Note

Interaction of Benzaldehyde to the Membrane Protein of *Escherichia coli*Kenji SAKAGUCHI,* Tokichi MIYAKAWA*
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A carcinostatic principle was isolated from the fig homogenate and identified as benzaldehyde.¹⁻³⁾ During the purification process, the active principle was assayed by its two biological activities; one is the enhancement of the incorporation of labeled tryptophan into *Escherichia coli* cells and the other is the carcinostatic activity against AC755 cancer implanted in mice. The both activities paralleled completely, and these two activities are commonly comprehensible from the view point of interaction of benzaldehyde with membrane proteins.¹⁾ The uptake of ¹⁴C-labeled tryptophan into *E. coli* was enhanced to the extent of 20~60%¹⁾ by the addition of benzaldehyde 0.1~0.001 μ g/ml, while the incorporation of glutamic acid, alanine, thymine and adenine decreased to 70% of its original uptake (Table I). Colistin, which is a natural surface active agent,

consistently inhibited the incorporation of the amino acids and organic bases tested probably because of its destructing effect to the cell membrane. The efficiency of benzaldehyde at low concentration is noteworthy because the increase of its concentration to 10 μ g/ml or more in the incorporation experiments have resulted in the loss on both enhancing and decreasing effect.

The change of incorporation efficiency is apprehensible by the change of biomembrane which includes active transport system of amino acids and bases. Therefore, the attachment of labeled benzaldehyde on the *E. coli* cell membrane was studied. The exponentially growing cells were harvested, suspended in the cold mineral-glucose medium,⁴⁾ added with ¹⁴C-benzaldehyde 3.75 μ Ci/nmol/ml and chloramphenicol 40 μ g/ml and incubated 15 min, at 37°C, and washed three times with distilled water. The cells thus treated adsorbed about 1% of labeled benzaldehyde inoculated. The outer-membrane fraction and cytoplasmic membrane fraction were separated from the burst protoplasts after Mizushima and Yamada.⁵⁾ The radioactivity remained only on the slower sedimenting peak consisted with cytoplasmic membrane of *E. coli* cells (Fig. 1). The separation of two membrane fractions were assigned by the test of succinate dehydrogenase activity.⁶⁾

In order to study further the selective binding of labeled benzaldehyde to the cell-membrane proteins, the isolated outer and cytoplasmic membrane fractions were incubated in a phosphate buffer containing ¹⁴C-benzaldehyde 20 μ Ci per ml, washed, and electrophoresed by a slab acrylamide gel of discontinuous system.⁷⁾ Figure 2 shows that the interaction of benzaldehyde with the membrane protein is not uniform but some proteins selectively bound with the aldehyde. Even some protein bands did not bind with the adduct at all. The

TABLE I. EFFECT OF BENZALDEHYDE TO THE UPTAKE OF AMONO ACIDS AND BASES INTO *E. coli* Cells

Substrate	Amount incorporated (cpm/%)				
	Control	Colistin	Benzaldehyde		
			5 μ g/ml	0.1 μ g/ml	0.01 μ g/ml
Thymine	2028 / 100	333 / 16	1399 / 69	1566 / 77	1891 / 93
Adenine	4433 / 100	420 / 9	3135 / 71	3684 / 83	4246 / 96
Glutamic acid	4067 / 100	0 / 0	2842 / 70	2779 / 68	3203 / 79
Alanine	6072 / 100	468 / 8	5283 / 87	5438 / 90	5838 / 96
Tryptophan ¹⁾	680 / 100	0 / 0	1076 / 158	915 / 134	808 / 119

The exponentially growing *E. coli* W3110 cells were harvested, washed with the cold 0.05 M Tris-HCl buffer pH 7.35, suspended in a mineral-glucose medium (CM medium)⁴⁾, containing glucose 0.2%, chloramphenicol 40 μ g/ml and ¹⁴C-labeled substrates 0.1 Ci per 4.9×10^{-5} mmol per ml, incubated for 30 min with shaking, and the radioactivity incorporated into the cells was counted on the membrane filter after harvesting and washing with the cold mineral-glucose medium.^{1,5)}

The incorporation of labeled substrates into *E. coli* cells at 30 min incubation were represented by cpm value and by percentage to the control experiments.

FIG. 1. Binding of ^{14}C -Benzaldehyde to the Membrane Fraction of *E. coli* Cells.

The membrane fraction of *E. coli* cells treated with ^{14}C -benzaldehyde were analysed by the sucrose density gradient from 55% to 35% (w/w), and the protein concentration of each fraction was determined by Folin's phenol reagent.⁶⁾ The cpm was assayed by a scintillation counter.

○, cpm of ^{14}C benzaldehyde; ●, protein concentration.

protein from cytoplasmic membrane retained much more amount of benzaldehyde compared to the outer-membrane protein. However, the latter also bound a small amount of the compound which probably resulted from the conformation change of the isolated membrane.

The aldehydes are known to form Schiff base adducts with free amino or sulfhydryl groups in the protein molecule.⁸⁾ Benzaldehyde and other aldehydes have been reported to enhance the ability of oxygen binding on sickle haemoglobin molecule.⁹⁾ They inhibit the Na^+ - and K^+ -activated, Mg^{++} dependent ATPase, which is a typical membrane protein.¹⁰⁾

The binding of benzaldehyde with the cytoplasmic and outer membrane protein of *E. coli* cells can be a possible elucidation why the benzaldehyde can affect to the incorporation ratio of substrates into the cell, because the membrane proteins are known to play major roles for the transportation of substrates. Another possibility is the inclusion of benzaldehyde into the lipid layer of cell membrane causing the change of permeability of the membrane, however, we have not found much amount of labeled benzaldehyde in outer-membrane fractions which of course contain much lipids.

FIG. 2. Covalent Labeling of Membrane Proteins with ^{14}C -Benzaldehyde.

To 1 ml of each suspension of isolated outer- and cytoplasmic membrane (300 μg protein) in 50 mm sodium phosphate buffer pH 7.4, 20 μCi of [carbonyl ^{14}C] benzaldehyde (specific radioactivity 3.96 mCi/mmol, Amersham) was added, incubated for 30 min at 37°C . With 9 ml of 50 mm sodium phosphate buffer (pH 7.4) the suspensions were centrifuged at $12,000 \times g$ for 30 min at 4°C , and washed twice. The pellet was solubilized by heating at 100°C for 1 minute in a 0.5 ml of buffer containing 62.5 mM Tris, 3% SDS, 1% β -mercaptoethanol and 10% sucrose, pH 6.8. One hundred μl of samples was subjected to SDS-polyacrylamide gel electrophoresis (7.5% acrylamide). The gel was stained with Coomassie brilliant blue. The fluorogram was prepared after Bonner and Laskey.¹²⁾ 1, Coomassie blue stained outer membrane proteins; 2, ^{14}C -benzaldehyde labeled outer-membrane proteins; 3, Coomassie blue stained cytoplasmic membrane proteins; 4, ^{14}C -benzaldehyde labeled cytoplasmic membrane proteins.

Benzaldehyde, a periactin, has been reported to be effective for the treatment of patients with various types of cancers.^{1,2)} The authors (T. M. and K. S.) also found the selective cytotoxicity of this substance and its derivatives¹¹⁾ against SV-40 virus transformed cultured rat fibroblast cells and not to the normal fibroblast cells, at the concentration of 10–50 μg per ml culture medium through the attachment of the substances to the cell surface protein.³⁾

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