measured as described previously (1). It was found that 7 to 10% of the total radioactivity was removed from the membrane vesicles by bovine serum albumin and appeared in the

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filtrate. No significant release of radioactivity was observed in the absence of bovine serum albumin. The analysis of labeled lipid materials released in the presence of bovine serum albumin gave approximately the following composition on the basis of radioactivities; free fatty acids 62%, lysophosphatidylethanolamine 34%, and phosphatidylethanolamine 4%.

In order to know the time course of the formation of these lipid materials, ^{14}C -acetate-labeled membrane vesicles were incubated for 30 min in the absence of bovine serum albumin. As shown in Table 1, the amount of fatty acids and lysophosphatidylethanolamine in the membrane vesicles increased with incubation

Table 1. Formation of fatty acids and lysophosphatidylethanolamine in membrane vesicles during preincubation period.

Formation of	Preincubation time (min)				
	0	5	10	15	30
	dpm of each fraction $\times 100$				
	total dpm of lipids in membrane vesicles				
Fatty acids	1.1	2.0	3.2	3.7	6.2
Lysophosphatidyl- ethanolamine	0.7	1.5	2.0	2.5	4.0

E. coli W2252 cells were grown in a nutrient broth medium containing sodium acetate [$1\text{-}^{14}\text{C}$] ($80\ \mu\text{Ci}$, $57\ \text{mCi/mmol}$). The Radiochemical Centre, Amersham, England) and membrane vesicles were prepared as described previously (1). ^{14}C -labeled membrane vesicles (0.4 mg protein and 49,560 dpm) were incubated at 25° for the indicated time. Phospholipids and fatty acids were extracted immediately after incubation by the method of BLIGH and DYER (5). Phospholipids and fatty acids were separated by thin-layer chromatography on a silica gel plate (E. Merck, Art. 5724, Darmstadt, West Germany) with a solvent system of chloroform-methanol-water (65:25:4, by vol.). Radioactive spots on the thin-layer plate were detected by autoradiography, and each spot was scraped off from the plate and its radioactivity was determined as described previously (1). Each fraction was identified from the *Rf* value.

Table 2. Effect of preincubation period and bovine serum albumin on serine uptake by membrane vesicles.

Preincubation time (min)	Uptake of serine ($\mu\text{mol/mg protein/min}$)		Ratio <i>B/A</i>
	Without bovine serum albumin (<i>A</i>)	With bovine serum albumin (<i>B</i>)	
2	69	141	2.04
15	78	173	2.21
30	56	173	3.09

The uptake of serine was measured as described previously (1). Where indicated, bovine serum albumin (1.8% final concentration) was added 2 min before the start of the uptake reaction.

Table 3. Effect of myristic acid and lysophosphatidylethanolamine on serine uptake by membrane vesicles.

Concentration of myristic acid and lysophosphatidylethanolamine (mM)	Uptake of serine (pmol/mg protein/min)		
	Control	With myristic acid	With lysophosphatidylethanolamine
0	108	—	—
0.04	—	68 (37) ^a	109 (−1) ^b
0.08	—	42 (61)	100 (7)
0.16	—	27 (75)	94 (13)

^a Numbers in parentheses indicate degree of inhibition (%) of serine uptake by the addition of these compounds.

^b Minus indicates stimulation.

time, although detectable amount of these materials was present at zero time. This suggests the action of phospholipase A in the membrane vesicles.

The effect of preincubation of the membrane vesicles on the transport activity of serine and the effect of bovine serum albumin were then examined. As shown in Table 2, the activity of serine uptake decreased and the extent of stimulation of serine uptake by bovine serum albumin increased with incubation time.

As shown in Table 3, the addition of myristic acid to the membrane vesicles severely inhibited the uptake of serine, while the addition of lysophosphatidylethanolamine caused no significant inhibition.

It was concluded that the stimulative effect of bovine serum albumin on the activity of amino acid uptake by membrane vesicles was partly due to the removal of fatty acids by serum albumin. Fatty acids were formed during the preparation and preincubation of membrane vesicles.

Besides bovine serum albumin, the effect of various proteins on the activity of serine uptake was examined. Although bovine serum albumin was a potent stimulator of serine uptake, methylated bovine serum albumin (Sigma Chemical Co., St. Louis, Mo., U.S.A.) at the concentration of 0.1 to 2.7% completely inhibited serine uptake. Gelatin (Koso Chemical Co., Tokyo), casein (from milk, Wako Pure Chemicals Co., Tokyo), and chymotrypsinogen (Sigma) at the concentration of 0.9% inhibited serine uptake by about 50%, while bovine globulin (Sigma) at the concentration of 0.9 to 2.7% stimulated it slightly (10%).

Membrane vesicles from *E. coli* ML 308–225 (generously supplied by Dr. H. R. Kaback of the Roche Institute of Molecular Biology, Nutley, New Jersey, U. S. A.) were also examined to investigate the effect of bovine serum albumin on the activity of serine uptake. A similar stimulation was also observed with the membrane vesicles from this organism.

Bovine serum albumin stimulated serine uptake significantly even with membrane vesicles preincubated for a short period which contained only a small amount of fatty acids. Therefore, the mechanism of stimulation of transport activ-

ities by bovine serum albumin could not be explained by a mere removal of fatty acids from membrane vesicles. In fact, we have shown previously (3) that the addition of bovine serum albumin decreased the proton permeability of membrane vesicles, stimulated the uptake of ^3H -triphenylmethylphosphonium ion, and also stimulated the uptake of serine driven by an artificial membrane potential introduced by K^+ -diffusion *via* valinomycin. Therefore, bovine serum albumin added to the membrane vesicles may play a role in stabilizing membrane potential produced by the electron transport system or by K^+ -valinomycin.

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