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Repression and Inhibition of Transport Systems for Branched-Chain Amino Acids in *Salmonella typhimurium*

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Kinetics of the transport systems common for entry of L-isoleucine, L-leucine, and L-valine in *Salmonella typhimurium* LT2 have been analyzed as a function of substrate concentration in the range of 0.5 to 45 μM. The systems of transport mutants, KA203 (*ilvT3*) and KA204 (*ilvT4*), are composed of two components; apparent K_m values for uptake of isoleucine, leucine, and valine by the low K_m component are 2 μM, 2 to 3 μM, and 1 μM, respectively, and by the high K_m component 30 μM, 20 to 40 μM, and 0.1 mM, respectively. The transport system(s) of the wild type has not been separated into components but rather displays single K_m values of 9 μM for isoleucine, 10 μM for leucine, and 30 μM for valine. The transport activity of the wild type was repressed by L-leucine, α-ketoisocaproate, glycyl-L-isoleucine, glycyl-L-leucine, and glycyl-L-methionine. That for the transport mutants was repressed by L-alanine, L-isoleucine, L-methionine, L-valine, α-ketoisovalerate, α-keto-β-methylvalerate, glycyl-L-alanine, glycyl-L-threonine, and glycyl-L-valine, in addition to the compounds described above. Repression of the mutant transport systems resulted in disappearance of the low K_m component for valine uptake, together with a decrease in V_{max} of the high K_m component; the kinetic analysis with isoleucine and leucine as substrates was not possible because of poor uptake. The maximum reduction of the transport activity for isoleucine was obtained after growing cells for two to three generations in a medium supplemented with repressor, and for the derepression, protein synthesis was essential after removal of the repressor. The transport activity for labeled isoleucine in the transport mutant and wild-type strains was inhibited by unlabeled L-alanine, L-cysteine, L-isoleucine, L-leucine, L-methionine, L-threonine, and L-valine. D-Amino acids neither repressed nor inhibited the transport activity of cells for entry of isoleucine.

It is known that isoleucine, leucine, and valine enter into microbial cells through stereospecific transport systems. In *Escherichia coli*, evidence for multiplicity of the transport systems has been provided by kinetic analysis of entry of the branched-chain amino acids as a function of substrate concentration (6, 12, 19, 24) and by genetic analysis using transport-defective and -derepressed mutants (2, 7, 8). The transport activity of cells is repressible by certain amino acids, especially L-leucine (3, 4, 7, 9, 10, 15, 17, 19, 20, 22, 23). The entry of branched-chain amino acids into cells is inhibited by the presence of several amino acids in the medium (7, 8, 12, 16, 20, 22, 24).

To elucidate the nature of the transport systems for branched-chain amino acids in *Salmonella typhimurium* LT2 by genetic and biochemical approaches, Kiritani (11) previously isolated several *ilvT* derivatives, defective in the transport, from an isoleucine-valine-requiring mutant, KA931 (*ilvC8*). We attempted to study the nature of the *ilvT* mutation extensively, by substituting the *ilvC8* locus in the double mutants with the *ilvC*$^+$ locus of the wild-type strain. In this report, we present data of the kinetic analysis of transport activities of the branched-chain amino acids in the resulting *ilvT* and wild-type strains. We also show that entry of isoleucine in cells is inhibited by alanine, cysteine, leucine, methionine, threonine, or valine and that the transport activity of cells is repressed by several glycyl dipeptides as well as by amino acids, although *ilvT* and wild-type strains respond differently to these substances.

MATERIALS AND METHODS

Bacterial strains. The wild-type strain of *S. typhimurium* LT2 and its derivatives were used. Strains KA203 (*ilvT3*) and KA204 (*ilvT4*), defective in the active transport of branched-chain amino acids, were derived from strains CE4 (*ilvT3 ilvC8*)

and CE5 (*ilvT4 ilvC8*), respectively, as indicated below. The genetic and biochemical characters of CE4 and CE5 were described previously (11).

Media. For transport experiments, bacteria were grown in the minimal medium described previously (11). When required, the medium was supplemented with additions as indicated in the text. For an agar medium 1.5% agar was added.

Isolation of *ilvT* prototrophic strains. The *ilvT* prototrophs were isolated after transduction of strains CE4 and CE5 (*ilvT ilvC8*) with P22 phages. The transduction mixture was prepared following the method described previously (11). To obtain *ilvT ilvC8*$^+$ recombinants from a cross between a wild-type donor and strains CE4 or CE5, 0.1-ml volumes of the transduction mixtures were spread on minimal agar plates. After 48 h of incubation at 37°C, colonies that appeared on these plates were isolated and purified by successive single colony isolations. KA203 and KA204, derived from CE4 and CE5, respectively, were thus obtained; the presence of the *ilvT* character was verified by uptake assay for branched-chain amino acids.

Transport assays. Transport was measured by the method described previously (11). For preparing cell suspensions for transport assays, an overnight bacterial culture in minimal medium was diluted one-twentieth with fresh medium or the medium containing appropriate supplements, and cells were grown on a shaker at 37°C. When the optical density of the culture reached 0.3 to 0.4 at 660 nm in a Shimadzu-Bausch and Lomb Spectronic 20 spectrophotometer (about 3×10^8 to 4×10^8 cells/ml), the bacteria were harvested, washed twice by centrifugation with minimal medium, and suspended in minimal medium containing 100 μg of chloramphenicol per ml.

K_m and V_{max} values were calculated by double-reciprocal plotting, $1/V$ and $1/S$, where S is concentration of the substrate expressed in micromoles per liter and V is micromoles of labeled amino acid incorporated per 0.5 min/g of cells (dry weight).

Reagents. Amino acids (Kyowa Hakko Co.) were all L-form, unless otherwise mentioned. D-Amino acids were obtained from Tokyo Casei Industries, Ltd., dipeptides from Tokyo Casei Industries, Ltd., and α-keto acids from Sigma Chemical Co. Uniformly ^{14}C-labeled L-amino acids were obtained from the Radiochemical Centre, Amersham, England.

RESULTS

Kinetics of the entry of branched-chain amino acids into transport mutants and wild type. To elucidate the kinetic behavior of the transport systems of *S. typhimurium*, the initial rate of uptake of labeled branched-chain amino acids was measured in the concentration range of 0.5 to 45 μM. As shown in Fig. 1A and B, a double-reciprocal analysis of the data indicates that the transport systems of valine in *ilvT* mutants are heterogeneous, whereas the system(s) of wild type appears to be homogeneous (Fig. 1C). Apparent K_m and V_{max} values calculated according to the formula of Neal (13) are shown in Table 1. Two transport systems with K_m values of about 1 μM and 0.1 mM for uptake of valine were found in *ilvT* mutants. The K_m for wild type, 30 μM, does not coincide with any

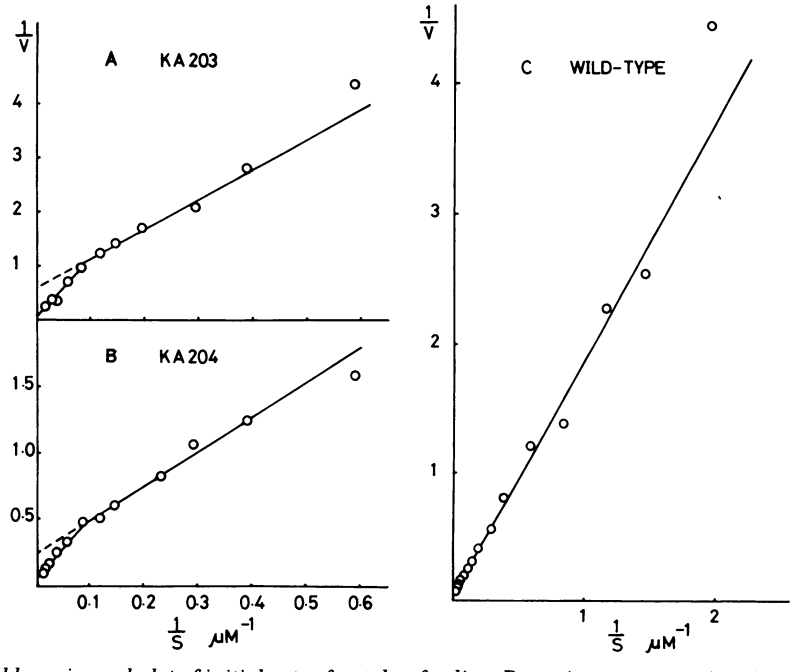

FIG. 1. *Double-reciprocal plot of initial rate of uptake of valine. Bacteria were grown in minimal medium.*

TABLE 1. *Apparent K_m and V_{max} for the transport systems*[a]

Strain	Amino acid in minimal medium	Isoleucine				Valine				Leucine			
		K_m		V_{max}		K_m		V_{max}		K_m		V_{max}	
		1	2	1	2	1	2	1	2	1	2	1	2
KA203	None	1.7	31.0	0.8	1.6	0.7	116	1.1	16.0	1.5	19.1	0.9	1.0
	L-Alanine	NM[b]		NM			75		5.9	NM		NM	
	L-Isoleucine	NM		NM			71		5.9	NM		NM	
	L-Leucine	NM		NM			101		6.7	NM		NM	
	L-Methionine												
	L-Valine	NM		NM			91		7.0	NM		NM	
KA204	None	1.5	34.9	1.6	1.5	1.2	115	1.9	18.1	2.5	43.2	1.7	1.7
	L-Alanine	NM		NM			73		5.3	NM		NM	
	L-Isoleucine	NM		NM			82		10.0	NM		NM	
	L-Leucine	NM		NM			104		10.0	NM		NM	
	L-Methionine	NM		NM			74		3.9	NM		NM	
	L-Valine	NM		NM			85		12.5	NM		NM	
Wild type	None	9.0		8.1		31.6		12.9		10.4		10.8	
	L-Alanine	10.6		7.7		37.6		18.2		13.9		10.6	
	L-Isoleucine	8.7		6.4		26.6		12.3		13.9		7.9	
	L-Leucine	7.3		3.1		27.4		6.2		13.3		3.9	
	L-Methionine	8.7		5.0		39.4		11.1		12.1		10.0	
	L-Valine	9.3		6.2		33.9		12.2		18.0		9.1	

[a] Bacteria were grown in minimal medium with or without 3 mM of a supplement as indicated in the table. Data for K_m (micromolar) and V_{max} (micromoles per 0.5 min/g of cells) are average of three experiments.

[b] NM indicates that K_m and V_{max} values are not measurable because of poor entry of radioisotope in cells.

of the values obtained with *ilvT* mutants. With regard to entry of isoleucine and leucine, similar results were obtained (Table 1). Although double-reciprocal plots of the uptake of branched-chain amino acids in wild type have yielded linear lines, one cannot rule out the possibility that the low and high K_m systems similar to those in *ilvT* mutants are not separated under the present experimental condition.

Repression of the transport activity by L-amino acids and α-keto acids. Transport activity of cells for branched-chain amino acids is repressed in the presence of various amino acids, though *ilvT* mutants and wild-type strain responded differently. As illustrated in Fig. 2, repression of the transport activity of KA204 was observed when cells were grown in the presence of alanine, isoleucine, leucine, methionine, or valine, whereas the repression of wild type was provoked by leucine and slightly by isoleucine, methionine, or valine. In general, the maximum repression of the transport activity in cells was achieved at a concentration of more than 1 mM of these amino acids, except methionine. Essentially identical results were obtained with KA203 cells (data not shown). The other 13 amino acids tested did not repress transport activity. As presented in Fig. 3, *ilvT* mutant cells grown in the presence of leucine lost the activity of the low K_m component for valine transport. Results of kinetic analysis of the transport activity in cells grown in various media are listed in Table 1. The low K_m component for entry of valine was not found in cells of *ilvT* mutants grown on alanine, isleucine, leucine, methionine, or valine, and V_{max} values of the high K_m component were reduced. Since the amounts of isoleucine and leucine taken up in repressed cells of *ilvT* mutants were so small, the kinetic analysis of the active transport was not possible (Fig. 4). V_{max} values for entry of branched-chain amino acids in wild-type cells were reduced when grown with leucine, but not when grown with other amino acids (Table 1).

Although entry of isoleucine in cells was repressed by particular L-amino acids as mentioned above, the entry was not significantly repressed by their D-isomers (Table 2). Among α-keto acids examined, α-ketoisocaproate, a precursor of leucine, was a potent repressor of the transport activity for isoleucine, and α-keto-β-methylvalerate and α-ketoisovalerate, precursors of isoleucine and valine, respectively, repressed the transport activity of KA204, but did not significantly repress that of wild type.

Uptake of labeled isoleucine in *ilvT* mutants decreased after cell multiplication in minimal medium supplemented with alanine, isoleucine, leucine, or valine, whereas that in the wild type decreased significantly in the presence of leucine (Fig. 5). Full repression of the transport activity was attained within 3 h after

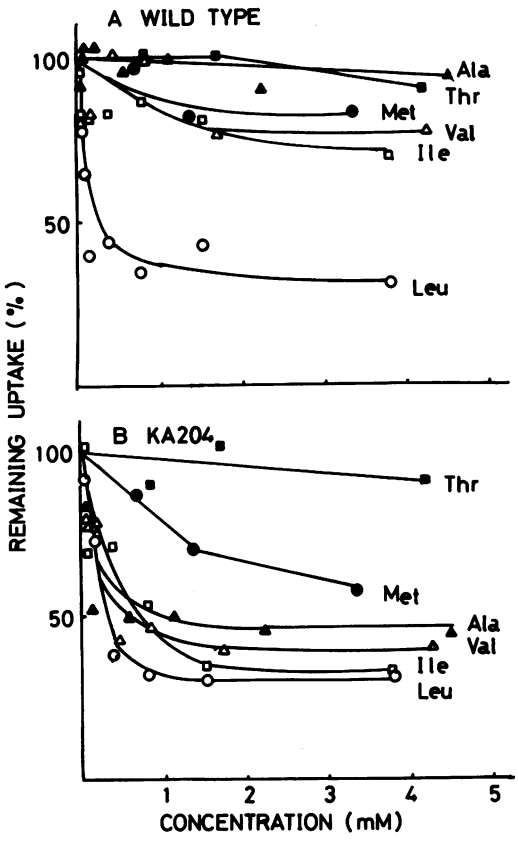

FIG. 2. *Effect of amino acid concentration on repression of [^{14}C]isoleucine uptake. Uptake of [^{14}C]isoleucine by bacteria grown in the presence of the indicated amino acid was measured at 0.5 min; the concentration of [^{14}C]isoleucine was 30 μM and the specific activity was 1.3 × 10^7 cpm per μmol. Uptake of [^{14}C]isoleucine by cells of wild type and KA204 grown in minimal medium was 4.2 and 1.0 μmol per 0.5 min per g of cells, respectively. Abbreviations: Ala, L-alanine; Ile, L-isoleucine; Leu, L-leucine; Met, L-methionine; Thr, L-threonine; Val, L-valine.*

transferring cells in these media. Since the doubling time of cells in these media is about 1 h, the maximal repression was reached after growth of cells for two to three generations. Derepression of the transport activity for entry of isoleucine occurred after removal of repressors, and the activity in cells reached a steady state after 2 to 3 h (Fig. 6). The process of derepression was sensitive to chloramphenicol.

Repression by dipeptides. Dipeptides are taken up by bacterial cells through a transport system(s) distinct from that of free amino acids and cleaved into component amino acids by intracellular peptidase (1, 5, 14, 21). When strain KA204 was grown on glycyl-L-alanine, glycyl-L-isoleucine, glycyl-L-leucine, glycyl-L-methionine, glycyl-L-threonine, or glycyl-L-valine, the transport activity for isoleucine was repressed (Fig. 7B). The activity of wild type was also repressed by each of these dipeptides, except for glycyl-L-alanine, though glycyl-L-threonine and glycyl-L-valine were weak repressors (Fig. 7A). Results obtained with KA203 were not different from those with KA204. In general, the amounts of dipeptides required to induce maximum repression were 1 to 3 mM. The repression of the transport was achieved after growing cells for two to three generations in the supplemented medium, and the process for derepression was sensitive to chloramphenicol (data not shown). When these data obtained with dipeptides were compared with those with amino acids, two differences were noted: (i) glycyl-L-threonine was an effective repressor of KA204, but threonine was not; glycyl-L-isoleucine and glycyl-L-methionine were potent repressors in wild type, but isoleucine and methionine were not; (ii) the degree of

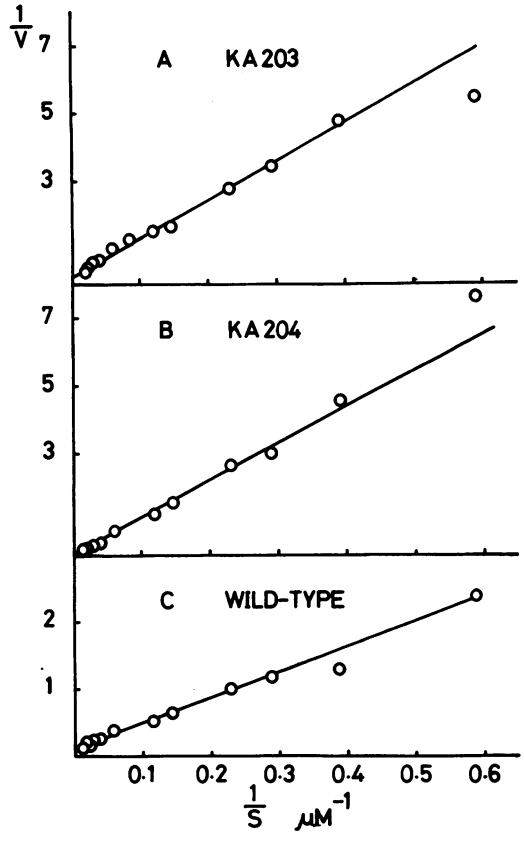

FIG. 3. *Double-reciprocal plot of initial rate of uptake of L-valine. Bacteria were grown in the presence of 1 mM L-leucine.*

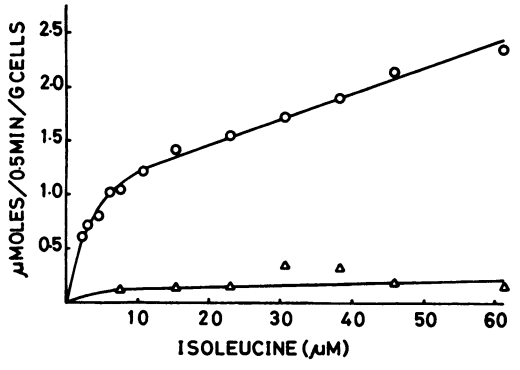

FIG. 4. *Uptake of [^{14}C]isoleucine in KA203 as a function of the concentration. Bacteria were grown in minimal medium* (○) *or in the medium supplemented with 1 mM* L-*leucine* (△). *Specific activity of [^{14}C]isoleucine was 1.2 × 10^7 cpm per μmol.*

TABLE 2. *Repression of transport activity for entry of isoleucine by* D-*amino acids or α-keto acids* [a]

Supplement in minimal medium	Concn (mM)	Uptake relative to control (%)		
		Wild type	KA203	KA204
D-Alanine	5.6	105	95	83
D-Isoleucine	3.8	98	100	108
D-Leucine	3.8	76	85	74
D-Methionine	3.4	81	72	100
D-Valine	4.3	86	86	86
α-Keto-β-methyl-valerate	3.3	77		55
α-Ketoisovalerate	4.3	79		29
α-Ketoisocaproate	3.8	41		5

[a] Initial uptake of [^{14}C]isoleucine by bacteria grown in the presence of the indicated substance was measured at 0.5 min; the concentration of [^{14}C]isoleucine was 30 μM, and the specific activity was 1.2 × 10^7 cpm per μmol. Cells of wild type, KA203, and KA204 grown in the absence of supplement took up 5.8, 1.9, and 1.9 μmol of isoleucine per 0.5 min/g of cells, respectively, and these control values were taken as 100%, respectively. Average values of two experiments are presented.

repression caused by dipeptides was greater than that by corresponding amino acids.

Kinetic analysis revealed that dipeptides mainly decreased the V_{max} values of isoleucine and valine uptake in the wild type, although glycyl-L-methionine did not repress the uptake of valine. K_m values for the transport of wild-type cells were not changed by these repressions, although K_m values for uptake of isoleucine and valine were elevated in cells grown on glycyl-L-leucine. When the transport systems of KA204 cells were repressed by dipeptides, the low K_m component for entry of valine disappeared and V_{max} values of the high K_m component decreased. A maximum reduction in V_{max} of 93% was recorded with cells grown on glycyl-L-leucine.

As shown in Table 4, transport activity of cells for arginine was not affected by the growth on amino acids or dipeptides, which were repressors for the transport systems of branched-chain amino acids. When KA204 and wild-type cells were grown in the presence of glycyl-L-glutamate or glycylglycine, no repression occurred in the transport activity for either isoleucine or arginine.

Inhibition of isoleucine uptake. Entry of labeled isoleucine into cells of *ilvT* mutants and wild type was inhibited by addition of unlabeled alanine, cysteine, isoleucine, leucine, methionine, threonine, or valine in the uptake

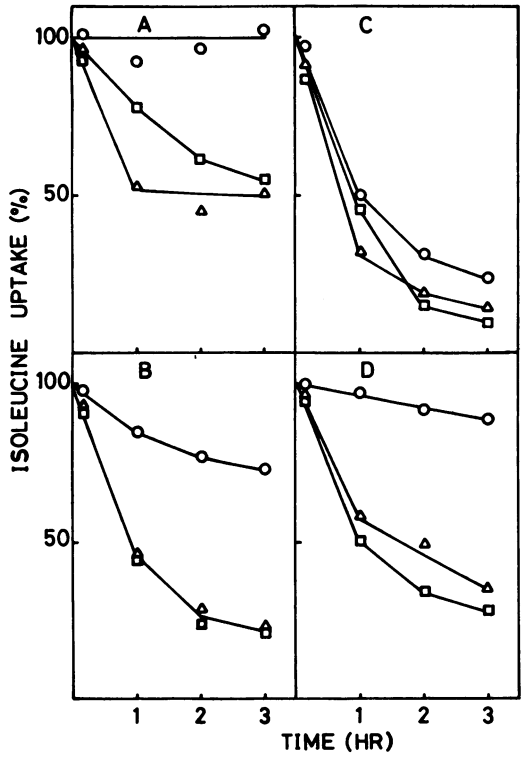

FIG. 5. *Time course for the repression of isoleucine uptake in the presence of 2 mM each of* L-*alanine (A),* L-*isoleucine (B),* L-*leucine (C), or* L-*valine (D). Bacteria grown in minimal medium were harvested in log phase, washed, and grown in the supplemented medium; cells were then sampled at intervals, washed twice, and suspended in minimal medium. Uptake of [^{14}C]isoleucine in the wild type* (○), *KA203* (△), *and KA204* (□) *for 0.5 min was measured; concentration of [^{14}C]isoleucine was 30 μM, and the specific activity 1.2 × 10^7 cpm per μmol. Uptake of [^{14}C]isoleucine by the wild type, KA203, and KA204 at 0 h was 4.7, 1.1, and 1.1 μmol per 0.5 min per g of cells, respectively.*

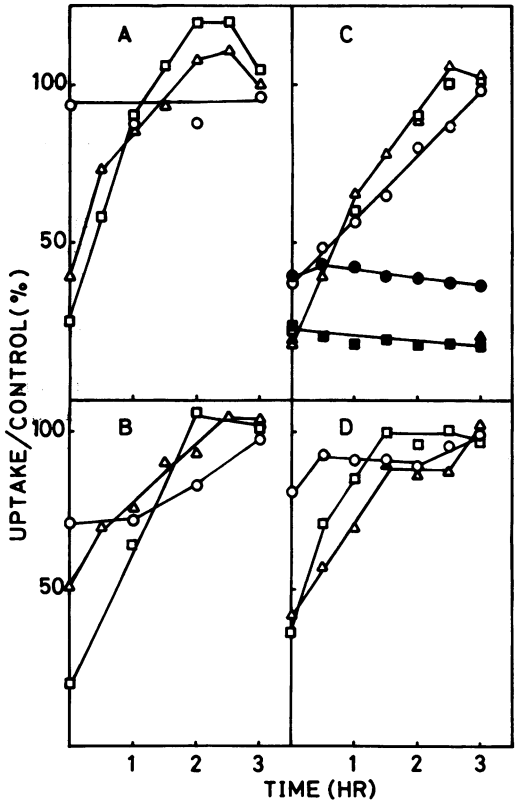

FIG. 6. *Time course for the derepression of isoleucine uptake after removal of* L-*alanine (A),* L-*isoleucine (B),* L-*leucine (C), and* L-*valine (D). Bacteria grown in supplemented media were harvested, washed twice, and grown in minimal medium, and samples were removed at intervals. Uptake of [^{14}C]isoleucine by bacteria for 0.5 min was measured; concentration of [^{14}C]isoleucine was 30 μM, and the specific activity was 1.2 × 10^7 cpm per μmol. In control experiments, uptake of [^{14}C]isoleucine by wild type, KA203 and KA204 cells grown in minimal medium was 5.0, 1.2, and 1.3 μmol per 0.5 min per g of cells, respectively. Symbols: ○, wild type; △, KA203; □, KA204 in the absence of chloramphenicol; ●, wild type; ▲, KA203; ■, KA204 in the presence of 100 μg of chloramphenicol per ml.*

mixture as indicated in Table 5. Although the inhibitory effect by branched-chain amino acids was noticeable at a concentration of 0.3 mM, the inhibition due to alanine, cysteine, methionine, and threonine became apparent at a concentration of 3 mM; the concentration was 100 times higher than that of labeled isoleucine in the uptake mixture. It is of interest that, in the form of a free amino acid or glycyl dipeptide, except for cysteine, these amino acids are potent repressors of the transport systems for branched-chain amino acids in *ilvT* mutants.

Since bacterial growth is strongly inhibited in the presence of more than 1 mM cysteine (data not shown), it is uncertain whether or not the inhibition of isoleucine uptake by cysteine is truly attributable to inhibition of the transport systems. The effect of a dipeptide con-

TABLE 3. *Apparent K_m and V_{max} for the transport systems*[a]

Strain	Dipeptide in minimal medium	[^{14}C]isoleucine		[^{14}C]valine	
		K_m	V_{max}	K_m	V_{max}
Wild type	Glycyl-L-alanine	7.9	8.6	29	11.3
	Glycyl-L-threonine	14.0	7.4	42	9.1
	Glycyl-L-valine	6.6	5.6	35	7.1
	Glycyl-L-methionine	11.5	5.2	30	10.0
	Glycyl-L-isoleucine	12.8	4.0	40	5.3
	Glycyl-L-leucine	21.2	3.4	56	5.2
KA204	Glycyl-L-alanine			134	6.3
	Glycyl-L-threonine			88	5.9
	Glycyl-L-valine			147	6.7
	Glycyl-L-methionine			90	5.0
	Glycyl-L-isoleucine			181	6.3
	Glycyl-L-leucine			75	1.3

[a] Bacteria were grown in minimal medium supplemented with 3 mM dipeptide as indicated in the table. Data for K_m (micromolar) and V_{max} (μmol/0.5 min per g of cells) are average value of two separate experiments.

TABLE 4. *Repression of transport systems for arginine and isoleucine by amino acids or dipeptides*[a]

Supplement in minimal medium	Concn (mM)	Uptake relative to control (%)			
		Wild type		KA204	
		Arg[b]	Ile	Arg	Ile
L-Alanine	3.4			100	46
L-Isoleucine	2.3			107	35
L-Leucine	2.3	99	45	94	25
L-Methionine	3.4			105	60
L-Valine	2.6			97	44
Glycyl-L-alanine	2.1			107	28
Glycyl-L-glutamate	4.9	107	112	99	105
Glycylglycine	5.4	102	100	96	98
Glycyl-L-isoleucine	1.6	108	41	108	10
Glycyl-L-leucine	1.6	95	21	98	10
Glycyl-L-methionine	1.5	102	60	122	16
Glycyl-L-threonine	1.7			88	55
Glycyl-L-valine	1.7	106	60	106	25

[a] Initial uptake for 0.5 min by bacteria grown in minimal medium supplemented with an amino acid or a dipeptide was measured. The concentrations of [^{14}C]arginine and [^{14}C]isoleucine were 1.9 and 30 μM, respectively, and specific activities of these isotopes were 10^7 and 1.2 × 10^7 cpm per μmol, respectively. Cells of wild type and KA204 grown in minimal medium took up 2.4 and 2.7 μmol of arginine per 0.5 min/g of cells, respectively, and 4.8 and 1.2 μmol of isoleucine, respectively. These control values were taken as 100%, respectively. Average value of two experiments are presented.

[b] Abbreviations: Arg, [^{14}C]arginine; Ile, [^{14}C]isoleucine.

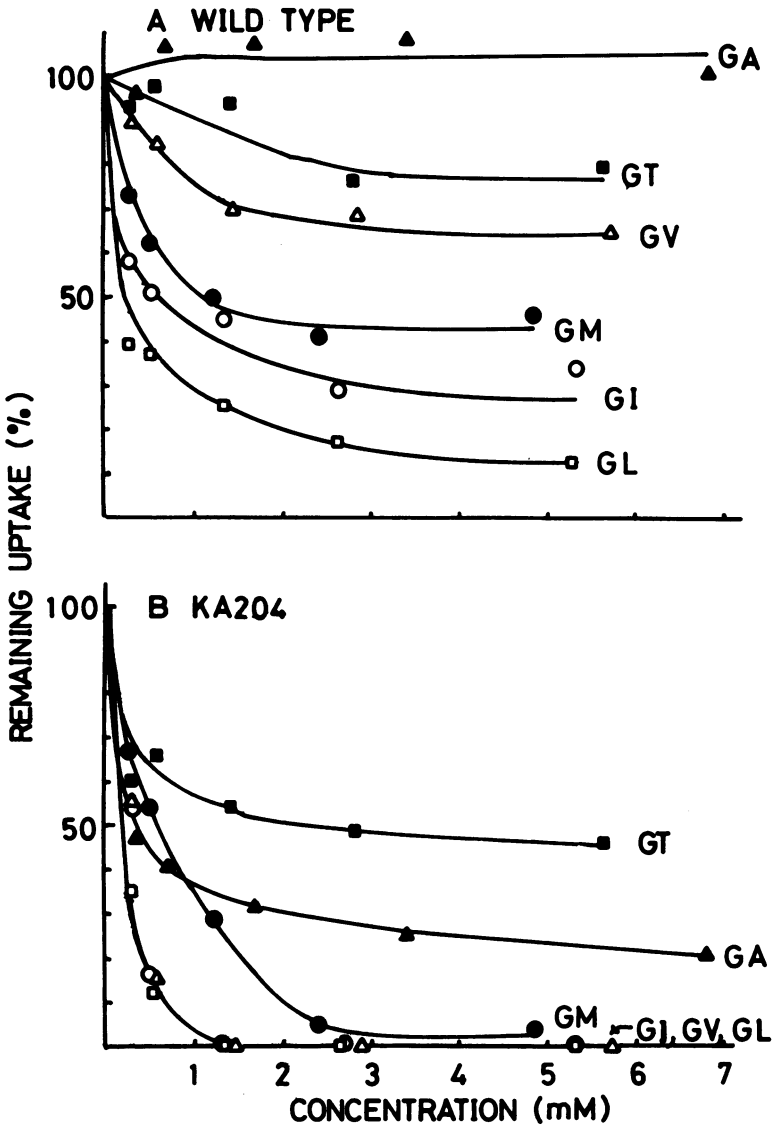

FIG. 7. *Effect of dipeptide concentration on repression of [^{14}C]isoleucine uptake. Uptake of [^{14}C]isoleucine by bacteria grown in the presence of the indicated dipeptide was measured at 0.5 min; the concentration of [^{14}C]isoleucine was 30 μM and the specific activity was 1.4 × 10^7 cpm per μmol. Uptake of [^{14}C]isoleucine by cells of wild type and KA204 grown in minimal medium was 5.5 and 1.6 μmol per 0.5 min per g of cells, respectively. Abbreviations: GA, glycyl-L-alanine; GI, glycyl-L-isoleucine; GL, glycyl-L-leucine; GM, glycyl-L-methionine; GT, glycyl-L-threonine; GV, glycyl-L-valine.*

taining cysteine on the transport systems was not tested.

Entry of labeled isoleucine into cells of *ilvT* mutants and wild type was not inhibited by the addition of an unlabeled α-keto acid, a D-amino acid, or a dipeptide.

DISCUSSION

In the present investigation, *ilvT* mutants were shown to have multiple transport systems, whereas a single system(s) appeared to be present in the wild-type strain. If we assume the existence of multiple transport systems in *S. typhimurium* analogous to those in *E. coli* (6, 7, 12, 19, 24), we can infer that the transport systems of wild type cannot be distinguished in the concentration range of the amino acids used and display a combined, single K_m value. In the *ilvT* mutants, the transport systems can be differentiated because of the lack of the domi-

TABLE 5. *Inhibition of [^{14}C]isoleucine uptake by addition of unlabeled amino acids, α-keto acids, D-amino acids, or dipeptides*[a]

Unlabeled amino acid added	Concn (3 × xM)	Uptake relative to control (%)		
		Wild type	KA203	KA204
L-Alanine	10^{-3}	75	64	54
	10^{-4}	88	93	101
L-Arginine	10^{-3}	88	101	81
L-Aspartic acid	10^{-3}	87	94	87
L-Cysteine	10^{-3}	18	35	35
	10^{-4}	85	81	81
L-Glutamic acid	10^{-3}	83	95	100
Glycine	10^{-3}	80	91	81
L-Histidine	10^{-3}	93	92	98
L-Isoleucine	10^{-3}	3	3	8
	10^{-4}	19	26	26
L-Leucine	10^{-3}	2	5	8
	10^{-4}	15	22	27
L-Lysine	10^{-3}	85	88	81
L-Methionine	10^{-3}	39	62	55
	10^{-4}	96	83	83
L-Ornithine	10^{-3}	106	103	100
L-Phenylalanine	10^{-3}	85	81	84
L-Proline	10^{-3}	96	87	86
L-Serine	10^{-3}	99	95	110
L-Threonine	10^{-3}	64	42	28
	10^{-4}	94	80	90
L-Tryptophan	10^{-3}	108	100	87
L-Tyrosine	10^{-3}	85	83	89
L-Valine	10^{-3}	2	18	10
	10^{-4}	40	53	51
α-Keto-β-methylvalerate	10^{-3}	108	83	80
α-Ketoisovalerate	10^{-3}	114	87	83
α-Ketoisocaproate	10^{-3}	118	91	66
D-Alanine	10^{-3}	82	80	83
D-Isoleucine	10^{-3}	89	81	81
D-Leucine	10^{-3}	82	81	82
D-Methionine	10^{-3}	80	94	85
D-Valine	10^{-3}	90	92	97
Glycyl-L-alanine	10^{-3}	82	89	80
Glycyl-L-isoleucine	10^{-3}	137	100	84
Glycyl-L-leucine	10^{-3}	164	83	74
Glycyl-L-methionine	10^{-3}	94	81	81
Glycyl-L-threonine	10^{-3}	73	83	83
Glycyl-L-valine	10^{-3}	118	80	80

[a] In the presence of the indicated substance, uptake of [^{14}C]isoleucine by bacteria grown in minimal medium was measured at 0.5 min. The concentration of [^{14}C]isoleucine was 30 μM and the specific activity was 1.4×10^7 cpm per μmol. Cells of the wild type, KA203, and KA204 in the absence of additions took up 3.7, 1.0, and 1.1 μmol of isoleucine per 0.5 min per g of cells, respectively, and these values were taken as 100%, respectively. The average values of two experiments are presented.

nating high-activity system. Further study on this problem is now under way.

When data on uptake of branched-chain amino acids by *ilvT* mutants are compared with those obtained with the parent double mutants (*ilvT ilvC8*) described in the previous report (11), it is found that the *ilvT* mutants take up two- to ninefold more amino acids than the parents. Since minimal medium was used for growth of the *ilvT* mutants and the medium for the parents was supplemented with large amounts of isoleucine and valine (11), low initial rate of uptake of the branched-chain amino acids by the parent cells was probably attributed to repression of the transport activity. In fact, the activity of *ilvT* mutants is repressed by alanine, isoleucine, leucine, methionine, and valine. These amino acids have been reported as repressors of the transport systems for branched-chain amino acids in *E. coli* (4, 7, 9, 10, 23) and leucine in *S. typhimurium* (18). Occurrence of repression is verified in the wild

type and *ilvT* mutants in the present study, because (i) more than two generations of bacterial growth in the presence of repressor substance is necessary for the maximum reduction of the transport activity, and (ii) growth in minimal medium is required to reverse the repression.

α-Keto acids, precursors of branched-chain amino acids, behave just as the corresponding respective amino acids in repression of the transport in *ilvT* mutants and wild-type cells. It is not known whether repression is provoked directly by the α-keto acids or indirectly by the amino acids produced through cellular metabolism.

In *ilvT* mutants, valine uptake by the low K_m component of the transport systems is sensitive to repression, but the uptake by the high K_m component is relatively resistant. These properties of the transport system could not be demonstrated with isoleucine and leucine as substrates because the uptake by repressed cells was so poor. When data from kinetic analysis of the valine uptake obtained with *ilvT* mutants under unrepressed and repressed conditions are compared with those from *E. coli* described by Wood (24), the low K_m component appears to correspond to the LIV-II system and the high K_m component corresponds to the nonspecific system. However, it is probable that K_m values of the transport systems in *ilvT* mutants have been altered by the *ilvT* mutation.

It has been reported that dipeptides are taken up in bacterial cells through a distinct transport system(s) and cleaved into component amino acids by the action of an intracellular peptidase (1, 5, 14, 21). Based on this evidence, it is inferred that the effect of dipeptides on repression of the transport of branched-chain amino acids is indirect and that the component amino acid produced by cleavage of the dipeptide plays a main role on the repression. Although isoleucine and methionine per se are not identified as repressors for wild type (threonine in the case of *ilvT* mutants), they become repressors in the form of a dipeptide. This implies that permeability of cell membrane for dipeptides or the pool size of cells is greater than that for free amino acids. Failure to repress the transport activity of the wild type with alanine as well as glycyl-L-alanine indicates that (i) alanine can repress the altered transport systems of *ilvT* mutants, but cannot repress the normal system(s) of wild type or (ii) a minor component of the transport systems in wild type is sensitive to alanine, but the apparent K_m value for uptake of branched-chain amino acids is not affected by the repression because of a negligible contribution of the minor component to the transport activity.

Entry of labeled isoleucine into *ilvT* mutant and wild-type cells was inhibited by the addition of unlabeled alanine, cysteine, isoleucine, leucine, methionine, threonine, or valine in the uptake system. These results are consistent with those reported in the transport of leucine in *E. coli* by Templeton and Savageau (22). It appears that amino acids capable of repressing the transport systems of cells can also compete with branched-chain amino acids for the entrance into cells.

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