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DEOXYCYTIDINE UPTAKE BY ISOLATED MEMBRANE VESICLES FROM *ESCHERICHIA COLI* K 12

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SUMMARY

1. Two different deoxycytidine-uptake systems were found in isolated membrane vesicles of *Escherichia coli* K 12 cells: one was not significantly inhibited by guanine nucleosides, the other was markedly inhibited by these nucleosides.

In isolated membrane vesicles of a showdomycin-resistant mutant (Shm^r-001), the former system was no longer detectable but the latter was found to remain active.

2. The deoxycytidine uptake by these systems was stimulated by reduced phenazine methosulfate, D(−)-lactate, succinate, and NADH, but not by ATP, phosphoenolpyruvate, or phosphoribosyl pyrophosphate.

The reduced phenazine methosulfate-stimulated uptake of deoxycytidine by these systems was markedly inhibited by sodium azide.

3. Among sulfhydryl reagents tested, *N*-ethylmaleimide did not significantly inhibit the uptake of deoxycytidine by these systems, even at very high concentrations; *p*-chloromercuribenzoic acid did inhibit uptake.

4. The antibiotic showdomycin strongly inhibited the uptake of deoxycytidine by membrane vesicles of *Escherichia coli* K 12 but only slightly inhibited that by membrane vesicles of the mutant (Shm^r-001). Further, showdomycin produced a rapid efflux of deoxycytidine from previously loaded membrane vesicles of *Escherichia coli* K 12.

These results suggest that showdomycin is transported by the deoxycytidine transport system which is not inhibited by guanine nucleosides in isolated membrane vesicles of *Escherichia coli* K 12.

INTRODUCTION

The transport of the antibiotic showdomycin [2-(β-D-ribofuranosyl)maleimide] in *Escherichia coli* K 12 appears to be closely related to that of a wide variety of nucleosides[1].

We have isolated showdomycin-resistant mutants from *Escherichia coli* K 12[2]. These mutants have an impaired ability to take up not only showdomycin but also a number of nucleosides, including deoxycytidine, cytidine, uridine, deoxythymidine, adenosine, deoxyadenosine and deoxyguanosine.

Preliminary studies[3] with one of these showdomycin-resistant mutants, Shm^r-001, suggested that its reduced ability to take up showdomycin and various nucleosides is attributable to defects in the cytoplasmic membrane.

Two deoxycytidine transport systems have been found in *Escherichia coli* K 12: one has a high affinity for deoxycytidine and is not significantly inhibited by guanine and hypoxanthine nucleosides; the other has a low affinity for deoxycytidine and is markedly inhibited by these nucleosides.

In the showdomycin-resistant mutant, the former system is no longer detectable, but the latter system remains active. The former system, or at least a part of the system, seems to be common to a wide variety of nucleosides and also showdomycin.

The present investigation is an extension of these earlier studies and provides more direct evidence that the system involved in the uptake of nucleosides and showdomycin is located on the cytoplasmic membrane of *Escherichia coli* K 12, and that reduction in the ability of the showdomycin-resistant mutant Shm^r-001 to take up nucleosides and showdomycin is due to an alteration in the cytoplasmic membrane.

MATERIALS AND METHODS

Preparation of cells

Escherichia coli K 12 and a showdomycin-resistant mutant, Shm^r-001 (derived from K 12)[2,3], were grown with aeration in a jar fermentor, using a glucose–mineral medium[4] supplemented with 0.025% (w/v) Difco casamino acid. Cells were harvested at approximately the end of the logarithmic phase of growth, and stored at −85 °C.

Preparation of membrane vesicles

Membrane vesicles were prepared from lysozyme and EDTA-induced spheroplasts of K 12 and Shm^r-001 essentially as described by Kaback[5]. They were stored at −85 °C in 0.1 M potassium phosphate buffer, pH 6.6, at concentrations of 1.0–2.3 mg protein per ml, and thawed just before use. Each membrane preparation was checked by microscopy. Contamination with whole cells was not observed.

Measurement of the uptake of deoxycytidine and other labeled compounds by membrane vesicles

Portions of the membrane suspensions (either 40 μl or 80 μl) were equilibrated at 25 °C for 10 min, then added to prewarmed tubes containing $MgSO_4$ (10 mM final concentration) and the respective labeled compounds (10–40 μM final concentration) as stated in the legends to the tables and figures. The mixtures (final volume of either 100 μl or 200 μl) were then incubated at 25 °C. After a given time, each reaction mixture was diluted with 2 ml of ice-cold 0.1 M LiCl solution, filtered through Millipore filters of 0.45-μm pore size, and washed with 4 ml of the same solution. The filters were dried and counted with a liquid scintillation spectrometer using toluene–phosphor solution (5 ml). In control experiments, the radioactive compound was added to the membrane suspension in 2 ml of ice-cold 0.1 M LiCl solution just prior to filtration. Correction was made to each experimental value by subtracting the corresponding value obtained in the control experiment.

Chemicals and reagents

Purified iodoacetic acid was kindly supplied by Dr N. Yoshida; cytosine arabinoside was purchased from the Upjohn Company; *N*-ethylmaleimide was from Nakarai Chemicals Ltd; phenazine methosulfate was from Calbiochem; the labeled

compounds were from the Schwarz BioResearch Corp.; the unlabeled nucleosides were from the Sigma Chemical Corp. and the Schwarz BioResearch Corp.

RESULTS

Ability of isolated membrane vesicles to take up deoxycytidine

It has previously been shown that the components responsible for specificity with respect to the two different deoxycytidine transport systems are not released from the cells by osmotic shock[3]. Kaback and his co-workers[6–20] have also demonstrated that cell-free membrane vesicle preparations essentially free from peri-cytoplasmic components, cytoplasmic constituents and most of the cell wall material can be used for the study of transport of amino acids and sugars. We thus decided to investigate the mechanism of deoxycytidine transport in membrane preparations from a showdomycin-resistant mutant (Shm^r-001) of *Escherichia coli* K 12 and from the parent strain. These preparations, in which the membrane vesicles had been physically separated from whole cells, did indeed show small but significant deoxycytidine uptake.

The effects of a series of electron donors on deoxycytidine uptake by the preparations are shown in Table I. Addition of ascorbate and phenazine methosulfate, an artificial electron-donor system, to membrane vesicles from the parent and mutant cells significantly enhanced the steady-state level of deoxycytidine accumulation. Other electron donors, such as D(−)-lactate, succinate and NADH, caused similar though weaker stimulation of deoxycytidine accumulation. Although

TABLE I

EFFECT OF VARIOUS ELECTRON DONORS ON THE UPTAKE OF [^{14}C]DEOXYCYTIDINE BY MEMBRANE VESICLES FROM PARENT AND MUTANT CELLS

Membrane suspensions from the parent (2.25 mg membrane protein per ml) or mutant cells (1.71 mg membrane protein per ml) were allowed to equilibrate at 25 °C for 10 min, then 0.08-ml samples were added to tubes containing $MgSO_4$ (10 mM final concentration), [2-^{14}C]deoxycytidine (60.3 Ci/mole, 22.7 μM final concentration) and one of the electron donors being tested (each at a final concentration of 20 mM except phenazine methosulfate, which was at 0.1 mM). Total volume of each incubation mixture was 0.2 ml. After a 2-min incubation at 25 °C, the membrane vesicles were filtered, washed, and their total radioactivity was determined. For further explanation, see text. PMS, phenazine methosulfate. Results are expressed as pmoles/mg membrane per 2 min.

Electron donor added (mM)	[^{14}C]*Deoxycytidine uptake*			
	Parent		*Mutant*	
	pmoles	*Ratio*	*pmoles*	*Ratio*
None	19.1	1.0	9.0	1.0
PMS (0.1) + ascorbate (20)	66.9	3.5	61.7	6.9
D(−)-Lactate (20)	43.4	2.3	25.6	2.9
Succinate (20)	37.0	1.9	12.3	1.4
NADH (20)	33.4	1.8	16.2	1.8

the data are not shown, it is noteworthy that energy sources, such as ATP, phosphoenolpyruvate, and 5-phosphoribosyl pyrophosphate, were all ineffective.

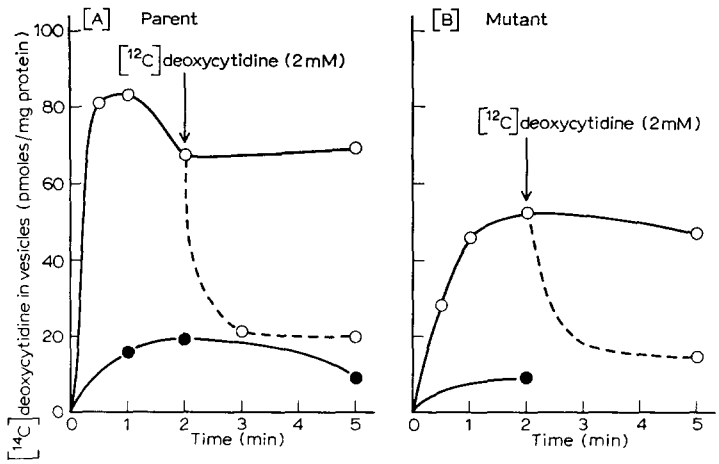

Fig. 1. (A) Membrane suspensions of the parent cells (2.25 mg membrane protein per ml) were allowed to equilibrate at 25 °C for 10 min, then 0.08-ml samples were added to tubes containing $MgSO_4$ (10 mM final concentration), and [2-^{14}C]deoxycytidine (60.3 Ci/mole, 22.7 μM final concentration). The mixtures were with (○—○) or without (●—●) sodium ascorbate (20 mM final concentration) and phenazine methosulfate (0.1 mM final concentration). Total volume of each incubation mixture was 0.2 ml. The incubations were continued at 25 °C for the indicated times, then terminated and assayed as described in Materials and Methods. The arrow indicates the time of addition of 2 mM unlabeled deoxycytidine (○—○). (B) Membrane suspensions of the mutant cells (1.71 mg membrane protein per ml) were allowed to equilibrate at 25 °C for 10 min, then 0.08-ml samples were added to tubes containing $MgSO_4$ (10 mM final concentration), and [2-^{14}C]deoxycytidine (47.4 Ci/mole, 21.1 μM final concentration). The mixtures were with (○—○) or without (●—●) sodium ascorbate (20 mM final concentration) and phenazine methosulfate (0.1 mM final concentration). Total volume of each incubation mixture was 0.2 ml. The incubations were continued at 25 °C for the indicated times, then terminated and assayed as described in Materials and Methods. The arrow indicates the time of addition of 2 mM unlabeled deoxycytidine (○—○).

With the demonstration that the membrane vesicles are capable of deoxycytidine uptake, it is of interest to compare the uptake by membrane vesicles from parent cells with the uptake by those of mutant cells. Fig. 1 shows typical curves for [^{14}C]-deoxycytidine uptake by membrane vesicles from the parent and mutant cells during incubation with ascorbate and phenazine methosulfate at 25 °C. The membrane vesicles from the parent cells took up [^{14}C]deoxycytidine very rapidly, reaching steady state within 1 min; whereas uptake by membrane vesicles from the mutant cells was slower, the uptake during the first 1 min been only 60% of that by the preparation from parent cells. It can also be seen that the addition of excess unlabeled deoxycytidine results in the rapid loss of the accumulated radioactivity from preloaded membrane vesicles from both parent and mutant cells.

As indicated in Table II, sodium azide effectively blocked the ascorbate–phenazine methosulfate-stimulated uptake of deoxycytidine by membrane vesicles from both parent and mutant cells.

TABLE II

EFFECT OF VARIOUS METABOLIC INHIBITORS ON THE UPTAKE OF [^{14}C]DEOXYCYTIDINE BY MEMBRANE VESICLES FROM PARENT AND MUTANT CELLS

Membrane suspensions from the parent (2.25 mg membrane protein per ml) or mutant cells (1.71 mg membrane protein per ml) were allowed to equilibrate at 25 °C for 10 min, then 0.04-ml samples were added to tubes containing $MgSO_4$ (10 mM final concentration), sodium ascorbate (20 mM final concentration), phenazine methosulfate (0.1 mM final concentration), [2-^{14}C]-deoxycytidine (60.3 Ci/mole, 22.7 μM final concentration with membrane suspensions from the parent, 49.3 Ci/mole, 21.3 μM final concentration with membrane suspensions from the mutant) and the cited amount of the metabolic inhibitor being tested. Total volume of the incubation mixture was 0.1 ml. After a 1-min incubation at 25 °C, the membrane vesicles were filtered, washed, their total radioactivity was determined. For further explanation, see text. Results are expressed as pmoles/mg membrane protein per min.

Inhibitor added (mM)	[^{14}C]*Deoxycytidine uptake*			
	Parent		*Mutant*	
	pmoles	%	*pmoles*	%
None	95.0	100.0	53.0	100.0
NaN_3 (50)	0.6	0.6	6.7	12.6
p-Chloromercuribenzoic acid (0.1)	31.7	33.4	11.0	20.8
Iodoacetic acid (2)	65.5	69.0	50.5	95.3

The sulfhydryl reagent, *p*-chloromercuribenzoic acid was a strong inhibitor of deoxycytidine uptake (Table II); but other sulfhydryl reagents, such as *N*-ethylmaleimide (Table III and Fig. 2) and iodoacetic acid (Table II), were not effective

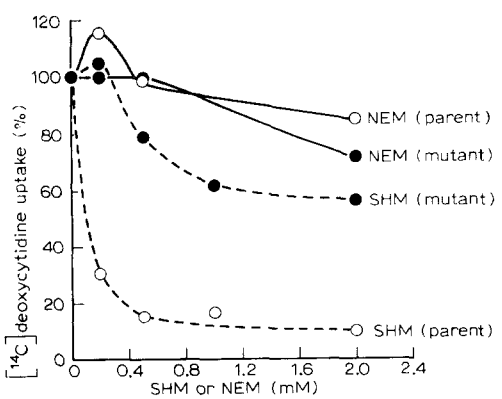

Fig. 2. Membrane suspensions of the parent (1.01 mg membrane protein per ml) or mutant cells (1.71 mg membrane protein per ml) were allowed to equilibrate at 25 °C for 10 min, then 0.08-ml samples were added to tubes containing $MgSO_4$ (10 mM final concentration), [2-^{14}C]deoxycytidine (47.4 Ci/mole, 21.1 μM final concentration), sodium ascorbate (20 mM final concentration), phenazine methosulfate (0.1 mM final concentration) and the cited amounts of showdomycin or *N*-ethylmaleimide. Total volume of each incubation mixture was 0.2 ml. After a 1-min incubation at 25 °C, the membrane vesicles were filtered, washed, and their total radioactivity was determined. For further explanation, see text. SHM, showdomycin; NEM, *N*-ethylmaleimide.

TABLE III

EFFECT OF SHOWDOMYCIN AND *N*-ETHYLMALEIMIDE ON THE UPTAKE OF [^{14}C]DEOXYCYTIDINE, [^{14}C]URIDINE AND [^{14}C]DEOXYTHYMIDINE BY MEMBRANE VESICLES FROM PARENT AND MUTANT CELLS

Membrane suspensions of the parent (2.25 mg membrane protein per ml) or mutant cells (1.71 mg membrane protein per ml) were allowed to equilibrate at 25 °C for 10 min, then 0.08-ml samples were added to tubes containing $MgSO_4$ (10 mM final concentration), sodium ascorbate (20 mM final concentration), phenazine methosulfate (0.1 mM final concentration), and one of the following labeled compounds: [2-^{14}C]deoxycytidine (47.4 Ci/mole, 21.2 μM final concentration); [2-^{14}C]uridine (52.9 Ci/mole, 18.9 μM final concentration); [2-^{14}C]deoxythymidine (46.9 Ci/mole, 21.3 μM final concentration); L-[U-^{14}C]proline (115.0 Ci/mole, 39.5 μM final concentration). The reaction mixtures were with or without showdomycin or *N*-ethylmaleimide (each at a final concentration of 500 μM). Total volume of the incubation mixture was 0.2 ml. After a 1-min incubation at 25 °C, the membrane vesicles were filtered, washed, and their total radioactivity was determined. For further explanation, see text.

Addition (μM)	[^{14}C]*Deoxycytidine uptake (%)*		[^{14}C]*Uridine uptake (%)*		[^{14}C]*Deoxythymidine uptake (%)*		[^{14}C]*Proline uptake (%)*
	Parent	*Mutant*	*Parent*	*Mutant*	*Parent*	*Mutant*	*Parent*
None	100*	100**	100***	100†	100††	100§	100§§
Showdomycin (500)	22.9	78.5	17.0	77.6	14.6	90.8	46.8
N-Ethylmaleimide (500)	105.8	100.3	88.4	100.6	107.0	101.9	23.9

* 100 = 110.7 pmoles/mg membrane protein per min.
** 100 = 49.0 pmoles/mg membrane protein per min.
*** 100 = 66.1 pmoles/mg membrane protein per min.
† 100 = 22.3 pmoles/mg membrane protein per min.
†† 100 = 81.3 pmoles/mg membrane protein per min.
§ 100 = 39.0 pmoles/mg membrane protein per min.
§§ 100 = 151.4 pmoles/mg membrane protein per min.

inhibitors. Under the same experimental conditions, uptake of [^{14}C]proline by the membrane vesicles from parent cells was markedly inhibited by *N*-ethylmaleimide (Table III).

Effect of other nucleosides on the uptake of deoxycytidine by membrane vesicles

Table IV shows that [^{14}C]deoxycytidine uptake by membrane vesicles from parent cells was markedly inhibited by pyrimidine and adenine nucleosides (cytidine, uridine, deoxythymidine, adenosine and deoxyadenosine) but was inhibited to a lesser degree by guanine nucleosides (guanosine and deoxyguanosine). In the membrane vesicles from mutant cells, however, [^{14}C]deoxycytidine uptake was markedly inhibited not only by the pyrimidine and adenine nucleosides but also by the guanine nucleosides. Xanthosine and cytosine arabinoside did not significantly inhibit [^{14}C]-deoxycytidine uptake by either preparation.

These results are analogous to those found with deoxycytidine transport systems in whole cells[3].

Fig. 3 shows in detail the effect of guanosine on the uptake of [^{14}C]deoxycytidine by membrane vesicles. Uptake by the membrane vesicles from parent cells

TABLE IV

EFFECT OF VARIOUS NUCLEOSIDES ON THE UPTAKE OF [^{14}C]DEOXYCYTIDINE BY MEMBRANE VESICLES FROM PARENT AND MUTANT CELLS

Membrane suspensions of the parent (2.25 mg membrane protein per ml) or mutant cells (1.71 mg membrane protein per ml) were allowed to equilibrate at 25 °C for 10 min, then 0.04-ml samples were added to tubes containing $MgSO_4$ (10 mM final concentration), sodium ascorbate (20 mM final concentration), phenazine methosulfate (0.1 mM final concentration), [2-^{14}C]deoxycytidine (60.3 Ci/mole, 22.7 μM final concentration), and one of the nucleosides being tested (each at a final concentration of 1 mM). Total volume of each incubation mixture was 0.1 ml. After a 1-min incubation at 25 °C, the membrane vesicles were filtered, washed, and their total radioactivity was determined. For further explanation, see text.

Nucleoside added (each 1 mM)	[^{14}C]*Deoxycytidine uptake* (%)	
	Parent	*Mutant*
None	100*	100**
Deoxycytidine	8.6	6.0
Cytidine	11.6	21.8
Uridine	17.5	23.3
Deoxythymidine	20.4	31.7
Adenosine	21.2	1.2
Deoxyadenosine	13.0	13.3
Guanosine	45.2	10.9
Deoxyguanosine	44.8	4.8
Xanthosine	59.3	70.7
Cytosine arabinoside	68.1	54.8

* 100 = 93.6 pmoles/mg membrane protein per min.
** 100 = 42.1 pmoles/mg membrane protein per min.

decreased with increasing guanosine concentration to about 40% of the maximal value, no further decrease being observed. Uptake by the membrane vesicles from mutant cells was less than 0.5 of that by vesicles from parent cells, and was strongly inhibited by guanosine. These results suggest that at least two systems may function for deoxycytidine transport: one which is not inhibited by guanosine and another which is inhibited; and that membrane vesicles from mutant cells retain only the latter system. This is consistent with the results of a similar experiment with whole cells[3].

Effect of showdomycin on the uptake of deoxycytidine by membrane vesicles

Reduced phenazine methosulfate-stimulated [^{14}C]deoxycytidine uptake by membrane vesicles from parent cells was largely inhibited by the antibiotic showdomycin, whereas such uptake by membrane vesicles from mutant cells was not greatly affected by the antibiotic (Fig. 2). Since [^{14}C]deoxycytidine uptake was only slightly inhibited by *N*-ethylmaleimide, an analogue of the maleimide moiety of showdomycin, the susceptibility of the [^{14}C]deoxycytidine-uptake mechanism of membrane vesicles from parent cells to showdomycin would not seem to be due to the sulfhydryl-reactive action of the antibiotic. Similar inhibitory effects of showdomycin were observed on the uptake of [^{14}C]uridine and [^{14}C]deoxythymidine by membrane

vesicles from parent cells (Table III). The uptake of these two compounds was also not significantly susceptible to *N*-ethylmaleimide.

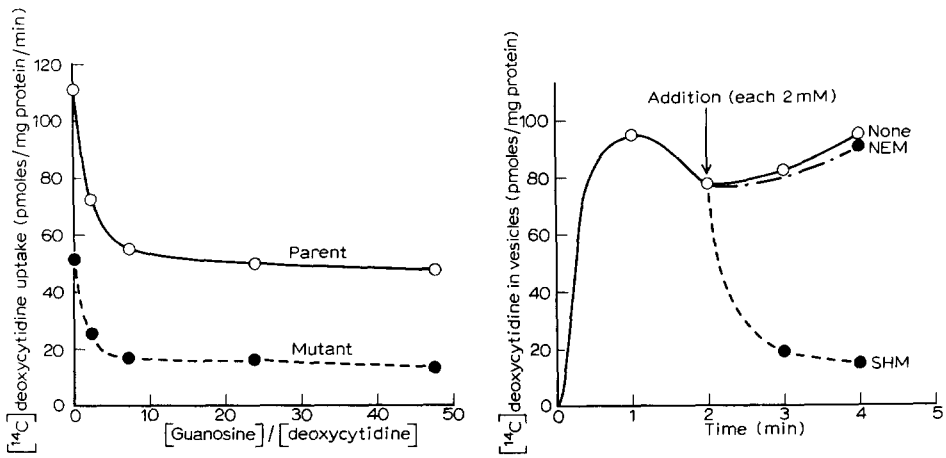

Fig. 3. Membrane suspensions of the parent (2.25 mg membrane protein per ml) or mutant cells (1.71 mg membrane protein per ml) were allowed to equilibrate at 25 °C for 10 min, then 0.08-ml samples were added to tubes containing $MgSO_4$ (10 mM final concentration), [2-^{14}C]deoxycytidine (47.4 Ci/mole, 21.1 μM final concentration), sodium ascorbate (20 mM final concentration), phenazine methosulfate (0.1 mM final concentration), and the cited amounts of guanosine. Total volume of each incubation mixture was 0.2 ml. After a 1-min incubation at 25 °C, the membrane vesicles were filtered, washed, and their total radioactivity was determined. For further explanation, see text.

Fig. 4. Membrane suspensions of the parent cells (2.25 mg membrane protein per ml) were allowed to equilibrate at 25 °C for 10 min, then 0.04-ml samples were added to tubes containing $MgSO_4$ (10 mM final concentration), [2-^{14}C]deoxycytidine (60.3 Ci/mole, 22.7 μM final concentration), sodium ascorbate (20 mM final concentration), and phenazine methosulfate (0.1 mM final concentration). Total volume of each incubation mixture was 0.1 ml. The incubations were continued at 25 °C for the indicated times, then terminated and assayed as described in Materials and Methods. The arrow indicates the time of addition of 2 mM showdomycin (●---●) or 2 mM *N*-ethylmaleimide (●–·–●). SHM, showdomycin; NEM, *N*-ethylmaleimide.

It can also be seen that the addition of showdomycin resulted in rapid loss of the majority of the accumulated radioactivity (derived from [^{14}C]deoxycytidine) from the preloaded membrane vesicles from the parent cells, whereas the addition of *N*-ethylmaleimide to these membrane vesicles did not cause significant loss of the accumulated radioactivity (Fig. 4).

These results suggest that showdomycin behaves as a nucleoside analogue at the membrane vesicles from the parent cells and is transported by their deoxycytidine transport system.

DISCUSSION

Earlier studies with whole cells[3,4] pointed to the involvement of two different systems in transport of deoxycytidine in *Escherichia coli* K 12. However, we were unable to obtain direct answers as to the location of the systems and the way in which

they function. We now have found that these deoxycytidine transport systems are present in isolated membrane vesicles from *Escherichia coli* K 12 cells.

The specificity of the transport systems in the membrane vesicles was determined by measuring the inhibition of uptake of labeled deoxycytidine by membrane vesicles by other unlabeled nucleosides.

One of the transport systems was strongly inhibited by pyrimidine and adenine nucleosides but not significantly inhibited by guanine nucleosides; the other system was strongly inhibited by not only pyrimidine and adenine nucleosides but also by guanine nucleosides. The former system (designated temporarily as the first deoxycytidine transport system) was not observed in the membrane vesicles from cells of the showdomycin-resistant mutant, Shm^r-001, while the latter system (designated temporarily as the second deoxycytidine transport system) was retained.

This behavior is analogous to that of the deoxycytidine transport systems studied in whole cells, indicating that the transport systems of deoxycytidine in *Escherichia coli* K 12 cells are membrane bound.

The deoxycytidine uptake by membrane vesicles was markedly stimulated by the addition of electron donors, such as reduced phenazine methosulfate, D(−)-lactate, succinate and NADH. Reduced phenazine methosulfate-stimulated uptake of deoxycytidine by membrane vesicles was markedly inhibited by sodium azide.

It is clear from these observations that the deoxycytidine transport by membrane vesicles involves oxidation of the electron donors and the transfers of electron. This is consistent with similar findings[7–13,17,21] on the transport of various amino acids, sugars and succinate.

It has been postulated by Kaback and his co-workers[9,10,14,15] that transport carriers for amino acids and sugars may be sulfhydryl group-containing proteins which may also act as intermediates in electron transfer reactions of the respiration chain. The uptake of deoxycytidine by membrane vesicles was significantly inhibited by *p*-chloromercuribenzoic acid, a sulfhydryl reagent, but not markedly by other sulfhydryl reagents, such as *N*-ethylmaleimide and iodoacetic acid. This indicates either that the deoxycytidine carriers do not contain a free sulfhydryl group, or that access of *N*-ethylmaleimide and iodoacetic acid to a sulfhydryl group present is hindered.

Rayman *et al.*[21] reported that succinate uptake by membrane vesicles from *Escherichia coli* K 12 is completely unaffected by *N*-ethylmaleimide but is severely inhibited by *p*-chloromercuribenzoate. Our results with deoxycytidine resemble their findings obtained with succinate with respect to insusceptibility to *N*-ethylmaleimide.

The antibiotic showdomycin markedly inhibited the uptake of labeled deoxycytidine by membrane vesicles from *Escherichia coli* K 12 cells at low concentrations (Fig. 2), and the addition of showdomycin caused rapid loss of the majority of the accumulated radioactivity (derived from [^{14}C]deoxycytidine) from preloaded membrane vesicles of *Escherichia coli* K 12 cells (Fig. 4). These effects of showdomycin would not be due to the sulfhydryl-reactive action of its maleimide moiety, because similar effects were not observed when *N*-ethylmaleimide, an analogue of the maleimide moiety of showdomycin, was added to the same membrane vesicles.

These results suggest that showdomycin is transported by the deoxycytidine transport system in the membrane vesicles from *Escherichia coli* K 12 cells.

Since the uptake of deoxycytidine by the membrane vesicles from showdomycin-

resistant mutant cells was only slightly inhibited by showdomycin (Fig. 2 and Table III), the deoxycytidine transport system involved in transport of showdomycin must be lost or inactivated in the membrane vesicles from mutant cells.

As the first deoxycytidine transport system is no longer present in the membrane vesicles of mutant cells, it is very likely that it is the first deoxycytidine transport system which is responsible for the transport of showdomycin in the membrane vesicles from *Escherichia coli* K 12 cells.

The deoxycytidine uptake by the membrane vesicles from parent cells was largely inhibited by uridine and deoxythymidine (Table IV). Further, though in the membrane vesicles from parent cells not only [^{14}C]deoxycytidine uptake but also [^{14}C]uridine and [^{14}C]deoxythymidine uptake were markedly inhibited by showdomycin, uptake by the membrane vesicles from the mutant cells were essentially unaffected by the antibiotic (Table III). These results suggest that the first deoxycytidine transport system can transport uridine and deoxythymidine, in addition to deoxycytidine and showdomycin.

It would seem that in the transport system of the membrane vesicles from showdomycin-resistant mutant (Shm^r-001) cells, a component(s) of the first deoxycytidine transport system has been lost or inactivated. This conslusion strongly supports our previous consideration[3] that the showdomycin-resistant mutation is attributable to defects in the nucleoside transport system (designated as the common transport system in a previous paper[3]) located on the cytoplasmic membrane of *Escherichia coli* K 12 cells.

If out of two transport systems for deoxycytidine the first is inhibited by showdomycin while the second is not, then the antibiotic should inhibit only a part of the uptake of deoxycytidine by the membrane vesicles from parent cells.

However, it is shown that showdomycin inhibits all uptake by the membrane vesicles from parent cells. Although resolution of this paradox must await further studies on the mechanism of uptake of deoxycytidine by the membrane vesicles, possible explanations for these facts may be either that the second deoxycytidine transport system is repressed by showdomycin which entered into the vesicles by the first deoxycytidine transport system, or that deoxycytidine which entered into the vesicles by the second deoxycytidine transport system rapidly effluxes from the vesicles in the presence of the antibiotic.

Preliminary results recently obtained in our laboratory revealed that the isolated membrane vesicles from parent cells show activity for taking up [^{14}C]showdomycin which is markedly stimulated by reduced phenazine methosulfate, and is strongly inhibited by deoxycytidine and adenosine but not significantly by guanosine, and that the isolated membrane vesicles of the mutant (Shm^r-001) show no significant activity for taking up [^{14}C]showdomycin, regardless of the presence of reduced phenazine methosulfate.

These results strongly support the conclusion that showdomycin is transported by the first deoxycytidine transport system but not by the second deoxycytidine transport system.

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