JOURNAL OF BACTERIOLOGY, Oct. 1973, p. 107-113

Vol. 116, No. 1
Printed in U.S.A.

The *hisP* Protein, a Known Histidine Transport Component in *Salmonella typhimurium*, Is Also an Arginine Transport Component

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Received for publication 3 May 1973

The *hisP* protein is an essential component of the high-affinity histidine transport system in *Salmonella typhimurium*. Our present studies demonstrate that this protein is also an essential component of an arginine transport system. Strains with a mutation in the *hisP* gene are unable to transport arginine for use as a sole nitrogen source. However, such strains have normal high-affinity transport of arginine, indicating that the *hisP* protein is not required for all arginine transport systems. Histidine does not appear to compete with arginine for transport through the *hisP* system, suggesting that the *hisP* protein may not be a "carrier" for these amino acids. The *hisJ* protein, a periplasmic histidine-binding protein, is known to function in conjunction with the *hisP* protein in the transport of histidine. The *hisJ* protein does not function with the *hisP* protein in arginine transport.

Two components, J and P, of the high-affinity histidine permease in *Salmonella typhimurium* have been well-defined (reviewed in reference 2). The genes for these components, *hisJ* and *hisP*, along with a regulatory site, *dhuA*, lie in a cluster on the *Salmonella* chromosome (3, 10). The J component is a periplasmic histidine-binding protein which has been purified and characterized biochemically (4, 11). Mutant strains lacking the J protein are defective in the high-affinity transport of L-histidine (3). In addition, they are unable to transport D-histidine, a poor substrate of the high-affinity permease, for use as a source of L-histidine (3).

The P component has been defined genetically and physiologically but not biochemically. The isolation of amber mutations in the *hisP* gene indicates that the P component is also a protein (5). Mutant strains lacking the P protein totally lack transport of histidine through the high-affinity permease (3, 16). As expected, such strains are unable to utilize D-histidine as a source of L-histidine (3, 10). In addition, they are resistant to the inhibitory histidine analogue hydrazino-imidazolyl-propionic acid (16), whereas strains lacking the J protein are not (3). Our working hypothesis is that the P protein functions after the J protein in the transport of histidine (3).

In this report we present evidence that the P protein (but not the J protein) also functions in a transport system for arginine.

MATERIALS AND METHODS

Chemicals. Amino acids were obtained from Sigma Chemical Co., Calbiochem, and Nutritional Biochemicals Corp. Histidinol and the peptide arginyl-arginine were obtained from Cyclo Chemical Co. Glycylglycylarginine was obtained from Fox Chemical Co. L-[^{3}H]arginine, sp act 7.0 Ci/mmol, was obtained from Schwarz/Mann. ICR372 was kindly donated by H. J. Creech. D-2-hydrazino-3-(4-imidazolyl) propionic acid (D-HIPA) was synthesized from L-histidine (17).

Bacterial strains. All strains were derived from *S. typhimurium* strain LT2 and are listed in Table 1. The selection of mutations in *hisP* by resistance to the inhibitory histidine analogue HIPA and the genetic analysis of such mutations have been described (5). Mutations in *hisP* were isolated in arginine auxotrophs in the presence of 0.1 mM glycylglycylarginine as arginine source. Phage (P22 int-4) grown on these mutants were used to transduce TA1852 (*hisFΔ-645 dhuA1 purF145*) to *pur*$^+$. Transductants which had received the *hisP* mutation were identified by HIPA resistance, and phage-sensitive clones were isolated as described previously (15).

The isolation and genetic analysis of *dhuA* mutations and *hisJ* mutations have also been described (3). (*hisJ* mutations have been isolated only in *dhuA*-containing strains.)

TA2164 (*metE338 hut*$^+$) was constructed from

TABLE 1. *Bacterial strains*

Strain	Genotype	Description of *hisP* mutation
TA1772	(Wild-type)	
TA1857	(Wild-type)	
hisP1661	*hisP1661*	Amber
TA1858	*hisP1661*	Isogenic with TA-1857
TA2520	*hisP5666*	ICR-induced
TA1866	*hisPΔ5651*	Deletion
TA1867	*hisPΔ5670*	Deletion
TA831	*hisFΔ645*	
TA2500	*hisFΔ645 hisP5665*	Spontaneous
TA2502	*hisFΔ645 hisP5667*	ICR-induced
TA271	*hisFΔ645 dhuA1*	
TA1014	*dhuA1*	
TA1008	*hisFΔ645 dhuA1 hisP5503*	Spontaneous
TA1195	*dhuA1 hisP5503*	
TA1650	*dhuA1 hisJ5601*	
TA1802	*dhuA1 hisJ5601 hisP5583*	Spontaneous
TA1803	*dhuA1 hisJ5601 hisP5584*	ICR-induced
TA1768	*hisFΔ645 dhuA1 hisJ5617*	
TA2813	*argB69 hisP5676*	Spontaneous
TA2814	*arg509 hisP5677*	Spontaneous
TA3125	*hisFΔ645 hisP5676*	
TA3126	*hisFΔ645hisP5677*	
TA1852	*hisFΔ645 dhuA1 purF145*	
TA1853	*dhuA1 purF145*	
TA2164	*metE338 hut*$^+$	
TA3116	*dhuA1 purF145 gal*$^-$	
TA3117	*dhuA1 purF145 hut*$^+$	
TA3118	*hut*$^+$	
TA3119	*hisP1661 hut*$^+$	
TA3120	*dhuA1 hut*$^+$	
TA3121	*dhuA1 hisJ5601 hut*$^+$	
TA3122	*dhuA1 hisP5503 hut*$^+$	
TA3129	*aroP734 hut*$^+$	
TA3130	*hisP1661 aroP735 hut*$^+$	

metE338 by transducing it to growth on histidine as a nitrogen source (with citrate as carbon source) with phage P376 grown on *S. typhimurium* 15–59 (7, 12; J. Broach, unpublished results). TA3116 (*purF145 dhuA1 gal*$^-$) was constructed from TA1853 (*purF145 dhuA1*) by selecting for resistance to 2-deoxygalactose with glycerol as the carbon source (K. Floyd, personal communication). TA3117 (*purF145 dhuA1 hut*$^+$) was then constructed from TA3116 by transducing it to growth on galactose as sole carbon source with phage grown on TA2164, and screening for a transductant able to grow on histidine as a nitrogen source (with succinate as carbon source) (7, 12). Whenever the strain contained the *purF145* mutation, adenine and thiamine were present.

A set of isogenic *hut*$^+$ strains with mutations in the *dhuA hisJ hisP* gene cluster (TA3118 to TA3122) was constructed by transducing TA3117 to prototrophy with phage grown on the appropriate strains and screening the transductants for the presence of mutations in the histidine permease cluster. Strains with a mutation in *hisP* were identified by HIPA resistance, and strains with a mutation in *hisJ* or a wild-type permease region were identified by resistance to azaserine in the presence of tryptophan (G. F. Ames, unpublished results).

Spontaneous mutations in *aroP*, a gene for the aromatic permease, were isolated in TA3118 and TA3119 by resistance to azaserine (and simultaneous resistance to 5-methyltryptophan) as described previously (1).

Growth of bacterial strains. Bacterial strains were grown in a minimal salts medium containing (per liter): K_2SO_4, 1.0 g; $K_2HPO_4 \cdot 3H_2O$, 17.7 g; KH_2PO_4, 4.7 g; $MgSO_4\ 7H_2O$, 0.1 g; and NaCl, 2.5 g. Glucose, glycerol, or citrate at a final concentration of 0.4 to 0.6% was used as carbon source, as indicated. Arginine, arginylarginine, histidine, or NH_3 at a final concentration of 2.5 to 10 mM was used as nitrogen source, as indicated. Cultures were incubated at 37 C in a New Brunswick rotary shaker with vigorous aeration. Bacterial growth was monitored turbidimetrically by measuring absorbancy at 650 nm. For determination of growth rates on various nitrogen sources, an inoculum culture of each strain was grown overnight in medium containing both NH_3 and the nitrogen source to be tested at a concentration of 10 mM (10 mM NH_3 is enough to allow full growth of the culture). The following day the cultures were centrifuged, washed with minimal salts, resuspended, and used as inocula at a dilution of $1/100$ or $1/50$.

Growth of strains on various nitrogen sources was tested on petri plates by the disk method (15). In this method, a layer of soft agar containing the carbon source and 10^8 bacteria is first poured over the surface of a minimal salts agar plate. A filter paper disk impregnated with the nitrogen source is then placed in the center of the plate. Strains which are able to use the nitrogen source grow in a circular zone around the disk. A variation of this method, in which many strains can be tested on a single plate, is the radial streak method (15). In this method a disk impregnated with the nitrogen source (or other requirement) is placed in the center of a plate containing both salts and carbon source, and a number of clones are streaked radially toward the disk.

Transport assays. The transport of ^{3}H-arginine was assayed by the growing cells method (1) in which incorporation of low concentrations of external amino acid into protein is measured.

RESULTS

Utilization of arginine as a nitrogen source: (i) hisP mutants. Strains with a mutation in the *hisP* gene were tested for their ability to use a variety of amino acids as sole nitrogen source (9). The *hisP* mutations tested included spontaneous mutations, two spontaneous dele-

tions, and mutations induced with the frameshift mutagen ICR372 (6). The tests were done by the disk method (see Materials and Methods) with 0.4% glucose as carbon source and 3 μmol of amino acid in the disk as nitrogen source. Strains with a *hisP* mutation failed to grow on arginine as sole nitrogen source (Table 2). They grew normally on other amino acids known to be used as a nitrogen source by *S. typhimurium* (aspartate, glutamate, glutamine, serine, cysteine, and alanine), giving growth zones of the same diameter and same apparent density as control strains.

Failure of strains with a *hisP* mutation to utilize arginine as a nitrogen source was confirmed in liquid culture (Fig. 1). With 0.4% glucose as carbon source and 10 mM arginine as nitrogen source TA1772, a wild-type control, has a doubling time of 7.7 h. A strain with a *dhuA* mutation, TA1014, has a slightly faster doubling time, 6.5 h. (Strains with a *dhuA* mutation produce elevated amounts of normal J protein [3, 4].) A strain with a mutation in *hisP*, TA2520, and a strain with the double mutation *dhuA hisP*, TA1195, do not grow on this medium (doubling time greater than 35 h). Similar results were obtained with other carbon sources (Table 3). With glycerol or citrate as carbon source, the doubling time of wild-type and *dhuA* strains is 5 h, and again strains with a mutation in *hisP* have a doubling time greater than 35 h. The failure of *hisP* strains to utilize arginine does not appear to be due to an inhibition by it since the growth of such strains on NH_3 as nitrogen source is not inhibited by a fivefold molar excess of arginine (Table 3).

TABLE 2. *Growth of hisP-containing and control strains on arginine as a nitrogen source*[a]

Strain	Genetic constitution	Growth on glucose-arginine
TA1772	Wild-type	+
hisP1661	*hisP*	−
TA1014	*dhuA*	+
TA1195	*dhuA hisP*	−
TA1866	*hisP* (Δ)	−
TA1867	*hisP* (Δ)	−
TA1650	*dhuA hisJ*	+
TA1768	*dhuA hisJ*	+
TA1802	*dhuA hisJ hisP*	−
TA1803	*dhuA hisJ hisP*	−

[a] The test was done on petri plates by the disk method with 0.4% glucose as carbon source and 3 μmol of arginine or other amino acid in the disk as nitrogen source. Plates were scored after 48 h at 37 C. Control amino acids on which all strains grew equally well were aspartate, glutamate, glutamine, serine, cysteine, and alanine.

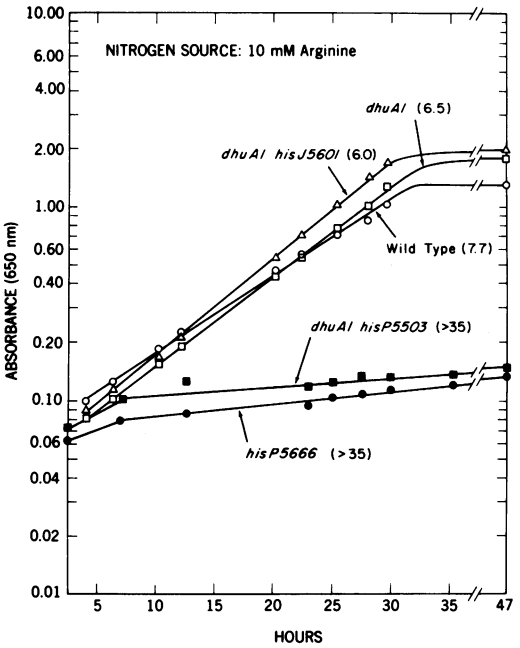

FIG. 1. *Growth rates of various strains on arginine as sole nitrogen source. Growth rates were determined as described in Materials and Methods. The carbon source was 0.4% glucose. The concentration of arginine was 10 mM. The doubling time for each strain is indicated in parenthesis after the strain designation. Wild-type, TA1772; hisP5666, TA2520; dhuA1, TA1014; dhuA1 hisJ5601, TA1650; dhuA1 hisP5503, TA1195.*

(ii) hisJ mutants. It was of interest to see whether the J protein was involved in arginine utilization since this protein binds arginine in vitro (4). Disk tests with two strains containing frameshift mutations in *hisJ* (TA1650 and TA1789) indicated that these strains utilized arginine normally (Table 2). (They also utilized normally the other amino acids tested.) As shown in Fig. 1, one of these strains, TA1650, has as good a doubling time on glucose-arginine as its parent strain TA1014. These results indicate that the J protein is *not* required for utilization of arginine as a nitrogen source.

hisP mutants have normal arginine metabolism. To demonstrate that the defect in arginine utilization caused by a *hisP* mutation is not in metabolism, *hisP* strains were tested for their ability to grow on the dipeptide arginylarginine as nitrogen source. It has been shown by Payne et al. that peptides have transport systems distinct from those for amino acids (reviewed in reference 13). All of the strains tested, including those with a mutation in *hisP*, were able to grow on the dipeptide (Table 4). As a control for the purity of the peptide, the strains

TABLE 3. *Doubling times of hisP-containing and control strains on arginine as a nitrogen source*[a]

Carbon source	Nitrogen source	Doubling time of strains (h)				
		TA1772 (wild-type)	TA2520 (*hisP*)	TA1014 (*dhuA*)	TA1650 (*dhuA hisJ*)	TA1195 (*dhuA hisP*)
0.4% Glucose	10 mM arginine	7.7	>35	6.5	6.0	>35
0.8% Glycerol	10 mM arginine	5.1	>35	4.9	5.0	>35
0.6% Citrate	10 mM arginine	5.2	>35	5.0	4.5	>35
0.6% Glucose	5 mM NH_3	0.80	0.81			
0.6% Glucose	5 mM NH_3 + 5 mM arginine	0.78	0.83			
0.6% Glucose	5 mM NH_3 + 50 mM arginine	0.83	0.83			

[a] Growth conditions are described in Materials and Methods.

TABLE 4. *Growth of hisP-containing and control strains on the dipeptide arginylarginine as nitrogen source*[a]

Nitrogen source	Growth of strains				
	TA1772 (wild-type)	TA2520 (*hisP*)	TA1014 (*dhuA*)	TA1650 (*dhuA hisJ*)	TA1195 (*dhuA hisP*)
Peptide (2 μmol)	+	+	+	+	+
Arginine (4 μmol)	+	−	+	+	−
Hydrolyzed peptide[b] (equivalent to 2 μmol)	+	−	+	+	−

[a] The test was done on petri plates by the disk method with 0.4% glucose as carbon source and the indicated amount of peptide or arginine in the disk as nitrogen source. Plates were scored after incubation at 37 C for 24 h.

[b] The peptide was hydrolyzed for 20 h at 110 C in 6 N HCl. This solution was then diluted and evaporated to dryness five times. The residue was dissolved in water, and the pH was adjusted to 4 with a small volume of 6 N NaOH.

were tested for their ability to grow on its acid hydrolysis products. As expected, strains with a *hisP* mutation were unable to grow on the hydrolyzed peptide, whereas all other strains grew normally.

Utilization of the dipeptide by *hisP* strains was confirmed in liquid culture with glucose as carbon source and 2.5 mM arginylarginine as nitrogen source. On this medium, both *hisP* strains (TA1858, TA1195) and control strains (TA1857, TA1014) have the same doubling time (about 4 h). These results indicate that the defect in arginine utilization caused by a *hisP* mutation is not due to a defect in arginine metabolism.

hisP mutants have normal high-affinity arginine transport. It was previously reported that a *hisP* mutation does not alter the high-affinity transport of arginine for incorporation into protein (16). Transport assays have been repeated with concentrations of arginine from 10^{-8} to 8×10^{-8} M (growing cells assay [1]). Both *hisP* strains and control strains have equal high-affinity transport of arginine with a K_m of about 5×10^{-8} M (calculated from data in Table 5). Rosen has described a specific high-affinity transport system for arginine (K_m 2.6×10^{-8} M) in *Escherichia coli* (14).

To confirm that a *hisP* mutation does not greatly affect the transport of arginine to satisfy an auxotrophic requirement, *hisP* mutations were isolated in arginine auxotrophs as described in Materials and Methods. (The tripeptide glycylglycylarginine was used as arginine source in this selection since tripeptides are transported by an oligopeptide permease [13].) The resulting *hisP* strains, TA2813 and TA2814, and their parent strains were tested for ability to grow on limiting amounts of arginine by the radial streak method (see Materials and Methods). The tests were done on petri plates with glucose as carbon source, NH_3 as nitrogen source, and 0.01 to 1.0 μmol of arginine in the disk to satisfy the auxotrophic requirement. As the amount of arginine in the disk was decreased, the growth of all strains was limited, but there was no detectable difference between *hisP* strains and their parent strains. To show that the *hisP* mutations were not leaky, they were transduced into a histidine auxotroph, as described in Materials and Methods, and it was demonstrated that they caused a decreased

growth of this auxotroph on limiting amounts of histidine. As expected, both the original *arg⁻ hisP* strains and the *his⁻ hisP* strains obtained by transduction were unable to utilize arginine as a nitrogen source.

Competition between histidine and arginine. The above results demonstrate that the *hisP* protein is not required for high-affinity arginine transport. Since this protein is required for high-affinity histidine transport (K_m 3 × 10^{-8} M) (3), histidine was tested as an inhibitor of growth on arginine. (Histidine cannot be used as a nitrogen source by *S. typhimurium* strain LT2.) TA1772 (wild-type), TA1014 (*dhuA*), and TA1650 (*dhuA hisJ*) were plated on 0.4% glucose with 1 or 5 mM arginine as nitrogen source, and a disk containing 5 μmol of histidine was placed in the center of each plate (Fig. 2A). Histidine did not inhibit growth on arginine under these conditions.

Utilization of histidine as a nitrogen source: (i) hisP mutants. *S. typhimurium* strain LT2 is unable to utilize histidine as a nitrogen source. If a more efficient promoter for the genes of the histidine degradation pathway is introduced into this strain, it acquires the ability to utilize histidine as a nitrogen source with carbon sources other than glucose (7, 12). A histidine-utilizing strain was constructed, and various mutations in the *dhuA hisJ hisP* gene cluster were then introduced into this strain as described in Materials and Methods. The resulting isogenic *hut*⁺ strains (TA3118 through TA3122) were tested for their ability to utilize histidine as a nitrogen source. All of the strains tested, including those with a *hisP* mutation, had a doubling time of 2.8 to 3.2 h on a medium containing 10 mM histidine as nitrogen source and 0.6% succinate as carbon source. Thus, a *hisP* mutation has no effect on histidine utilization under these conditions. (As expected, *hut*⁺ strains containing a *hisP* mutation were unable to utilize arginine as nitrogen source.)

(ii) aroP mutants. The aromatic permease is a lower-affinity transport system for histidine (K_m 10^{-4} M) and a high-affinity transport system for the aromatic amino acids (K_m about 10^{-7} M) (1). Mutations in the aromatic permease (*aroP*) were isolated in TA3118 (*hut*⁺) and TA3119 (*hut*⁺ *hisP*) as described in Materials and Methods. Mutations in *aroP* decreased the growth rate of TA3119 from 3.0 to 7.0 h but did not affect the growth rate of TA3118.

Competition between phenylalanine and histidine. Since mutations in *aroP* decreased the growth rate of *hisP* strains on histidine as a nitrogen source, phenylalanine, a high-affinity substrate of the aromatic permease, was tested as an inhibitor of growth on histidine. (Phenylalanine cannot be used as a nitrogen source.) TA3118 (*hut*⁺) and TA3119 (*hut*⁺ *hisP*) were plated on 0.4% succinate with 1 or 5 mM histidine as nitrogen source, and a disk containing 1 μmol of phenylalanine was placed in the center of each plate (Fig. 2B). Zones of inhibition (about 5.0 cm in diameter) were clearly visible for both strains. These zones were turbid for TA3118 and were clearer for TA3119. Mutations in *aroP* eliminated phenylalanine inhibition in both strains.

DISCUSSION

The transport of arginine for utilization as a nitrogen source is dependent on the *hisP* gene product, one of the components of the high-affinity histidine permease. Strains with a mutation in the *hisP* gene are specifically unable to utilize arginine as nitrogen source, but they utilize a variety of other amino acids normally. The defect in arginine utilization is not due to a defect in metabolism since *hisP* strains are able

TABLE 5. *Uptake of* ^{3}*H-arginine measured by incorporation into protein*[a]

Arginine concn (M × 10^6)	Rate (counts/min)		Optical density at 650 nm		Rate (μmol per min per g dry wt)		Ratio of rates TA1858/TA1857 (%)
	TA1857 (wild-type)	TA1858 (*hisP*)	TA1857	TA1858	TA1857	TA1858	
1	6,300	4,020	0.0078	0.0048	0.65	0.68	104
2	11,800	6,900	0.0081	0.0051	1.18	1.09	92
4	15,100	9,400	0.0085	0.0053	1.44	1.43	100
8	18,600	10,100	0.0088	0.0055	1.71	1.49	87

[a] The amount of external arginine incorporated into protein was measured at 30-s intervals for 2 min, and rates of uptake (counts per minute) were determined from plots of this data. (At the lower concentrations of arginine, 30-s and 1-min points were used to calculate rates.) The optical density of the culture at the time of assay was determined as described previously (1) and was converted to grams dry weight (1 optical density unit = 4.7 × 10^{-4} g dry wt).

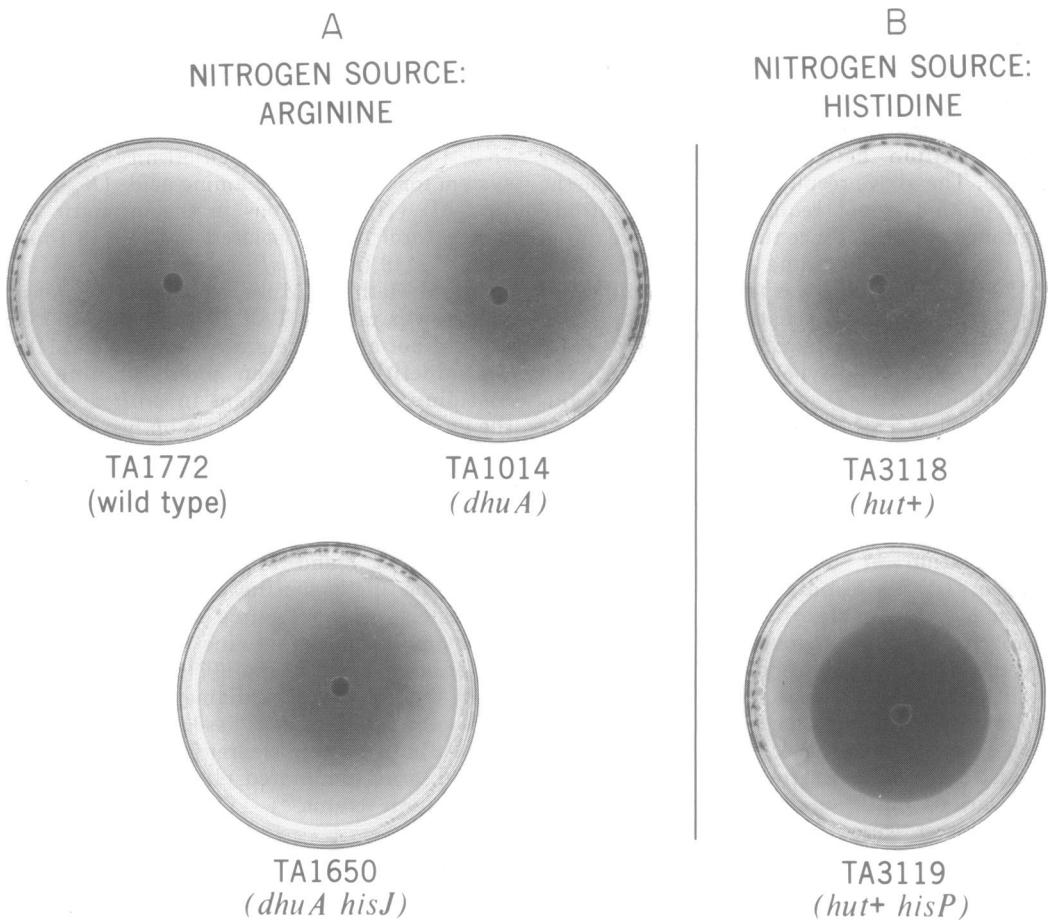

FIG. 2. *A, Effect of histidine on the utilization of arginine (1 mM) as a nitrogen source. Histidine (5 μmol in the disk) does not inhibit (see text). Plates were incubated for 48 h at 37 C. B, Effect of phenylalanine on the utilization of histidine (1 mM) as a nitrogen source in hut⁺ strains. Phenylalanine (1 μmol in the disk) inhibits even in hisP⁺ strains (see text). Plates were incubated for 48 h at 37 C.*

to utilize the dipeptide arginylarginine as a nitrogen source with the same doubling time as their parent strains. This peptide presumably enters the cell through a peptide transport system (13).

Strains with a *hisP* mutation have normal high-affinity transport of arginine, indicating that the *hisP* protein is not a component of all arginine transport systems. The growth of arginine auxotrophs is not affected by a *hisP* mutation. Calculations (not shown) indicate that the rate of arginine uptake in a *hisP* mutant is adequate to allow its utilization as a nitrogen source at the growth rate observed for the wild-type. (Yield experiments show that all four nitrogens of arginine can be utilized.) Apparently, *hisP* mutants fail to grow on arginine as a nitrogen source because they do not maintain a large enough pool for rapid functioning of the arginine degradative enzymes.

The *hisJ* gene product, a periplasmic histidine-binding protein, does not appear to function in conjunction with the *hisP* protein in the transport of arginine. Strains with a mutation in the *hisJ* gene show no defect in the utilization of arginine as a nitrogen source and, in fact, grow somewhat faster on arginine than do their parent strains (Fig. 1). This suggests the possibility that another binding protein specific for arginine might function with the *hisP* protein in arginine transport.

Growth studies indicate that histidine does not compete with arginine for transport through the *hisP* system. This lack of competition suggests that the *hisP* protein may not be a "carrier" (8) for these amino acids but may have some other function in the transport process.

To test the effect of permease mutations on

the utilization of histidine as a nitrogen source, strains able to degrade histidine (*hut*$^+$) were constructed. Single mutations in *hisP* or in *aroP* (the aromatic permease) do not affect the doubling time on histidine as a nitrogen source. However, strains with a double mutation *hisP aroP* have a longer doubling time than their parent strains. Phenylalanine, a high-affinity substrate of the aromatic permease, can be shown to inhibit growth on histidine even in *hisP*$^+$ strains.

ACKNOWLEDGMENTS

We thank B. N. Ames, J. Broach, and L. Kier for helpful discussion and criticism during the course of this work.

This study was supported by Public Health Service grant AM12121 (to G.F.A.) from the National Institute of Arthritis and Metabolic Diseases. S.G.K. was a postdoctoral fellow supported by Public Health Service fellowship 1 FO2 GM53347-01 from the National Institute of General Medical Sciences.

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