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Histidine and Aromatic Permeases of *Salmonella typhimurim*

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Mutants defective either in the histidine permease (*hisP*) or in the aromatic permease (*aroP*) were isolated in *Salmonella typhimurium* and were characterized. The *hisP* locus had a 49% linkage to *purF* by phage transduction. The *aroP* locus was close to *proA*. Merozygotes diploid for the *hisP* gene were constructed by episomal transfer, and *hisP*$^+$ was dominant over *hisP*. The properties of merozygotes are described and discussed. A method for the selection of revertants of *hisP* mutants was devised. By this method, one of the *hisP* mutants was characterized as an amber mutant. The specificity of the aromatic permease was investigated by using as substrates analogues of the aromatic amino acids and of histidine.

Some properties of the active transport of histidine and of the aromatic amino acids in *Salmonella typhimurium* have been previously presented (1, 12). Preliminary results demonstrated that amino acids are incorporated into lipidic compounds, and the possible role of these compounds in transport has been discussed (2). This paper describes further studies on the genetics and properties of active transport mutants.

Two permeases are capable of transporting histidine in *S. typhimurium:* a specific histidine permease and an aromatic permease (1, 12). The histidine permease (which is highly specific for histidine and has a K_m of 8×10^{-8} M) also transports the histidine analogue, D(+)-α-hydrazinoimidazolepropionic acid (HIPA), which is a powerful inhibitor of growth of *S. typhimurium* (13). A mutant resistant to inhibition by HIPA was selected and demonstrated to have a defective histidine-specific permease (12). Mutants of this type are designated *hisP*.

In this paper, we describe additional characteristics of the *hisP* mutants, including the map position of the *hisP* locus, a method for selecting revertants of *hisP* mutations, the dominance of *hisP*$^+$ over *hisP*, the phenotype of episome-containing strains which carry either two copies of the wild-type *hisP*$^+$ gene or only an episomal *hisP*$^+$ gene, and the isolation of amber *hisP* mutations.

The general aromatic permease (1) transports phenylalanine, tyrosine, tryptophan, histidine, and numerous analogues of each of these amino acids. The affinity for the aromatic amino acids is very high (the K_m values are all about 10^{-7} M), whereas the affinity for histidine is very much lower (K_m, 10^{-4} M) than that of the histidine permease. The aromatic permease also transports a glutamine analogue, azaserine, which inhibits the growth of *S. typhimurium*. Mutants with a defective aromatic permease have been isolated as strains resistant to inhibition by azaserine and have been designated *aroP* (1). (Those azaserine-resistant mutants (*aza*) having an altered aromatic permease are now designated *aroP*. Mutant *aza-3* (1) is now designated *aroP504*.)

Mapping of the *aroP* locus and additional properties of the general aromatic permease are also reported.

MATERIALS AND METHODS

Strains. All strains used were derived from *S. typhimurium* strain LT-2. Strain SR305 (HfrA, *gal-50, hisD23*) was obtained from the collection of M. Demerec. Multiply marked strain SL751 (*ile-405 proA46 purC7 str-r rha-461 fla-56 iM-10 fim*$^-$) was obtained from the collection of B. A. D. Stocker. Histidine permease mutant *hisP1650* (12) and aromatic permease mutant *aroP504* (1) have been previously described. All other strains were obtained from P. E. Hartman and B. N. Ames.

Double-mutants TA235 (*hisHB22 hisP1657*) and TA242 (*hisHB22 hisP1661*) were constructed by selecting for HIPA-resistant mutants of strain *hisHB22* on medium containing 3×10^{-4} M HIPA and 3×10^{-3} M L-histidinol (histidinol is not transported by the histidine permease). All other strains containing either *hisP* or *aroP* mutations were constructed by selection for HIPA or azaserine resistance, respectively, with the appropriate parental strains. TA 236 (*ile-405 proA46 purC7 str-r rha-461 fla-56 iM-10 fim*$^-$ *hisP1655*) and TA237 (*ile-405 proA46*

purC7 str-r rha-461 fla-56 iM-10 fim⁻ aroP505) were derived from SL751.

Strain TR11 (*aroD5 cysC1112*/F'32 *aroD*⁺ *dsd*) was constructed by infecting SB259 (*aroD5 cysC1112*) with the F'32 episome. The *Escherichia coli* episome F'32 was isolated and kindly donated by E. McFall (8). Strains TR134 (*purF145 hisP1653*/F'32 *dsd*) and TR135 (*purF145*/F'32 *dsd*) were constructed by infecting strains *purF145 hisP1653* and *purF145*, respectively, with the F'32 episome.

Growth of bacterial strains. All strains were grown in the minimal medium E (14) with 0.5% glucose added as a carbon source, and appropriate supplements for auxotrophic strains. The cultures were incubated at 37 C in a New Brunswick rotary shaker. Bacterial growth was monitored turbidimetrically by measuring the absorbancy of the culture at 650 nm. In our spectrophotometer, an absorbance of 0.500 corresponds to a bacterial density of 4×10^8 cells/ml and to 235 μg of cells (dry weight) per ml.

Assays. The uptake of ^{14}C-histidine was determined by the growing-cells method of Ames (1).

Resistance to analogues was tested on a petri plate by streaking clones radially (from the center to the periphery) and placing in the center a filter paper disc impregnated with the analogue (on unsupplemented plates: 0.02 μmole of azaserine, 0.5 μmole of HIPA, or 0.1 μmole of 5-methyltryptophan; discs on plates supplemented with 3×10^{-3} M L-histidinol were impregnated with 5 μmoles of HIPA). Colonies which grew near the center were scored as resistant.

Selection of revertants of histidine permease mutants. About 0.1 ml of a culture of the double-mutant (bearing deletion *hisHB22* and a *hisP* mutation) was spread on a minimal glucose plate containing 10^{-2} M phenylalanine and 10^{-5} M histidine. A very small crystal of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was placed in the center, and the plates were incubated for about 4 days.

Test for the presence of amber suppressors. A method for determining the presence of amber suppressors has been described by Berkowitz et al. (5). In our experiments, colonies on the reversion plates prepared as described above were replicated on minimal plates, containing 3×10^{-3} M L-histidinol and 1% lactose as the carbon source with 0.1 ml of the tester strains (SB391 or SB392) spread on them. As a control, colonies on the reversion plates were first printed on the same medium without tester strain. The replicate plates were incubated for about 4 days. Strain SB391 has the following genotype, *his644*/F' *lacX82*. Strain SB392 has the following genotype, *his644*/F' *lacU281*. Both F' lac mutations are UAG mutations.

Genetic tests. Conjugation experiments were performed by the method of Sanderson and Demerec (11). All crosses were uninterrupted matings of 3-hr duration.

Transfer of episomes was performed by spreading together on a selective plate 0.1 ml each of the episome-containing and the recipient strains.

Transduction tests were performed by spreading together on a selective plate 0.1 ml of an overnight culture of the recipient strain and approximately 10^9 phage (P22 or P22-L4) which had been prepared on the donor strain. P22-L4 is a nonlysogenizing mutant of P22; it was isolated and donated by H. O. Smith.

Materials. ^{14}C-L-histidine (about 300 μc/μmole) was purchased from New England Nuclear Corp., Boston, Mass. HIPA (13) and α-hydrazino-4-(*p*-hydroxyphenyl) propionic acid were gifts of F. A. Kuehl, Jr., of Merck Sharp and Dohme Research Laboratories, Rahway, N.J. Azaserine (O-diazoacetyl-L-serine) was obtained from E. P. Anderson. L-Histidinol and 5-methyl-DL-tryptophan were purchased from Cyclo Chemical Corp., Los Angeles, Calif., and Mann Research Laboratories, New York, N.Y. respectively. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wisc. The 1-amino-2-(4-hydroxyphenyl) ethyl phosphonic acid and 1-amino-2-phenylethyl phosphonic acid were purchased from Calbiochem, Los Angeles, Calif.

RESULTS

Location of the hisP gene. The mutation responsible for HIPA resistance was located between the histidine operon and the *purC* locus. This was determined by the following conjugation experiment. Strain SR305 (HfrA, gal⁻, his⁻, *str-s*) was mated with TA236 (*hisP*⁻, ile⁻, pro⁻, ade⁻, *str-r*). Selection was made singly for each auxotrophic marker, and the recombinants were tested for possession of the other, unselected markers. A more accurate location was obtained by two-point and three-point transduction tests with markers in the region of interest. Two-point transduction tests (Table 1) indicated that *hisP* mutations are very closely linked to *purF*. A 49% linkage was found between *hisP1650* and *purF145* and about 0.3% linkage between *hisP1650* and *aroD5*. Since *purF* and *aroD* are known to be about 10% cotransducible, the order *aroD purF hisP* was demonstrated. Three-point transduction tests confirmed this order (Table 2). Cross I indicates the order, *aroD purF hisP*; cross II indicates the order, *hisT*

TABLE 1. *Cotransduction of hisP with various markers*[a]

Recipient	Recombinants selected	Recombinants carrying donor *hisP* allele
		%
purF145	Pur⁺ (234)	49.0
aroD5	Aro⁺ (291)	0.3
cysA20	Cys⁺ (91)	<1.1
guaA1	Gua⁺ (119)	<0.8

[a] Strain *hisP1650* was used as the donor. The wild-type transductants were scored for HIPA resistance by the radial streak method. Numbers in parentheses indicate the number of colonies scored.

TABLE 2. *Three-point transduction tests*

Cross[a]	Unselected recombinant types[b]	No. of crossovers required for indicated gene order			Recombinants	
		pur hisP aro	*pur aro hisP*	*aro pur hisP*	Per cent	Total no.
I	*aroD hisP*	2	2	2	0.3	1[c]
	aroD hisP$^+$	4	2	2	8.4	24
	aroD$^+$ *hisP*	2	4	2	55.3	158
	aroD$^+$ *hisP*$^+$	2	2	2	36	103
		purF hisP hisT	*purF hisT hisP*	*hisT purF hisP*		
II	*hisT hisP*$^+$	2	2	2	13.6	15
	hisT hisP$^-$	4	2	2	33.6	37
	hisT$^+$ *hisP*$^+$	2	4	2	40.0	44
	hisT$^+$ *hisP*$^-$	2	2	2	12.8	14

[a] For cross I, the donor was *aroD5 hisP1654* and the recipient was *purF145*. For cross II, the donor was *hisT1207* and the recipient was *purF145 hisP1653*. For both crosses I and II, selection was for *Pur*$^+$.

[b] The *hisP* marker was scored for by testing the HIPA resistance by the radial streak method.

[c] This class is low because the two donor markers, *aroD* and *hisP* are only weakly cotransducible (*see* Table 1) and thus are seldom carried by the same transducing fragment.

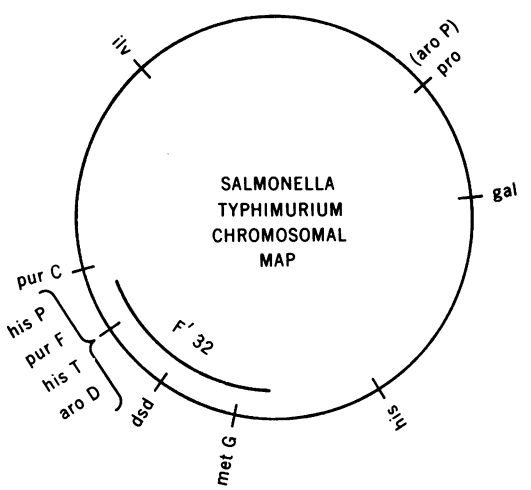

FIG. 1. *Chromosomal map of S. typhimurium.*

purF hisP. Since *hisT* is already known to be between *aroD* and *purF* (10), we inferred the gene order *aroD hisT purF hisP* (Fig. 1).

Properties of strains which are diploid for the hisP gene. The *hisP*$^+$ allele carried by the *E. coli* F'32 episome was found dominant to a chromosomal *hisP* mutation (Table 3). This was determined by transferring the F'32 episome from strain TR11 (*aroD5 cysC1112*/F'32 *dsd*) to the double-mutant *aroD5 hisP1654*. Selection was made for growth on minimal medium. The resulting colonies were merozygotes, carrying mutant alleles of *aroD* and *hisP* in the chromosome and wild-type alleles in the episome. These colonies were then tested for sensitivity to inhibition by HIPA, and all were sensitive (Table 3). This phenomenon demonstrates the dominance of the episomal *hisP*$^+$ gene over the mutant chromosomal *hisP* gene. The segregants which had lost F'32 (Table 3) had regained their requirement for aromatic amino acids simultaneously to resistance to HIPA. Thus, the phenotype of the merozygote is due to a dominance effect and not to recombination or reversion of any markers.

The effect of an added permease gene was to increase the sensitivity to HIPA inhibition (Fig. 2). Strain TR135, which was constructed by introducing the F'32 episome (*hisP*$^+$) into a *hisP*$^+$ strain, carries two wild-type *hisP*$^+$ genes and is supersensitive to inhibition by HIPA when compared with the wild-type. In addition to the much larger zone of inhibition for the diploid strain (TR135), it is clear that no resistant mutants appear, whereas strains with only one *hisP*$^+$ gene, such as wild-type or TR134 (*hisP*$^-$/F'*hisP*$^+$), yield many resistant colonies. This is to be expected, because a single *hisP*$^+$ gene is sufficient to confer HIPA sensitivity (*hisP*$^+$ being dominant over *hisP*), and simultaneous mutations to resistance in both genes (in TR135) would be extremely rare.

TR134 has a slightly larger zone of inhibition than wild-type, which suggests an intermediate level of permease activity. The presence of several episomal copies per cell [previously observed in other cases (6)] could be responsible for such

TABLE 3. *Properties of hisP mutants and dominance of episomal hisP+*

Strain	No. of isolates tested	Sensitivity to HIPA[a]	Medium used for test	Growth on minimal medium
LT-2 (wild-type)	1	S	Aromatic or minimal	+
hisP1650	1	R	Aromatic or minimal	+
hisP1654 aroD5	1	R	Aromatic	−
aroD5 hisP1654/F'32 *aroD+ hisP+*	24	S	Minimal	+
hisP1654 aroD5[b]	7	R	Aromatic	−

[a] S, sensitive; R, resistant.
[b] Derived from merozygotes by loss of F'32.

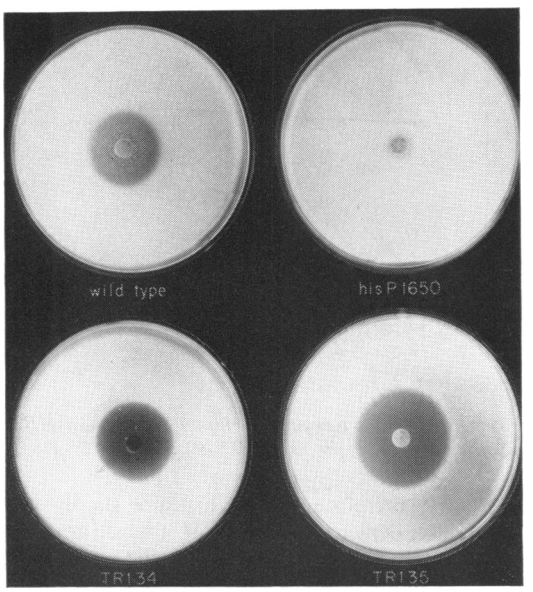

FIG. 2. *Response to inhibition by HIPA. T.R134 is purF145 hisP1653/F'32hisP+. TR135 is purF145 hisP+/F'32 hisP+. Plates contain minimal medium and 0.5 μmole of HIPA on the disc. No resistant colonies appeared in the inhibited zone of TR135, even after several days of incubation.*

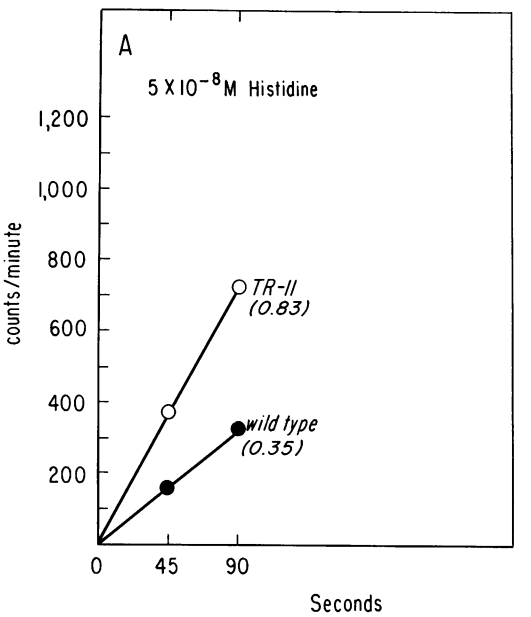

FIG. 3. *Assay of histidine permease activity in merozygote TR11 (aroD5 hisP+ cysC1112/F'32 hisP+). The assay was performed as described by Ames (1). Cysteine (2 × 10⁻⁴ M) was present during growth and assay. The specific activity of the histidine was 8.4 × 10⁷ counts per min per μmole as assayed in a gas-flow counter (Nuclear Chicago Corp., Des Plaines, Ill.). The concentration of the bacterial suspensions at time-zero was 7.3 and 7.8 μg (dry weight) per ml of wild-type and TR11, respectively. Numbers in parentheses indicate the rate of uptake [in μmoles of histidine per minute per gram (dry weight)]. The rate of uptake in TR11 was corrected for the slower growth rate (65 min as opposed to 58 min for the wild-type).*

increased permease activity. In fact, direct assay of permease activity in strains TR134, TR135, and the wild-type showed that HIPA sensitivity increased with increasing permease activity; TR135 (*hisP+*/F'*hisP+*) and TR134 (*hisP*/F'*hisP+*) had about 2 and 1.5 times the wild-type permease level, respectively (Fig. 3). Strain TR11, which is a merodiploid with two copies of the *hisP+* gene (like TR135), had more than twice the wild-type level of histidine permease activity.

The absence of HIPA-resistant mutants in merodiploids having two wild-type copies of the *hisP+* region (TR135, TR11) also demonstrates that the frequent, spontaneous, HIPA-resistant mutants occurring in the wild-type are all located in the chromosomal region homologous to F'32 genes and probably are all in the *hisP* gene (or genes).

Selection of revertants of hisP mutants. Histidine is transported by two different permeases,

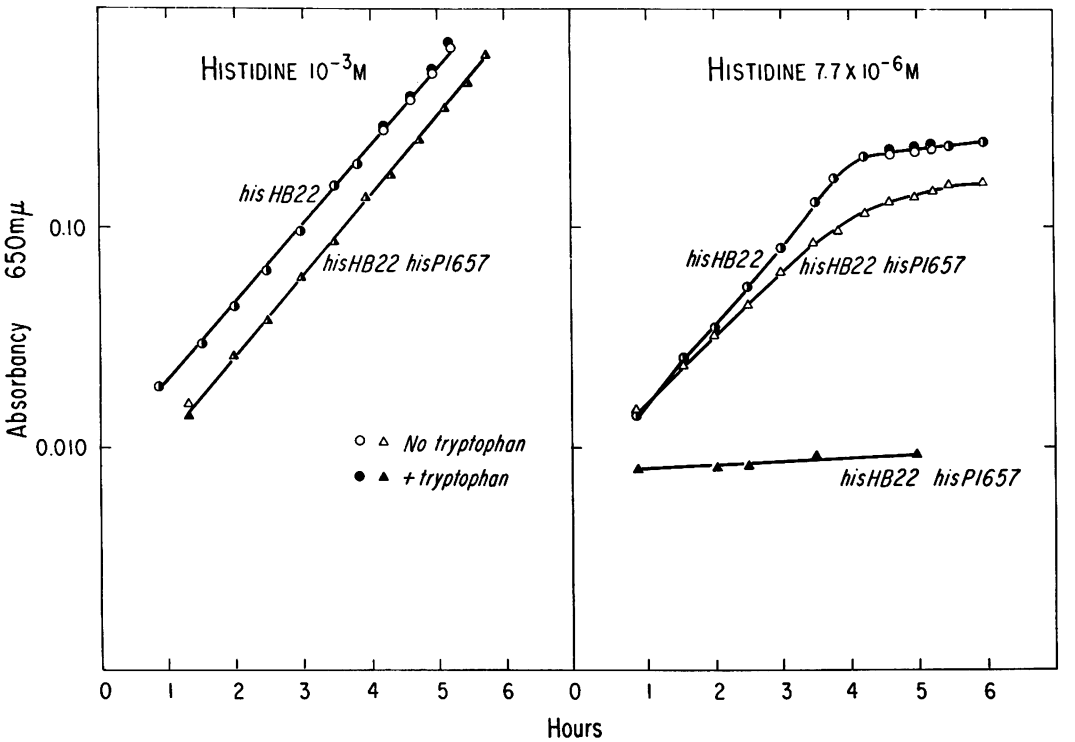

FIG. 4. *Effect of a histidine permease mutation on the growth of a histidine-requiring mutant.* (○, ●) *hisHB22* (△, ▲) *hisHB22 hisP1657. Open symbols, no tryptophan. Solid symbols, tryptophan* (10^{-3} M) *added.*

the histidine-specific permease and the aromatic permease (1). Therefore, a histidine-requiring strain which lacks the histidine-specific permease (*hisHB22 hisP1657*) must depend on the second (aromatic) permease or on diffusion as a means of obtaining exogenous histidine. Transport of histidine through the aromatic permease can be inhibited by phenylalanine, tyrosine, and tryptophan. Figure 4 shows the growth response of mutants *hisHB22* and *hisHB22 hisP1657* to histidine in the presence and absence of tryptophan. At high exogenous concentration of histidine (10^{-3} M), mutant *hisHB22 his P1657* grew as well as *his HB22* (which has an intact histidine-specific permease) even with the addition of tryptophan; at such high concentration, histidine can presumably enter the cell by diffusion. At low histidine concentration (7.7×10^{-6} M), *hisHB22* grew normally until the histidine was completely exhausted, at which point growth terminated abruptly; since the histidine-specific permease was utilized, tryptophan had no effect on its growth. On the other hand, the histidine uptake in *hisHB22 hisP1657* depended exclusively on the aromatic permease which had a much lower affinity for histidine [(K_m(His) = 10^{-4} M)]; therefore, as the histidine supply was gradually depleted, growth of this strain was increasingly limited. Moreover, in the presence of tryptophan, the double-mutant (*hisHB22 hisP-1657*) was unable to grow, since histidine uptake was completely prevented; mutation (*hisP1657*) had eliminated the histidine-specific permease, whereas excess tryptophan prevented the general aromatic permease from transporting histidine.

Inhibition of strain *hisHB22 hisP1657* by tryptophan can also be demonstrated on a petri plate. A lawn of *hisHB22 hisP1657* on a plate containing 3×10^{-5} M histidine was inhibited by tryptophan diffusing from a filter paper disc (Fig. 5). Revertants of *hisP* mutants, and *hisP*+ recombinants between *hisP* mutants, can be selected as colonies that are resistant to tryptophan inhibition. This selection permits both a recombinational analysis of the *hisP* gene and the classification of permease mutants by studying their reversion (5, 15).

Presence of amber mutations in hisP gene(s). The isolation of amber *hisP* mutants demonstrated that this gene(s) codes for a protein. All the available independent *hisP* mutants containing *hisHB22* were reverted with methyl-nitro-

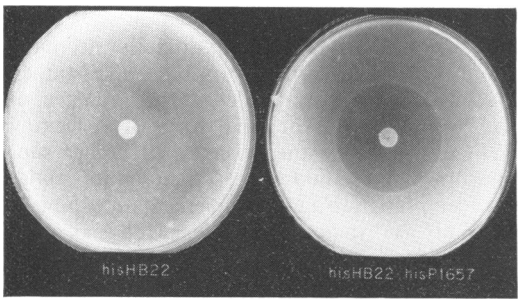

FIG. 5. *Effect of tryptophan on the growth of a histidine-requiring mutant with a defective histidine permease. Plates contain minimal medium supplemented with 3 × 10⁻⁵ M histidine. The filter disc in the center contains 1.0 μmole of tryptophan. Upon further incubation, colonies appear in the zone of inhibition of hisHB22 hisP1657.*

nitrosoguanidine on medium with low histidine plus phenylalanine; the revertants were checked for the presence of amber suppressors with the tester strains SB391 and SB392 (5). Reversion plates from several mutants repeatedly gave a positive response on the tester strains, whereas others were consistently negative.

Mutant *hisHB22 hisP1661*, which gave a high percentage of revertants containing an amber suppressor (determined by the positive response on SB392), was characterized further as an amber mutant by the following experiments. The revertants that apparently had an amber suppressor were purified from the reversion plate and again gave a positive response with both tester strains. They were also sensitive to HIPA by the radial streak test (as predicted for a suppressed *hisP* mutation). The presence of the original *hisP1661* mutation in one of these revertants was demonstrated by growing phage on it and by transducing strain *purF145* (to which *hisP* has 49% linkage) to wild-type. The Pur$^+$ transductants obtained were tested for resistance to HIPA. As expected, approximately 50% of the transductants were resistant to HIPA. The above experiments demonstrated that the revertant of *hisHB22 hisP1661* contains a suppressor mutation that suppresses both the *hisP* mutation and the *lac* amber mutation (in the tester strain) and that the reversion of the *hisP* mutation is not due to a change in the *hisP* gene.

Location of the aroP gene. The aromatic permease gene (*aroP*) was located in the proximity of the *proA* gene by mating strain SR305 (HfrA, gal$^-$, his$^-$, *str-s*) with TA237 (ile$^-$, pro$^-$, pur$^-$, *str-r*, *aroP*); the recombinants were tested for resistance to azaserine. No gene has been found which is cotransducible with *aroP*; less than 2% cotransducibility was observed between azaserine resistance and the following markers: *argF*, *leu500*, *purH*, *argA*, *purA*, *pyrB*, *ara*, *proA*, *pan*.

Properties of the aromatic permease. The specificity of the aromatic permease was investigated to gain information about the interaction between substrate and permease. A survey of the characteristics of the known substrates of the aromatic permease indicated that the nature of the side chain can vary considerably, although some aromatic character seems necessary. In fact, the following compounds, which have quite a variety of substitutions in the side chain, have been shown to be substrates of the aromatic permease (1): the natural amino acids, tyrosine, phenylalanine, tryptophan, and histidine; the amino acid analogues, 3-pyrazolealanine, 2-thiazolealanine, β-2-thienylalanine, β-3-furylalanine, *o*-, *m*-, and *p*-fluorophenylalanine, *o*-aminophenylalanine, 5-methyl-tryptophan, 2-methyl-histidine, among many others. Azaserine is unusual because, although it does not have a ring structure, it is a substrate.

This permease had no stringent requirement for the presence of the carboxyl group. In fact, analogues of the aromatic amino acids containing a phosphonic acid group instead of a carboxyl group were transported as demonstrated by the following facts. (i) Both the tyrosine and the phenylalanine phosphonate derivatives were very good inhibitors of growth of the wild-type, and tryptophan completely reversed this inhibition. (ii) Mutants resistant to inhibition by either of these analogues had simultaneously acquired resistance to azaserine, which is an indication of a defective aromatic permease. (iii) Aromatic permease mutant, *aroP504*, was resistant to the phenylalanine phosphonate analogue. (iv) Dopamine, which can be considered an analogue lacking completely the carboxyl group, reversed the inhibition caused by azaserine. There is no evidence that dopamine actually entered the cells, but its reversal of azaserine inhibition indicated that it can interfere with the action of the aromatic permease, and it gives indirect evidence that this permease does not require a carboxyl group for recognition of substrate.

Direct evidence that the aromatic permease transports amines or amino-alcohols was sought by testing the following compounds for inhibitory action: tyramine, tryptamine, phenylethylamine, tyrosinol, tryptophol, and phenylalaninol. Either they did not inhibit growth, or, if they did, the inhibition was achieved at very high concen-

trations (10^{-2} M or higher), and strain *aroP504* was as sensitive as the wild-type. At such high concentrations, several permeases or diffusion might also be involved in transport, besides, possibly, the aromatic permease. This could explain the sensitivity of *aroP504* to these compounds. Therefore, it was impossible to confirm unequivocally or exclude that compounds lacking a carboxyl group are substrates of the aromatic permease.

The hydrazino analogues of both tyrosine and histidine (HIPA) are transported by the aromatic permease because tryptophan partially reversed their inhibition of growth and because of the partial resistance of *aroP504* to inhibition by hydrazino tyrosine. Therefore, the aromatic permease is able to handle compounds in which hydrazino groups substitute for amino groups.

DISCUSSION

This paper further characterizes the specific histidine permease and the aromatic permease.

The map position of *hisP* has been accurately established, and it is dissimilar to that of any of the presently known histidine regulatory and biosynthetic genes (3, 4). This suggests, although it does not exclude, that intermediates involved in the active transport of histidine do not have a regulatory function for the histidine biosynthetic system. It has been previously established (9) that it is not necessary for histidine to be transported by the specific permease to repress the histidine operon (as demonstrated in the constitutive *hisS* mutants, which have a defective histidine-activating enzyme).

The wild type allele *hisP*$^+$ is dominant over *hisP*, as expected when the mutated gene no longer produces a functional protein.

The increased histidine permease activity in merozygotes which have two wild-type *hisP* loci indicates that the gene (or genes) which is limiting for the measurement of histidine transport is located in the section of chromosome covered by the F'32 episome. This does not exclude the possibility that other genes involved in histidine transport are present in other parts of the chromosome. However, this seems unlikely because (i) all *hisP* mutants which have been isolated map in the *hisP* region; and (ii) a merozygote with two wild-type *hisP* loci does not yield any resistant mutants. Therefore, if there are other loci involved in histidine transport, mutation in these loci must be lethal or not responsive to our selection methods.

The use of double-mutants, histidine-requiring and *hisP*, has supplied the means of selecting for revertants and recombinants of *hisP* mutants because of the inhibition by aromatic amino acids when growing on limiting histidine. This has allowed us to detect the presence of amber mutants in the *hisP* locus by checking *hisP* revertants for the existence of amber suppressors. This method will also be used for studying recombination and complementation among different *hisP* mutants. The occurrence of *hisP* amber mutants indicates that the product of this locus is a protein.

The amber mutant, *hisP1661*, when introduced into an otherwise wild-type genome, does not cause any decrease in growth rate. Therefore, it is concluded that the product of the *hisP* gene is completely dispensable under these conditions.

The location of the *hisP* and *aroP* genes in the bacterial map indicates that no linkage exists between these two permease genes. A gene apparently involved in transport of glutamate has been mapped in *E. coli* (7) and has a position quite distant from that of either *hisP* or *aroP* (assuming that the positions of these genes are analogous in *E. coli* and *S. typhimurium*, as are the positions of most other genes). The gene for arginine permease has been located near *serA* in *E. coli* (W. Maas, *personal communication*). No other amino acid permease has been mapped in either *E. coli* or *S. typhimurium*, but from this limited knowledge, it seems that no specific site exists for the common location of all permease genes. In addition, the *hisP* gene is unlinked to the histidine operon.

The aromatic permease has a broad specificity. The fact that it can transport phosphonic acid derivatives of the aromatic amino acids makes unlikely the possibility that the biochemical mechanism involves a carboxyl activation.

Both the histidine-specific permease (12) and the aromatic permease can transport α-hydrazino analogues of the amino acids. Apparently, no irreversible reaction occurs between these compounds and the components of the transport mechanism because the analogues are released on the inside of the cell where the inhibitory activity occurs. This phenomenon suggests that pyridoxal phosphate is not involved in the transport system because HIPA reacts with it irreversibly (12).

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