サルモネラ菌の分岐鎖アミノ酸高親和輸送系に関与するlivA遺伝子の座位

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Location of *livA* gene participating in the high-affinity transport of branched-chain amino acids in *Salmonella typhimurium* LT 2

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ABSTRACT

A structural gene responsible for the high-affinity transport system of branched-chain amino acids, *livA*, is located at 76-77 min, near *xyl*, on the genetic map of *Salmonella typhimurium*. Although the regulatory gene, *livR*, has been located in the same region as the *livA*, linkage relationship between the *livA* and *livR* genes is not yet known. The *livA* mutation does not affect the activity of leucine-isoleucine-valine-threonine binding protein. Isoleucine-valine requiring mutants can take up enough amounts of these amino acids for growth through only the low-affinity transport system(s), even if the high-affinity system is defective.

1. INTRODUCTION

In *Salmonella typhimurium*, branched-chain amino acids are mainly taken up through two general, specific transport systems, high-affinity (LIV-I) and low-affinity-(1) (LIV-II) systems (Kiritani and Ohnishi 1978). In *brnQ* mutants defective in the LIV-II system, transport activity of the low-affinity-(2) system (LIV-III) becomes detectable, whereas in wild-type strains the activity of LIV-III is concealed with that of the LIV-II system.

The *brnQ* gene and its regulator gene, *gleR* (Ohnishi and Kiritani 1978), have been cloned in a vector plasmid, pBR322 (Ohnishi *et al.*, submitted for publication). The LIV-I system utilizes the leucine-isoleucine-valine-threonine (LIVT) binding protein (Ohnishi *et al.* 1980; Ohnishi and Kiritani 1983). Mutants defective in the LIV-I system have been isolated from the *brnQ* mutant (Kiritani and Ohnishi 1978). With one of the double transport-defective mutants, KA261, we attempted to locate the mutation locus affecting transport activity of the LIV-I system on the *Salmonella* genetic map. We designated the mutation locus in KA261 as *livA*, which has tentatively been referred to as *liv-261* (Kiritani and Ohnishi 1978). We also present some biological properties of an *livA brnQ*$^+$ strain defective only in the LIV-I system.

Table 1. *Bacterial strains*

Strain	Genotype	Source or reference
Wild-type		Kiritani, 1974
KA931	*ilvC*8	Kiritani, 1974
CE4	*ilvC*8 *brnQ*3	Kiritani, 1974
KA203	*brnQ*3	Kiritani and Ohnishi, 1977
KA261	*ilvC*8 *brnQ*3 *livA*1	Kiritani and Ohnishi, 1978
KA266	*brnQ*3 *livA*1	Kiritani and Ohnishi, 1978
MA545	*ilvC*8 *brnQ*3 *livA*1 *xyl*	*xyl* mutant of KA261 mutagenized with EMS
MA230	*ilvC*8 *livA*1	*brnQ*$^+$ transductant of KA 261 from KA931
MA105	*leuBCD*39 *ara*-7 HfrK7	*thr*$^+$ revertant of SA949 supplied by SGSC*

* SGSC: Salmonella Genetic Stock Centre.

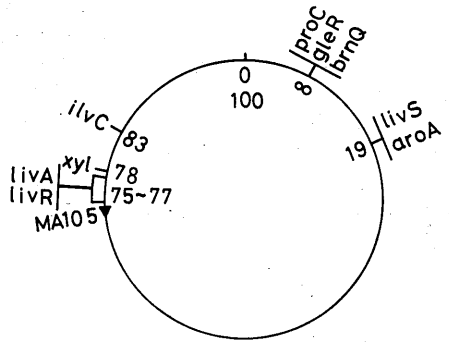

Fig. 1. Map positions (minutes) of relevant markers, and the point of origin and direction of transfer of HfrMA105. The *livR* and *livS* genes regulate the transport mediated by both the LIV-I and LIV-II systems (Ohnishi *et al.* 1983; Murata-Matsubara *et al.* 1985).

2. MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are all derivatives of *Salmonella typhimurium* LT 2 and listed in Table 1. Strain MA105 is a spontaneous *thr*$^+$ revertant of SA949 (Sanderson *et al.* 1972); the threonine-requiring trait has been lost during storage in the stab culture. Fig. 1 shows the map positions of the relevant markers according to Sanderson and Roth (1983).

Media. Penassay broth (Antibiotic medium 3, Difco Laboratories) as a nutrient broth, and minimal medium (Kiritani 1974) were used in this study. When D-xylose was used as a sole carbon source in the minimal medium, D-xylose (5 mg/ml) substituted for glucose (Xylose-minimal medium). When required, following supplements were added to the minimal medium; L-iso-

leucine (10 μg/ml), L-valine (20 μg/ml), glycyl-L-isoleucine (20 μg/ml), glycyl-L-valine (40 μg/ml), glycyl-L-leucine (20 μg/ml), Ca-pantothenate (1 μg/ml) and Casamino Acids (0.1%). For an agar medium, 1.5% agar was added.

Conjugation and transduction. Conjugation were carried out by the method described previously (Kiritani 1974). In crosses between HfrMA105 and recipient MA545, Ilv^+ recombinants were selected on minimal agar medium, Liv^+ on the medium containing isoleucine and glycylvaline, and Xyl^+ on xylose-minimal agar medium containing glycylisoleucine, glycylvaline and pantothenate. In the crosses, about 1.5×10^8 donor and 7.5×10^8 recipient cells in exponential phase in nutrient broth were mixed. P 22-mediated transduction was carried out according to the method described previously (Kiritani 1974).

Transport and binding assays. Transport assays were performed on exponentially growing cells as described previously (Ohnishi *et al.* 1980). An overnight culture grown in minimal medium containing glycylisoleucine, glycylleucine, glycylvaline, pantothenate and Casamino Acids was diluted 40-fold with the medium without Casamino Acids, and the bacteria were grown to the exponential phase. Apparent Km and Vmax values of the transport were calculated according to the formula of Neal (1972). Binding activities to isoleucine, leucine and histidine in osmotic-shock fluids of cells were determined by the equilibrium dialysis method described previously (Ohnishi *et al.* 1980).

Chemicals. Labeled amino acids were purchased from Amersham Co.. All other chemicals were commercial materials of analytical grade.

3. RESULTS AND DISCUSSION

Genetic mapping. To determine the *livA* locus on the chromosome, MA545 was crossed with HfrMA105 and the mating was interrupted at intervals. As seen in Fig. 2, Liv^+, Xyl^+ and Ilv^+ recombinants first appeared on their selective media at 9, 11 and 18 min, respectively, after mixing the donor and recipient cells. By measuring entrance time of these marker genes several times, the distance between *livA* and *xyl* genes was estimated to be 1 to 2 min. These results indicate that *livA*, *xyl* and *ilvC* loci can be arranged in this order. The marker order was also confirmed by examining frequency of unselected markers among Ilv^+ recombinants. As shown in Table 2, frequencies of $livA^+$ and xyl^+ markers among 72 Ilv^+ recombinants were 19.4 and 68.1%, respectively. A recombinant class, $ilvA^+$ *xyl* $ilvC^+$, which should arise as a consequence of double crossovers, was present in the fewest num-

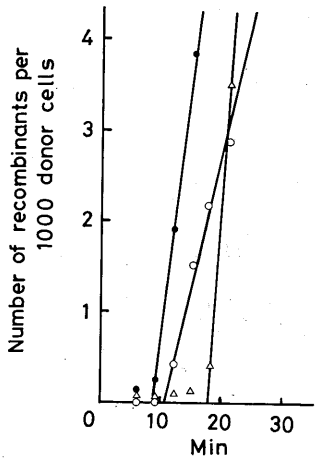

Fig. 2. Time of entry of *livA*$^+$, *xyl*$^+$ and *ilvC*$^+$ loci. Average numbers of Liv$^+$ (●), Xyl$^+$ (○) and Ilv$^+$ (△) recombinants on duplicated plates are plotted.

Table 2. *Frequency of unselected markers among Ilv$^+$ and Xyl$^+$ recombinants derived from the cross between HfrMA105 and recipient MA545*

Selected marker (Interrupted time)	Recombinant class *livA*	*xyl*	*ilvC*	No. of recombinants[a]		LIV-I uptake (μmoles/min/g cells)[b]	Frequency of unselected markers (%) *livA*$^+$	*xyl*$^+$	*ilvC*$^+$
ilvC$^+$ (24 min)	+	−	+	3 (4.2)	−	0.27-1.75	19.4	68.1	100
	+	+	+	11 (15.3)					
	−	−	+	20 (27.8)	−	0.01-0.04			
	−	+	+	38 (52.8)					
xyl$^+$ (15 min)	+	+	−	13 (27.1)		0.27-1.30	27.1	100	—
	−	+	−	35 (72.9)		0.01-0.04			

a) Values in parentheses indicate percent of recombinants.
b) Uptake is expressed in μmoles per minuete per gram of dry weight of cells. The assay mixture contained 0.2 μM L-(^{14}C)isoleucine (specific activity; 2.8×10^8 cpm/μmole).

bers. When the mating was interrupted at 15 min, 48 Xyl$^+$ recombinants isolated randomly carried *livA*$^+$ at a frequency of 27.1%, and no *ilvC*$^+$.

For detailed studies on isoleucine transport of Liv$^+$ recombinants, 43 *livA*$^+$ *ilvC* recombinants were isolated and tested for its isoleucine uptake at 0.2 and 9.6 μM, and the results are presented in Table 3. Transport activities of these recombinants assayed at 0.2 μM were comparable to or higher than those of CE4 and KA931, indicating that the recombinants acquired *livA*$^+$ gene by conjugation. Since the activities of some recombinants were very high at 9.6 μM, it was suspected that these cells restored normal transport activity in the

Table 3. *Isoleucine transport activity of Liv⁺ recombinants*[a)]

Recombinant or strain	Relevant genotype *livA*	*brnQ*	Isoleucine uptake at[b)] 0.2 μM (μmoles/min/g cells)	9.6 μM) (μmoles/min/g cells)
Liv⁺	+	−	0.38—3.85	0.86—6.62
MA 545	−	−	0.03	0.19
CE 4	+	−	0.54	1.61
KA 931	+	+	1.05	10.14

a) Forty-three Liv⁺ recombinants, which required isoleucine and glycylvaline for growth, were examined for isoleucine uptake.

b) Uptake of L-(^{14}C)isoleucine by bacteria in 20 sec was measured; specific activities of the labeled isoleucine were 2.8×10^8 cpm/μmole at 0.2 μM and 2.3×10^7cpm/μ mole at 9.6 μM.

Table 4. *Apparent Km and Vmax for the transport system*

Recombinant or strain	Relevant genotype *livA*	*brnQ*	Isoleucine* LIV-I Km	LIV-I Vmax	LIV-II Km	LIV-II Vmax	LIV-III Km	LIV-III Vmax
Liv⁺ no. 1	+	−	0.1	0.3	ND**		86.9	6.0
no. 2			0.1	0.8	ND		112.8	6.3
no. 3			0.1	4.5	ND		34.9	6.2
CE4	+	−	0.3	1.0	ND		34.4	2.9
MA545	−	−	ND		ND		25.0	0.7
MA230	−	+	ND		5.4	4.9	ND	
KA931	+	+	0.5	2.1	5.0	7.8	ND	

* Initial uptake of L-(^{14}C)isoleucine by bacteria in 20 sec was measured; the specific activity of (^{14}C)-isoleucine was 2.6×10^8 cpm/μmole in the concentration range of 0.1 to 2.3 μM, and 2.2×10^7 cmp/μmole in that over 2.3 μM. Km; μM, Vmax; μmoles/min/gram of dry weight of cells.

** ND: not detectable.

LIV-II system. As shown in Table 4, kinetic analysis of the transport systems revealed that three representatives of the *livA*⁺ recombinants still maintained the defect in the LIV-II system, despite of high activity in the LIV-III system or in both the LIV-I and LIV-III systems.

It is concluded that the *livA* mutational site is located in the region of 76 to 77 min on the genetic map (Fig. 1), where the regulatory *livR* gene has been located (Ohnishi *et al.* 1983). Co-transduction study to elucidate linkage relationship between the *livA* and *livR* loci, however, could not be performed, because the reversion frequency of the *livA*1 mutation in MA545 is high (about 10^{-7}). It is possible that *livA* and *livR* genes are involved in one

Table 5. *Binding activities in osmotic-shock fluids**

Strain	Genotype	Glycyl-L-leucine (3 mM) in growth medium	L-Histidine	L-Leucine (pmoles/mg protein)	L-Isoleucine
Wild-type		−	326 (1)	176 (0.54)	269 (0.83)
		+	257 (1)	5 (0.02)	5 (0.02)
KA 203	*brnQ*3	−	131 (1)	43 (0.33)	85 (0.65)
KA 266	*brnQ*3 *livA*1	−	169 (1)	56 (0.33)	110 (0.65)
		+	254 (1)	3 (0.01)	20 (0.08)

* Average values of two experiments are presented.
Numbers in parentheses indicate normalized values taking the binding activity for histidine as 1.0.
The specific activity of L-(^{3}H) histidine was 7×10^8 cpm/μmole, and that of L-(^{14}C) leucine or L-(^{14}C)isoleucine was 4 to 5×10^8 cpm/μmole.

Table 6. *Generation time (min) of transport mutants in various media*

Strain	Transport system		Minimal medium supplemented with*			
	LIV-I	LIV-II	IVP	IgV	IgVgLP	gIgVgLP
MA230	−	+	85	100	95	80
KA931	+	+	60	60	50	50
CE4	+	−	>300	90	100	80
KA261	−	−	>300	>300	>300	85

* Abbreviations: I; isoleucine, V; valine, P; pantothenate, gI; glycyl-L-isoleucine, gL; glycyl-L-leucine, gV; glycyl-L-valine.

operon. In *Escherichia coli* K-12, it was found that three genes, *livJ*, *livH* and *livG*, governing the LIV-I system, are clustered at 76 min on the *E. coli* genetic map (Sanderson and Oxender 1977; Nazos *et al.* 1986). Thus it is supposed that the *livA* and *livR* genes of *S. typhimurium* are located at a genetic region corresponding to that of the *livJ*, *livH* and *livG* of *E. coli*.

Activity of the binding protein of the livA mutant. Table 5 shows the activities of binding proteins for histidine, leucine and isoleucine, which were released in osmotic-shock fluids of cells. Upon comparing normalized values, the level of binding activity for branched-chain amino acids in KA266 was not significantly different from that in wild-type and KA203. The activity of KA266, like that of wild-type, was repressed by glycylleucine. It can be thus said that the *livA* mutation does not affect the binding protein for branched-chain amino acids.

Isoleucine transport and growth of the livA mutant. To test biological pro-

perties of the *livA* mutant, the *brnQ* gene in KA261 was replaced by the *brnQ*$^+$ allele from KA931. In the *ilvC livA brnQ*$^+$ transductant, MA230, the LIV-II system recovered normal activity, but the LIV-I system still remained defective (Table 4). As shown in Table 6, MA230 could grow in the medium containing isoleucine, valine and pantothenate, while CE4 and KA261 could not. The growth rate of MA230 was, lower than that of KA931, and not improved by addition of glycylisoleucine and glycylvaline, though these dipeptides can be incorporated into cells through the discrete dipeptide-transport system (Sussman and Gilvarg 1971). Thus the slow growing character of MA230 is not due to the defect in the LIV-I system but probably due to a factor in the genetic background. The result indicates that the isoleucine-valine requiring mutant of *S. typhimurium* can take up the branched-chain amino acids sufficient for its growth through the LIV-II transport system, even if the LIV-I system is defective. The result also corresponds well to that the transport mediated by the LIV-II system in KA931 contributes to about 80% of total isoleucine uptake (Table 4).

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