Biochimica et Biophysica Acta, 646 (1981) 309–319
Elsevier/North-Holland Biomedical Press

BBA 79348

URIDINE AND URACIL TRANSPORT IN *ESCHERICHIA COLI* AND TRANSPORT-DEFICIENT MUTANTS

SUMITRA ROY-BURMAN and DONALD W. VISSER

Department of Biochemistry, University of Southern California, School of Medicine, 2025 Zonal Avenue, Los Angeles, CA 90033 (U.S.A.)

(Received December 1st, 1980)
(Revised manuscript received April 14th, 1981)

Key words: Uridine transport; Uracil transport; Transport-deficient mutant; (E. coli)

Mutants of *Escherichia coli* K-12 which are defective in components of transport systems for uracil and uridine were isolated and utilized to characterize the transport mechanism of uracil and uridine. Mutant U^-, isolated from a culture of the parent strain, is resistant to 5-fluorouracil and is deficient in the uracil transport system. Mutant UR^-, isolated from a culture of the parent strain, is resistant to a low concentration of showdomycin and lacks the capacity to transport intact uridine. Mutant U^-UR^- isolated from a culture of mutant U^-, is resistant to a low concentration of showdomycin and is defective in both uracil and intact uridine transport processes. Mutant UR^-R^- was isolated from a culture of mutant UR^-, and is resistant to a high concentration of showdomycin. This mutant is defective for transport of intact uridine and in addition lacks the transport system for the ribose moiety of uridine. Characteristics of uracil and uridine transport in parent and mutant cells demonstrate the existence of specific transport processes for uracil, intact uridine and the uracil and ribose moieties of uridine. Mutants U^- and UR^-, which are defective for uracil transport, lack uracil phosphoribosyltransferase activity and retain a small but significant capacity to transport uracil. The data support the conclusion that uracil is transported by two mechanisms, the major one of which requires uracil phosphoribosyltransferase activity, while the other process involves the transport of uracil as such. The characteristics of uridine transport in parent and mutant strains show that, in addition to transport as the intact nucleoside, uridine is rapidly cleaved to the uracil and ribose moieties. The latter is transported into the cell by a process which, in contrast to transport of intact uridine, does not require an energy source. The uracil moiety is released into the medium and is transported by the uracil transport system. Whole cells of the parent and mutant strains differ in their ability to cleave uridine even though cell-free extracts of all the strains have similar uridine phosphorylase activity. The data implicate a uridine cleavage enzyme in a group transport of the ribose moiety of uridine, a process which is nonfunctional in mutants which lack the capacity to transport the ribose moiety of uridine. A common transport component for this process and the transport of intact uridine is indicated by similarities in the inhibitory effects of heterologous nucleosides on these processes.

Introduction

Two processes have been reported for the transport of nucleosides in bacteria. One of these, the transport of intact nucleoside, has been firmly established by the demonstration that strains of *Escherichia coli,* which cannot metabolize cytidine and uridine, retain the capacity to transport and concentrate these nucleosides several hundred-fold intracellularly [1,2]. These findings provide clear confirmation of other reports, indicating the existence of this trans-

Abbreviation: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

port system [3–7]. Intact nucleoside transport is energy-dependent and, in the case of uridine or cytidine, is energized by either electron transport or by ATP hydrolysis via $(Ca^{2+} + Mg^{2+})$-ATPase [8].

Another transport process for nucleosides has been reported in which the ribose and base moieties are transported separately. Evidence has been presented that uridine, adenosine and inosine are cleaved by appropriate nucleoside phosphorylases in *Salmonella typhimurium* [9,10], *E. coli* [9] and *Bacillus cereus* [11] in a translocation process whereby the ribose moiety is transported into cells or membrane vesicles while the base remains in the medium. Rader and Hochstadt [10] have proposed a transmembranal orientation of nucleoside phosphorylases as a possible mechanism for this process. Transport of the base moiety released from nucleoside cleavage may occur as the free base [4,12,13] or may require interaction of the base with an appropriate membrane-associated phosphoribosyltransferase which converts these bases into their monophosphates as they are transported into the cell [9,10]. Transport of the base moiety of the nucleosides released into the medium requires an energy source [4,8], whereas transport of the ribose moiety into the cell does not [8].

The transport processes for the purines and pyrimidines released into the medium from nucleosides are distinct from those involved in nucleoside transport [7,12]. Therefore, the observed uptake of radioactivity from ^{14}C-base labeled nucleosides is not a true measurement of intact nucleoside transport because the values include an indeterminate uptake of the base moiety subsequent to nucleoside cleavage. This problem has been circumvented by the use of mutants lacking nucleoside metabolizing enzyme [1,14] or by measurement of transport in mutants of *E. coli* which lack functional components involved in the transport of the free base or the intact nucleoside [7]. The latter approach has been utilized in the present study of uracil and uridine transport. Mutants of *E. coli* K-12 were isolated which are resistant to 5-fluorouracil and showdomycin and were shown to be defective in various components of uracil and uridine transport systems. The antibiotic, showdomycin, was chosen because it is closely related to uridine structurally [15,16] and is known to require the transport system for uridine before exerting its inhibitory effects as a nonspecific sulfhydryl reagent [3]. These mutants were utilized in transport studies at conditions which allow quantitation of the two transport processes for uridine and the determination of transport characteristics which are common to both.

There are conflicting conclusions regarding the mechanism involved in the transport of uracil in *E. coli*. Some reports [9,10,17] support the concept that uracil is transported in *E. coli* by a group translocation process involving uracil phosphoribosyltransferase in which uracil is converted to UMP during the transport process. Other workers have provided evidence [4,13] that the transport of uracil in *E. coli* may function without direct involvement of uracil phosphoribosyltransferase enzyme activity. In the present study an attempt was made to resolve these divergent conclusions by the use of several mutants of *E. coli* K-12 which are deficient in uracil phosphoribosyltransferase enzyme activity.

Materials and Methods

Materials. [8-^{14}C]Guanine was obtained from Schwarz/Mann and [U-^{14}C]adenosine was obtained from Amersham. All other ^{14}C-labeled compounds were obtained from New England Nuclear. Tetrahydrouridine was supplied by Dr. Harry B. Wood, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute.

Isolation of mutants and growth of cells. Mutant strains were derived from *E. coli* KL333Hfr, *leu*$^-$, *lacZ*$^-$, designated to KL333 (Table I). The mutant colonies were isolated by plating 10^7 to 10^8 cells in minimal medium containing glucose [18] and either 5-fluorouracil or showdomycin. The frequency of mutation for each of the mutant types was within the range, indicating a single genetic alteration. Each of the mutants was identified as HfR positive and retained dependence upon leucine for growth. The ability of mutations to be transferred by phase p*1* mediated transductions was tested by the method of Lennox [19]. The mutations were transducible in each of the strains. Twenty or more colonies of each mutant were selected and tested for uridine phosphorylase, uridine kinase and uracil phosphoribosyltransferase enzyme activities. Each mutant was also tested for its ability to transport [2-^{14}C]uridine and [2-^{14}C]uracil at standard assay conditions. All of the

mutant colonies selected in the presence of the same inhibitor conditions exhibited identical transport characteristics and enzyme activities. More extensive transport studies were carried out with a randomly selected colony from each of the mutant types. A list of the mutant strains and their enzyme activities are given in Table I. Strain BS255 Hfr H, *Upp, Udp, deoR5, Cm1A,* strain SØ742 F⁻, *Udp, Udk* and strain SØ676 F⁻, *Udp* were obtained from Dr. A. Munch-Petersen [1,2].

All strains were grown aerobically at 37°C in minimal medium [18] with glucose as the energy source. Cells were harvested in mid-log phase and washed twice by centrifugation in minimal medium [18] without glucose.

Assay for uptake. Uptake was assayed as described previously [7] with certain modifications. The incubation mixture was preincubated with 5 mM glucose for 10 min prior to the addition of ^{14}C-labeled nucleosides or bases. The specific activities of radioactive uracil and nucleosides were as follows: [2-^{14}C]uracil, 2–50 Ci/mol; [2-^{14}C]uridine, 2–50 Ci/mol; [U-^{14}C]uridine, 20–50 Ci/mol; [8-^{14}C]-adenosine, 54 Ci/mol; [U-^{14}C]adenosine, 324 Ci/mol; [2-^{14}C]cytidine, 20 Ci/mol; [2-^{14}C]thymidine, 20 Ci/mol; [8-^{14}C]deoxyadenosine, 54 Ci/mol; [8-^{14}C]-guanosine, 20 Ci/mol; [8-^{14}C]guanine, 58 Ci/mol; [8-^{14}C]hypoxanthine, 55 Ci/mol; [8-^{14}C]adenine, 50 Ci/mol; and thymine, 10 Ci/mol. Uracil or heterologous nucleosides, when present, were added at the same time as the radioactive substrate. Tetrahydrouridine, an inhibitor of cytidine deaminase [4], was added just prior to the incubation period when cytidine was used as substrate or inhibitor. After incubation for the indicated period of time, the reactions were terminated, and the cells were collected on Millipore filters and washed once as described previously [20], except that dilution of the reaction mixtures and the washing procedures were carried out at room temperature. Measurement of radioactivity was carried out as described previously [3], except as noted below. In assays of uptake from [U-^{14}C]uridine, scintillation fluid was added directly to the wet filters immediately after the wash procedures and then the radioactivity was measured. This procedure prevented intracellular oxidation of the compounds derived from the ribose moiety of the [U-^{14}C]uridine [7].

Analysis of products in the medium. Radioactive components in the medium were analyzed as described previously [3], except that the medium was collected immediately after the indicated incubation time periods and heated in a boiling water bath for 2 min without prior dilution with minimal medium [18]. The products were routinely separated by thin-layer chromatography on PEI-cellulose plates with 86% butanol in water (v/v). Phosphorylated compounds remained at the origin in this system [7].

Enzyme assay. Cells were harvested in late-log phase and washed twice with minimal medium. Washed cells were suspended in 5 vol. of 50 mM potassium phosphate buffer (pH 7.5), sonically disrupted at 0°C and centrifuged at 10000 rev./min for 20 min as previously described [21]. The supernatant was used for measurement of uracil phosphoribosyltransferase, uridine phosphorylase and uridine kinase enzyme activities.

Uracil phosphoribosyltransferase enzyme activity was assayed by a procedure similar to that described for adenine phosphoribosyltransferase [21], except that 10 mM GTP was added to the reaction mixture [22]. Separation of uracil and UMP was carried out by thin-layer chromatography on PEI-cellulose plates using water as solvent. Uridine phosphorylase enzyme activity was measured by the procedure of Razell [23], except that [2-^{14}C]uridine was used and uridine and uracil were separated by thin-layer chromatography on PEI-cellulose plates with 86% butanol in water (v/v). Uridine kinase activity of the crude extract was measured by the procedure of Skold [24]. The products were separated by thin-layer chromatography on PEI-cellulose plates with water.

Results

Uracil uptake

A comparison of uracil uptake by various strains of *E. coli* K-12 is shown in Table II. Uracil uptake in mutants KL333U⁻, KL333U⁻UR⁻ and KL333UR⁻U⁻, each of which lacks uracil phosphoribosyltransferase activity (Table I), is less than 2% that observed in the parent strain, KL333. Values for uracil uptake and uracil phosphoribosyltransferase activity in KL333UR⁻ are similar to the corresponding values in the parent strain. Uptake of uracil in KL333UR⁻R⁻ is about 70% that in strain KL333, and uracil phos-

TABLE I

LIST OF STRAINS AND THE ACTIVITIES OF URACIL PHOSPHORIBOSYLTRANSFERASE, URIDINE PHOSPHORYLASE AND URIDINE KINASE

Enzyme activities were measured by methods described in the text

Strain	Activity (%)			
	Uracil phospho-ribosyltransferase	Uridine phosphorylase	Uridine Kinase	Derivative or source
KL333	100 (32) [f]	100 (90) [f]	100 (1.6) [f]	– [a]
KL333U⁻	3	100	100	KL333 [b]
KL333UR⁻	100	98	100	KL333 [c]
KL333U⁻UR⁻	3	100	107	KL333U⁻ [c]
KL333UR⁻U⁻	4	96	100	KL333UR⁻ [d]
KL333UR⁻R⁻	101	94	102	KL333UR⁻ [e]

[a] KL333, Hfr, *leu⁻-40*, *lacZ⁻* (Norkin, L.C. (1970) J. Mol. Biol. 51, 633–655).
[b] By selection for resistance to 2 μM 5-fluorouracil.
[c] By selection for resistance to low concentration of showdomycin (2 μg/ml).
[d] By selection for resistance to 2 μM 5-fluorouracil.
[e] By selection for resistance to high concentration of showdomycin (20 μg/ml).
[f] Activities of each enzyme in KL333 were taken as 100%. Figures in parentheses indicate specific activities (nmol of substrate converted/min per mg of protein).

phoribosyltransferase activity in both strains is similar. Uptake of adenine, guanine, thymine and hypoxanthine in strain KL333UR⁻R⁻ is about 30% to 50% lower than the corresponding uptake values observed in KL333 (Table II). Uptake values of these bases in all other strains are similar to those in KL333.

It was observed that the very low uptake of uracil in strain KL333U⁻ is stimulated several-fold in the presence of inosine or adenosine (Table III). The intracellular products formed from [2-^{14}C]uracil under the above conditions were analyzed and it was found that more than 90% of the radioactivity was present as UTP (data not shown). It is possible that the stimulatory effect is due to the generation of

TABLE II

UPTAKE OF URACIL, ADENINE, GUANINE, HYPOXANTHINE AND THYMINE BY VARIOUS STRAINS OF *E. COLI*

Uptake was measured under standard assay conditions with a 30-s incubation period. The final concentrations of various substrates in the incubation mixture were as follows: uracil, 0.5 μM; adenine, 4.3 μM; guanine, 0.91 μM; hypoxanthine, 5.0 μM; and thymine, 5.0 μM

Strain	Uptake (%) [a]				
	Uracil	Adenine	Guanine	Hypoxanthine	Thymine
KL333	100 (244) [b]	100 (947) [b]	100 (648) [b]	100 (1250) [b]	100 (13) [b]
KL333U⁻	1.8	100	107	n.d. [c]	96
KL333UR⁻	100	99	94	99	90
KL333U⁻UR⁻	0.6	106	48	n.d. [c]	123
KL333UR⁻U⁻	0.8	n.d. [c]	n.d. [c]	n.d. [c]	n.d. [c]
KL333UR⁻R⁻	70	56	67	48	n.d. [c]

[a] Values are expressed as percentage of uptake in KL333 (100%).
[b] Values in the parentheses represent pmol uptake/30 s.
[c] n.d., not determined.

TABLE III

EFFECT OF INOSINE AND ADENOSINE ON THE UPTAKE OF URACIL AND URIDINE BY KL333 AND KL333U⁻

Uptake was measured under standard assay conditions. 0.5 μM uracil or uridine was used

Substrate	Addition	Incubation	Uptake (pmol)	
			KL333	KL333U⁻
[2-^{14}C]Uracil	None	10 s	98	1
	4 mM inosine	10 s	87	14
	None	30 s	232	4
	4 mM inosine	30 s	198	33
	4 mM adenosine	30 s	190	21
	None	2 min	400	12
	4 mM inosine	2 min	319	43
	4 mM adenosine	2 min	391	48
	4 mM adenosine plus 30 μM CCCP	2 min	n.d. [a]	0.3
	4 mM adenosine plus 2 mM KCN	2 min	10	7
[2-^{14}C]Uridine	None	30 s	198	59
	4 mM inosine	30 s	39	15
	4 mM adenosine	30 s	8	1
	None	2 min	411	25.6
	4 mM inosine	2 min	128	2.2
	4 mM adenosine	2 min	25	2

[a] n.d., not determined.

intracellular ribose 1-phosphate from adenosine or inosine by the action of purine-nucleoside phosphorylase. The increase in ribose 1-phosphate concentration may facilitate the formation of uridine from uracil intracellularly by the action of uridine phosphorylase and the subsequent trapping of uridine as nucleotides in the cell. Uptake of uracil in the presence of adenosine is almost completely inhibited by KCN or CCCP (Table III).

It is also possible that the purine nucleosides may provide an extracellular source of ribose 1-phosphate which, upon reaction with [^{14}C]uracil, forms [^{14}C]uridine. In this case, the stimulatory effect of the purine nucleoside would result in an increase in the observed uptake of radioactivity from uracil which would represent uridine rather than uracil uptake. This possibility is eliminated by the finding (Table III) that uridine uptake is drastically reduced in the presence of adenosine or inosine. For example, in the presence of 4 mM adenosine, 1–2 pmol of uridine is transported, whereas 20–48 pmol of uracil is transported under the same conditions (Table III).

Uptake of nucleosides by strain KL333 and mutant strains

A comparison of uridine, guanosine, thymidine, cytidine, deoxyadenosine and adenosine uptake by the various strains of *E. coli* is given in Table IV. Uptake of radioactivity from [2-^{14}C]uridine by KL333UR⁻ is 77% as compared to the parent strain. This value is reduced to 6% in the presence of 1 mM uracil, a condition which was designed to limit uptake of radioactivity from [2-^{14}C]uracil formed in the medium from the uracil moiety of uridine. The same low level uptake (6%) from [2-^{14}C]uridine in KL333U⁻ is 30% that in the parent strain and is unchanged by the presence of uracil. The presence of uracil reduces uptake of radioactivity from [2-^{14}C]-uridine in the parent strain to 32% of uptake observed in the absence of uracil. This reduced uptake value approximates uptake of radioactivity from [2-^{14}C]uridine in KL333U⁻ in the absence of uracil. Uptake of cytidine is about 40% and 8% in KL333UR⁻ and KL333UR⁻R⁻, respectively, as compared to that in the parent strain KL333. Under simi-

TABLE IV

UPTAKE OF NUCLEOSIDES BY VARIOUS STRAINS OF *E. COLI*

Uptake was measured under standard assay conditions with 30-s incubation. The final concentration of the substrates were as follows: [2-^{14}C]uridine, 5 μM; [8-^{14}C]guanosine, 2 μM; [2-^{14}C]thymidine, 5 μM; [2-^{14}C]cytidine, 5 μM; [8-^{14}C]deoxyadenosine, 2 μM; and [8-^{14}C]adenosine, 2 μM. Assays for cytidine uptake were carried out in the presence of 205 μM tetrahydrouridine [1]

Substrate	Uptake (%) [a]				
	KL333	KL333U$^-$	KL333UR$^-$	KL333U$^-$UR$^-$	KL333UR$^-$R$^-$
Uridine	100 (932) [b]	30	77	6	28
Uridine plus 1 mM nonlabeled uracil	32	30	6	6	6
Cytidine	100 (900) [b]	95	40	n.d. [c]	8
Guanosine	100 (1590) [b]	96	108	104	46
Thymidine	100 (128) [b]	95	88	90	25
Deoxyadenosine	100 (1285) [b]	96	108	88	44
Adenosine	100 (1115) [b]	98	88	92	44

[a] Nucleoside uptake by KL333 taken as 100%.

[b] pmol/30 s.

[c] n.d., not determined.

lar conditions uptake of deoxycytidine in KL333UR$^-$ is about 42% that in strain KL333 (data not shown in any table). Uptake of adenosine, guanosine, deoxyadenosine and thymidine is not altered appreciably in any of the mutant strains except KL333UR$^-$R$^-$ which exhibits a defect in transport of all of the nucleosides tested.

Uptake of ribose moiety

In the absence of glucose the uptake of [2-^{14}C]-uridine is less than 1% of that observed in the presence of glucose in all strains tested (Table V; Ref. 7). Therefore, the uptake of radioactivity from [U-^{14}C]-uridine in the absence of glucose represents transport of the ribose moiety of uridine only (Table V). Transport of the ribose moiety of uridine, which is similar in KL333 and KL333U$^-$, is reduced to 33% in KL333UR$^-$ and is negligible in KL333UR$^-$R$^-$ (Table V). Uptake of the ribose moiety of uridine in parent and mutant strains is similar in the presence of glucose. Uptake of the ribose moiety of adenosine is similar in all strains except in KL333UR$^-$R$^-$, where it is reduced to 40% that in KL333 (Table V).

A comparison of the transport of intact uridine with the transport of ribose moiety of uridine is provided in studies of the inhibitory effects of heterologous nucleosides on these processes (Table VI). Mutant KL333U$^-$ was used to determine the inhibitory effects on the uptake of intact uridine because this mutant lacks the capacity to transport the uracil moiety of uridine. Therefore, the observed inhibitory effects of heterologous nucleosides on uptake of radioactivity from [2-^{14}C]uridine represent inhibition of intact uridine only. Mutant KL333U$^-$UR$^-$ was used for measurements of the inhibitory effects on the uptake of the ribose moiety of uridine only. The uptake of both intact uridine and the ribose moiety of uridine is linear at concentrations up to 5 μM uridine and at a 10-s time period [7]. Therefore, the inhibitory effects of heterologous nucleosides were determined under these standard conditions at which uptake is sensitive to the presence of inhibitors. The inhibitory effects of a 5-fold molar excess of adenosine, deoxyadenosine, thymidine, deoxyuridine, guanosine and deoxyguanosine on transport of intact uridine are similar to the corresponding effects on the transport of the ribose moiety of uridine (Table VI). Neither guanosine nor deoxyguanosine inhibits uridine transport, whereas these purine nucleosides inhibit transport of the ribose moiety 44% and 31%, respectively. Adenosine, deoxyadenosine, thymidine and deoxyuridine inhibit transport of intact uridine 40% or more, adenosine being the most potent (70%). In each case, the corresponding inhibitory effects of

TABLE V

COMPARISON OF UPTAKE OF BASE AND RIBOSE MOIETY OF URIDINE AND ADENOSINE BY VARIOUS MUTANTS OF KL333

The uptake was measured under standard assay conditions in the presence of 5 μM uridine or adenosine. Uptake of base moiety was obtained from the total uptake of radioactivity from [2-^{14}C]uridine or [8-^{14}C]adenosine. Uptake of the ribose moiety was obtained by subtraction of radioactivity due to retention of the base moiety from the total retention of radioactivity from [U-^{14}C]uridine or [U-^{14}C]adenosine

Strain	Addition 5 mM glucose	Uptake from uridine (%) [a]		Uptake from adenosine (%) [a]	
		Uracil moiety	Ribose moiety	Adenine moiety	Ribose moiety
KL333	–	2	119	1	162
	+	100 (700) [b]	100 (1568) [b]	100 (1228) [b]	100 (700) [b]
KL333U$^-$	–	1	118	2	164
	+	35	112	101	113
KL333UR$^-$	–	0.8	33	1	192
	+	77	35	118	118
KL333U$^-$UR$^-$	–	0.7	33	n.d. [c]	n.d. [c]
	+	3	30	n.d. [c]	n.d. [c]
KL333UR$^-$R$^-$	–	0.6	1	0.2	65
	+	22	2	26	n.d. [c]

[a] Uptake by KL333 in the presence of 5 mM glucose was taken as 100%.
[b] Uptake pmol/30 s.
[c] n.d., not determined.

TABLE VI

EFFECT OF HETEROLOGOUS NUCLEOSIDES ON THE UPTAKE OF INTACT URIDINE IN KL333U$^-$ AND UPTAKE OF RIBOSE MOIETY OF URIDINE IN KL333U$^-$UR$^-$

Uptake was measured under standard assay conditions. Uridine was present at 5 μM. Each heterologous nucleoside was present at 25 μM. Tetrahydrouridine (205 μM) was added when cytidine was present in the reaction mixture. [2-^{14}C]-Uridine was used for the determination of uridine uptake in KL333U$^-$ and [U-^{14}C]uridine was used for the determination of ribose moiety uptake in KL333U$^-$UR$^-$. Incubation was for 10 s

Inhibitor	Uptake (%) [a]	
	Uridine (KL333U$^-$)	Ribose moiety of uridine (KL333U$^-$UR$^-$)
None	100 (205) [b]	100 (270) [b]
Adenosine	29	40
Deoxyadenosine	36	54
Guanosine	108	56
Deoxyguanosine	91	69
Cytidine	7	40
Thymidine	54	57
Deoxyuridine	52	60

[a] Uptake in the absence of inhibitor was taken as 100%.
[b] pmol uptake/30 s.

these nucleosides on uptake of the ribose moiety of uridine are somewhat less than the corresponding effects on uridine uptake. Cytidine inhibits intact uridine uptake more than 90%, whereas uptake of the ribose moiety of uridine is inhibited about 60%.

The possible involvement of uridine phosphorylase on transport of the ribose and uracil moieties of uridine was investigated by determination of the amount of uridine, uracil and ribose 1-phosphate present in the medium after incubation of the parent and mutant strains in the presence of [U-^{14}C]uridine at standard assay conditions. It was found that even though uridine phosphorylase activity in cell-free homogenates of the parent and all mutant strains is similar (Table I), the degradation of uridine in the medium varies considerably. After 30 s incubation in KL333UR$^-$R$^-$, less than 5% of total uridine in the medium is degraded, whereas more than 60% uridine remaining in the medium is degraded in KL333, KL333U$^-$ and KL333UR$^-$. Uptake of ribose moiety was also measured in strains SØ743 and SØ676, which are defective in uridine phosphorylase. The uptake was found to be negligible in the presence or absence of glucose (data not shown).

Discussion

The results (Tables I and II) provide a partial resolution of the conflicting conclusions regarding the mechanism of uracil transport in *E. coli.* Previous evidence [9,10,17] that a major mechanism for uracil transport involves a group translocation process involving uracil phosphoribosyltransferase is consistent with the data (Tables I, II) showing that each of the 5-fluorouracil-resistant strains (KL333U$^-$) lacks uracil phosphoribosyltransferase and exhibits a limited ability to take up radioactivity from [2-^{14}C]-uracil. Subsequent to reports that *E. coli* B is capable of uracil transport even though this strain lacks uracil phosphoribosyltransferase activity [4], Burton [13] demonstrated the presence of this enzyme in *E. coli* B when the assay was carried out in the presence of GTP, a strong activator of the enzyme. We have confirmed these results. Therefore, it is clear that uracil phosphoribosyltransferase is important for uptake of radioactivity from [2-^{14}C]uracil at conditions wich measure initial rate of transport. The extent to which uracil phosphoribosyltransferase enzyme activity is utilized in a membrane-bound translocation process or, as suggested by Burton [13], is required for intracellular trapping of uracil as UMP, is not established by the present data.

A second system for the transport of uracil as such into the cell is also suggested by the data of Tables I and III. Although uracil uptake by KL333U$^-$ is only about 1% that in the parent strain at the 10-s time period, this relatively low value must represent transport since the radioactivity was found in the acid-soluble cell fraction predominantly as UTP. The stimulatory effect of inosine or adenosine on uracil uptake in KL333U$^-$ may be explained by an increase in the trapping of uracil as nucleotides by the sequential action of uridine phosphorylase and uridine kinase after transport of uracil into the cell. Acceleration of these reactions would be expected to result from an increase in the intracellular concentration of the limiting substrate, ribose 1-phosphate, derived from the purine nucleosides transported into the cells. This explanation is strengthened by the observation that in strain BS255, which is defective in both uracil phosphoribosyltransferase and uridine phosphorylase activities, uracil uptake is negligible and is not increased by either adenosine or inosine (data not shown in tables). An alternative possibility that uridine is formed extracellularly by interaction of uracil with ribose 1-phosphate produced from the purine nucleosides is untenable. Uridine formed extracellularly in this manner could be transported in KL333U$^-$, but the transport of any small amount of uridine formed extracellularly in this manner is strongly inhibited by the presence of the purine nucleosides (Table III). In addition, it has been shown that purine nucleoside phosphorylases in *E. coli* are intracellular enzymes [2]. Thus, two processes for uracil transport exist in *E. coli.* One process requires uracil phosphoribosyltransferase activity, the second process is quantitatively less important and involves transport into the cell as free uracil. The low level of uracil uptake observed in the KL333U$^-$ mutants may not be an accurate reflection of the quantitative importance of this process since extracellular uracil is not rapidly converted to uridine and its phosphates due to limitations of the required substrate, ribose 1-phosphate.

It is known that showdomycin, a nucleoside analog structurally related to uridine, exerts its inhibitory effect only after transport into the cell by the uridine transport system [3,20]. It is, therefore, plausible to expect that each of the KL333UR$^-$ mutants is resistant to the antibiotic because of a defect in uridine transport. It is also reasonable to expect that KL333U$^-$ strains, which lack uracil phosphoribosyltransferase activity and a major fraction of their ability to transport uracil (Tables I and II), retain their capacity to transport intact uridine. The data in Table IV clearly demonstrate that these expectations are correct even though, as compared to the parent strain, KL333U$^-$ shows a 70% decrease in uptake of radioactivity from [2-^{14}C]uridine, and KL333UR$^-$ loses only 23% of its uptake capacity for [2-^{14}C]uridine. The data of Table IV clearly shows that the observed capacity of strain KL333UR$^-$ to transport radioactivity from [2-^{14}C]uridine must result from the release of uracil by cleavage of uridine and the subsequent transport of this uracil moiety into the cell. When extracellular uracil is added, uptake of radioactivity from [2-^{14}C]uridine is reduced to 6%, the same low level of uptake observed in KL333U$^-$UR$^-$, which lacks the capacity for both uracil and intact uridine transport. The loss by KL333U$^-$ of 70% of its capacity to transport radioactivity from [2-^{14}C]uridine, as compared to the

parent strain, may be explained by the inability of KL333U⁻ to transport uracil released into the medium from uridine. The addition of exogenous uracil does not alter uptake of radioactivity from [2-^{14}C]-uridine in KL333U⁻, showing that uracil has no effect on transport of intact uridine. It may be concluded that uptake of radioactivity from [2-^{14}C]uridine in KL333U⁻ represents only transport of intact uridine, and that uptake of radioactivity from [2-^{14}C]uridine in the parent strain consists of the transport of intact uridine (23% to 30%) and the uracil moiety (70% to 77%) which is released into the medium from uridine. The production of uracil in the medium occurs extracellularly and not by the intracellular cleavage of uridine by uridine phosphorylase and subsequent release of uracil into the medium. This is demonstrated by the following data. In the absence of glucose uridine or uracil is not transported but a substantial amount of ribose moiety from uridine is transported (Table V; Refs. 7 and 8). Under this condition uracil moiety remains in the medium. In addition, uptake of [2-^{14}C]uridine by strain SØ676 measured under the assay condition of Table IV is inhibited about 70% by the addition of 1 mM uracil (data not shown in any table). Since this strain lacks uridine phosphorylase [1] these data may suggest the presence of a periplasmic or membrane-integrated uridine cleavage enzyme. Although transport of intact uridine is lost completely in strain KL333UR⁻, the transport of guanosine, adenosine, deoxyadenosine, thymine and various bases, including uracil, is not altered appreciably in this strain (Tables II, IV). Thus, the transport process for intact uridine is specific and separate from the transport systems for other nucleosides and bases.

The characteristics of [2-^{14}C]uridine and [U-^{14}C]-uridine uptake in strain KL333U⁻UR⁻ (Table V) clearly demonstrate the existence of a transport process for the ribose moiety of uridine. This strain lacks the capacity to transport either uracil or intact uridine, as evidenced by the lack of incorporation of radioactivity from either [2-^{14}C]uracil or [2-^{14}C]-uridine (Tables II and IV). Thus, uptake of radioactivity from [U-^{14}C]uridine in this strain must represent uptake from the ribose moiety of uridine only. These data strongly support previous evidence for the existence of a transport process for the ribose moiety of uridine by intact cells. The previous evidence was based on uptake of radioactivity from [U-^{14}C]-uridine in the absence of an energy source (Table V; Refs. 7 and 8) which is required for incorporation of radioactivity from either [2-^{14}C]uracil or [2-^{14}C]-uridine.

A relationship between transport of intact uridine and the ribose moiety of uridine is suggested by the observed defects of KL333UR⁻ and KL333U⁻UR⁻ for transport of the ribose moiety of uridine which accompanies the loss of intact uridine transport in these mutants (Table V). Uptake of the ribose moiety is lost completely in KL333UR⁻R⁻; is partially (65% to 70%) lost in KL333UR⁻ and KL333U⁻UR⁻ and is retained in KL333U⁻. This contrasts with an unimpaired transport of the ribose moiety of adenosine in KL333UR⁻ and KL333U⁻UR⁻ and only a partial defect in KL333UR⁻R⁻. This reduced capacity for transport of the ribose moiety of uridine by the KL333UR⁻ or KL333U⁻UR⁻ mutants is not related to a defect in uridine cleavage since KL333UR⁻ retains the capacity to transport the uracil moiety of uridine (Table IV). In addition, these mutants degrade extracellular uridine to the same extent as the parent strain KL333. In both parent and these mutants about 60% of uridine in the medium is degraded and is found as uracil and a phosphorylated derivative of ribose. A possible explanation for the loss of the ability to degrade extracellular uridine by strain KL333UR⁻R⁻, even though this mutant has the same uridine phosphorylase activity in cell-free extracts, is discussed below.

Involvement of nucleoside phosphorylase has been suggested previously in a group transport process of the ribose moiety of nucleoside in *E. coli* [9], *Salmonella typhimurium* [9,10] and *Bacillus cereus* [11]. This suggested mechanism is consistent with our finding that there is no appreciable uptake of the ribose moiety of uridine in strains SØ676 and SØ742, both of which are defective in uridine phosphorylase [2]. However, strain KL333UR⁻R⁻ contains the same level of uridine phosphorylase as that present in the parent strain (Table I), even though this mutant neither transports the ribose moiety of uridine nor degrades extracellular uridine appreciably in the medium (see Results). Thus, it is likely that either a cleavage enzyme other than uridine phosphorylase is involved in the ribose moiety transport or the orientation of uridine phosphorylase in the membrane of this strain may be altered in a manner which does not

allow cleavage of extracellular uridine. A transmembranal orientation of nucleoside phosphorylase has been suggested by Rader and Hochstadt [10].

Strain KL333UR$^-$ was isolated at a low concentration of showdomycin at a frequency of mutation indicating a single genetic alteration. Each of 12 mutants isolated exhibited identical transport properties, indicating a consistency in the genetic alteration. This indicates that resistance to showdomycin is the result of deletion of a single functional component which is essential for transport of intact uridine and, since transport of the ribose moiety is also impaired (Table V), this functional component may also be involved in the transport of the ribose moiety of uridine. A relationship between these two transport processes for uridine is also indicated by the similarities in the inhibitory effects of adenosine, deoxyadenosine, deoxyuridine and thymidine on the transport of intact uridine and the respective inhibitory effects on the transport of the ribose moiety of uridine (Table VI). These effects are of particular significance since the heterologous nucleosides are not transported by either of the processes involving uridine (Table IV). The data suggest that these heterologous nucleosides interfere with a component which is common to both transport processes involving uridine. Cytidine, one of the exceptions to the effects of the heterologous nucleosides, exerts a much more potent inhibitory effect on transport of intact uridine than that on the ribose moiety of uridine. An explanation for this result may be that cytidine and uridine, in contrast to other nucleosides, have been reported to share a common process for transport of these nucleosides [7]. Guanosine and deoxyguanosine, the other exceptions, inhibit uptake of the ribose moiety only (Table VI). An explanation for this is not apparent.

Strain KL333UR$^-$R$^-$ which was isolated from KL333UR$^-$ at a high concentration of showdomycin shows a defect in another component which is essential for transport of the ribose moiety of uridine and is involved in the transport of all heterologous nucleosides (Table IV). Thus, it appears that these mutants are characterized by a deletion of a transport component which is also involved in the transport of all nucleosides. These results may be explained by a mutation in a functional component involved in the second nucleoside transport system of *E. coli* K-12 [25]. This system is characterized by Komatsu and Tanaka [25] as the 'second deoxycytidine transport system' and by Munch-Petersen and co-workers [2,14] as the *nup G* system. Since this system transports all nucleosides including guanosine and deoxyguanosine a mutation in this system would exhibit a reduction in uptake of all nucleosides as observed in KL333UR$^-$R$^-$.

Acknowledments

This work was supported by a grant, PCM 77-09891, from the National Science Foundation. The authors wish to acknowledge the able technical assistance of Mrs. Rita Chakravorty.

References

1 Mygand, B. and Munch-Petersen, A. (1975) Eur. J. Biochem. 59, 365–372
2 Munch-Petersen, A. and Mygand, B. (1976) J. Cell. Physiol. 89, 551–560
3 Von Dippe, P.J., Roy-Burman, S. and Visser, D.W. (1973) Biochim. Biophys. Acta 318, 105–112
4 Von Dippe, P.J., Leung, K.-K., Roy-Burman, S. and Visser, D.W. (1975) J. Biol. Chem. 250, 3666–3671
5 Doskocil, J. (1974) Biochem. Biophys. Res. Commun. 56, 997–1002
6 Komatsu, Y. (1973) Biochim. Biophys. Acta 330, 206–221
7 Leung, K.-K. and Visser, D.W. (1977) J. Biol. Chem. 252, 2492–2497
8 Roy-Burman, S., Von Dippe, P.J. and Visser, D.W. (1978) Biochim. Biophys. Acta 511, 285–296
9 Hochstadt-Ozer, J. (1974) CRC Crit. Rev. Biochem. 2, 259–309
10 Rader, R.L. and Hochstadt, J. (1976) J. Bacteriol. 128, 290–301
11 Mura, U., Sgarrella, F. and Ipata, P.L. (1978) J. Biol. Chem. 253, 7905–7909
12 Roy-Burman, S. and Visser, D.W. (1975) J. Biol. Chem. 150, 9270–9275
13 Burton, K. (1977) Biochem. J. 168, 195–204
14 Munch-Petersen, A., Mygand, B., Nicolaisen, A. and Phil, N.J. (1979) J. Biol. Chem. 254, 3730–3737
15 Nishimura, H., Mayama, M., Komatsu, H.K., Shimaoka, N. and Tanaka, Y. (1964) J. Antibiotics, Ser. A 17, 148–155
16 Darnall, K.R., Townsend, L.B. and Robins, R.K. (1967) Proc. Natl. Acad. Sci. USA 57, 548–553
17 Hochstadt-Ozer, J. (1972) J. Biol. Chem. 247, 2419–2426
18 Davis, B.D. and Mingioli, E.S. (1950) J. Bacteriol. 60, 17–20

19 Lennox, E.S. (1955) Virology 1, 190
20 Roy-Burman, S. and Visser, D.W. (1972) Biochim. Biophys. Acta 282, 383–392
21 Hochstadt-Ozer, J. and Stadtman, E.R. (1971) J. Biol. Chem. 246, 5294–5303
22 Molloy, A. and Finch, L.R. (1969) FEBS Lett. 5, 211–213
23 Razell, W.E. (1967) in Methods in Enzymology (Grossman, L. and Moldave, K., eds.), Vol. 12, pp. 119–125, Academic Press, New York
24 Skold, O. (1960) J. Biol. Chem. 235, 3273–3279
25 Komatsu, Y. and Tanaka, K. (1973) Biochim. Biophys. Acta 311, 496–506