The Histidine-binding Protein J Is a Component of Histidine Transport

IDENTIFICATION OF ITS STRUCTURAL GENE, hisJ*

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SUMMARY

The histidine-binding protein J, previously shown to be involved in histidine transport in Salmonella typhimurium (AMES, G. F., AND LEVER, J. (1970) Proc. Nat. Acad. Sci. U. S. A. 66, 1096), is shown unequivocally to be the product of the hisJ gene. A hisJ mutant with an altered J protein and a correspondingly altered histidine transport has been isolated and characterized. The J protein from this strain has an increased temperature sensitivity, besides having altered chromatographic and electrophoretic mobilities. The in vivo effect of the altered J protein is expressed as an increased temperature sensitivity of histidine transport. Our data indicate that the hisJ gene is the structural gene for the J protein and that the J protein is an obligatory component of histidine transport.

As expected, there is an excellent correlation between the specificity of transport in the wild type strain and the specificity of binding of the wild type J protein for a variety of amino acids, amino acid analogues, and inhibitors.

Salmonella typhimurium transports L-histidine through at least five permeases with different patterns of specificity and affinity (1–3). The histidine permease with the highest affinity (J-P permease, with a Kₐ of about 10⁻⁸ M) has been shown to be composed of at least two proteins, J and P (3), and has been analyzed kinetically, biochemically, and genetically. The J protein is a periplasmic histidine-binding protein, released by osmotic shock (3, 4) and coded for by the hisJ gene (3). The P protein is essential for histidine transport by the J protein and is coded for by the hisP gene (2, 3) but has not been identified biochemically. The P protein is also necessary for the functioning of another histidine permease (the K-P permease, with a Kₐ of about 10⁻⁷ M), which works in parallel to the J-P system (3).

We demonstrated earlier a direct correlation between the activity of the histidine permease and the levels of the J protein (3). Strains with a mutation in the hisJ gene are defective in histidine transport and correspondingly lack the J protein. Strains with a mutation in the hisD gene, which is thought to be a control locus for histidine transport, have elevated histidine transport and are correspondingly elevated in the J protein. The proof that hisJ mutations are in the structural gene for the J protein, rather than in a control locus for the genes of histidine transport, was obtained by the characterization of a temperature-sensitive mutation which simultaneously alters both the J protein and transport through the J-P permease. This mutant is described in detail in this paper together with other evidence on the role of the J protein in transport. The J-P permease also transports other substances (among them b-histidine, L-arginine, L-2-hydrazino-3-(4-imidazolyl)propionic acid) with lower affinity; the correlation between the specificity of transport and the binding specificity of the J protein is presented.

The purification and some of the properties of the wild type J protein have been described (4).

EXPERIMENTAL PROCEDURE

Chemicals—All chemicals from commercial sources were of the highest purity available. Amino acids were obtained from Sigma, Calbiochem, and Nutritional Biochemicals Corporation. L-Lysine was obtained from Fox Chemical Company. O-Diazoacetyl-l-serine was obtained from the National Cancer Institute (Cancer Chemotherapy National Service Center, Bethesda, Md.). n-HIPA was synthesized from L-histidine (5). The product was homogeneous chromatographically, using paper chromatography in 1-propanol-1 N HCl (3:1, v/v), and 1-propanol-1 N NH₄Cl (3:1, v/v), with detection by imidazole spray (6) and ninhydrin.

L-[14C]Histidine was obtained from New England Nuclear Corporation, with a specific activity of 2.4 Ci per mmole. It was stored at -18°C in 50% ethanol. Histidine concentration was verified by amino acid analysis, and radiochemical purity was monitored by paper chromatography in the two 1-propanol systems above, using radioautography for detection.

Possible histidine contamination in amino acids and analogues used in the competition experiments was monitored using a Beckman/Spinco model 121 amino acid analyzer with expanded range. Imidazole pyruvic acid (Calbiochem), L-histidinol

* The abbreviations used are: n-HIPA, n-2-hydrazino-3-(4-imidazolyl)propionic acid; J⁺ protein, temperature-sensitive J protein; asasecine, O-diazoacetyl-l-serine.

4309
either by the dialysis or by the filtration method (3, 4). [3H]Histidine binding activity was assayed as already described (3, 4). [3H]Histidine was freed of contaminating histidine (0.01 μc) by column chromatography, using AG-50 W-X4 resin (Bio-Rad) in the NH₄⁺ form (8).

Assays—L-Histidine uptake was measured by the “growing cells” method (1) (10 to 20 μg, dry wt, of cells per ml), with the modification for the washing of the samples as already described (3). The J protein was purified, assayed, and characterized as described (3, 4). [3H]Histidine binding activity was assayed either by the dialysis or by the filtration method (3, 4).

Bacterial Strains and Media—All strains were derived from S. typhimurium strain LT-2 and are listed in Table I. Conditions for growth have been described (2). Except for TA271 which was obtained from T. Klopotowski (9), all strains were constructed in this laboratory and analyzed genetically by transduction with phage P22 int-4 as described earlier (2). Growth on n-histidine (1 μmole on disc) and sensitivity to n-HIPA (in the presence of L-histidinol for histidine auxotrophs) were assayed on Petri plates by the radial streak method (2).

Genetic Analysis of Bacterial Strains—Strains TA271 (dhuA1 hisF645) and TA1014 (dhuA1) have been described (3). Strain TA1768 (dhuA1 hisJ5617 hisF645) was obtained by mutagenizing TA271 with the frameshift mutagen, ICR-191 (10), and then selecting for a mutation in hisJ (such mutation renders the strain unable to grow on n-histidine, but still n-HIPA sensitive), as described previously (3). Mutation hisJ5617 so obtained was shown to be closely linked (about 88%) to dhuA1 by the same methods used for other hisJ mutations (3). Strain TA1789 (dhuA1 hisJ5617 hisJ5620 hisF645; briefly mentioned in (3)) was isolated as a revertant of TA1768, which was capable of growing on n-histidine at room temperature, but not at 40°C.

Strains TA1771 and TA1791 were obtained by transducing the hisJ revertant TA1791. A. Initial rates of uptake of L-histidine (micromoles per min per g, dry wt) as a function of temperature. B. L-histidine uptake through the J component as a function of temperature. The initial rates of uptake at each temperature by TA1771 (i.e. through the K component) are expressed as percentages, taking the correct rate of uptake by TA1014 as 100% at each temperature. The L-[3H]histidine concentration is 4 x 10⁻⁶ M.

results

Mutant Strain with Qualitative Alteration of Both J Protein and Histidine Transport—Strain TA1789 (dhuA1 hisJ5617 hisJ5620 hisF645) is a revertant of TA1768 (dhuA1 hisJ5617 hisF645) and was chosen for further study because of its temperature-sensitive phenotype. It carries two mutations in hisJ, both induced by the frameshift mutagen ICR-191 (10); the initial mutation, hisJ5617, causes loss of both the J component of transport and the J protein, and hisJ5620, a mutation at a second site in the hisJ gene, restores partial J protein function.

As a consequence of the double mutation in the hisJ gene, strain TA1789 (and its prototrophic derivative TA1791) has a temperature-sensitive J component of transport which can be analyzed by measuring either L-histidine uptake or the ability to grow on n-histidine. Concomitantly with these altered properties of transport, TA1789 (and TA1791) produces a J protein with several altered properties including an increased temperature sensitivity.

Genetic Mapping—Strain TA1789 (dhuA1 hisJ5617 hisJ5620 hisF645) was shown by the following genetic tests to contain the mutation, hisJ5620, responsible for the second site reversion, in the hisJ gene. Mutation hisJ5620 was first shown to be cotransducible (42%) with purF146, by growing phage on TA1789.

| Strain   | Genotypea | n-Histidine growth | n-HIPA resistance | J protein |
|----------|-----------|--------------------|-------------------|-----------|
| TA271    | dhuA1 hisF645 | +                  | Supersensitive | Elevated  |
| TA1014   |          |                   |                   |           |
| TA1768   | dhuA1 hisJ5617 hisF645 | -                  | Sensitive | Elevated  |
| TA1771   | dhuA1 hisJ5617 hisJ5620 hisF645 | +b | Sensitive | Absent    |
| TA1789   | dhuA1 hisJ5617 hisJ5620 hisF645 | -      | Absent     | Elevatedb |
| TA1791a  |           |                   |                   |           |

a Only the histidine-requiring strains, but not their prototrophic transductants, can be assayed for n-histidine growth. Only the hisF645 causes a defect in histidine biosynthesis.

b Temperature-sensitive phenotype.

c Preliminary results with this strain have been published previously (3).

*Fig. 1. Temperature sensitivity of L-histidine uptake in the hisJ revertant TA1791. A, initial rates of uptake of L-histidine (micromoles per min per g, dry wt) as a function of temperature. B, L-histidine uptake through the J component as a function of temperature. The initial rates of uptake at each temperature by TA1771 (i.e. through the K component) are expressed as percentages, taking the corrected rate of uptake by TA1014 as 100% at each temperature. The L-[3H]histidine concentration is 4 x 10⁻⁶ M. Histidine auxotrophs TA1768 and TA1789, respectively, to prototrophy with wild type phase.

Bacterial strains

| Strain | Genotype | n-Histidine growth | n-HIPA resistance | J protein |
|--------|----------|--------------------|-------------------|-----------|
| TA271  | dhuA1 hisF645 | +                  | Supersensitive | Elevated  |
| TA1014 |          |                   |                   |           |
| TA1768 | dhuA1 hisJ5617 hisF645 | -                  | Sensitive | Elevated  |
| TA1771 | dhuA1 hisJ5617 hisJ5620 hisF645 | +b | Sensitive | Absent    |
| TA1789 | dhuA1 hisJ5617 hisJ5620 hisF645 | -      | Absent     | Elevatedb |
| TA1791a|           |                   |                   |           |

a Only the histidine-requiring strains, but not their prototrophic transductants, can be assayed for n-histidine growth. Only the hisF645 causes a defect in histidine biosynthesis.

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Fig. 2. Growth on D-histidine (A) at 17° and (B) at 40°. All cultures were grown overnight at 17° in minimal medium containing \(7 \times 10^{-5} \) mM L-histidine; this histidine concentration is exhausted during growth of the cultures to stationary phase. At zero time each culture is diluted 50-fold in minimal medium containing \(2 \times 10^{-4} \) mM D-histidine and grown at the indicated temperature. The TA271 culture stops growing at optical density of about 0.500 because the D-histidine concentration is limiting.

and transducing the double mutant purF145 hisF645 to Pur+ on L-histidine medium and showing that 47 out of 111 of the Pur+ His- recombinants had a temperature-sensitive phenotype for D-histidine growth. The double mutation causing temperature sensitivity was then placed very close to dhuA1 (90% cotransducible) by the following cross. Phage grown on TA1789 was used to transduce the double mutant purF145 hisF645 to growth at 23° on D-histidine (0.3 mM), in the presence of adenine and thiamine (0.2 mM and 0.02 mM, respectively; adenine plus thiamine are required by strains containing the purF145 mutation); thus, the selection was only for dhuA1, and not for the temperature-sensitive mutation or for Pur+. The recombinants growing on these plates were then tested for D-histidine growth at 23° and at 40°: recombinants growing at both temperatures inherited only the dhuA1 mutation, those growing only at 23° inherited also the temperature-sensitive mutation. Ninety per cent (72 out of 80) of the D-histidine-growing recombinants were temperature sensitive; the remaining 10% grew well at either temperature, confirming the presence of dhuA1 in this strain. Thus mutation hisJ5617 is 42% cotransducible with purF145 and 90% cotransducible with dhuA1; these results firmly establish its position in the histidine permease cluster.2

2 It has been recently found (Govons and Ferro-Luzzi Ames, unpublished results) that the linkage between purF145 and the permease cluster is 2% when purF145 is the unselected marker in the transduction experiments, as opposed to a linkage of about 40% when Pur+ is the selected marker. These data could be explained if purF145 were a large deletion.

Studies on Histidine Transport in Mutant Strain

Temperature Sensitivity of L-Histidine Uptake—Fig. 1, A and B, show that increasing temperature causes a decay in the rate of uptake of labeled L-histidine through the temperature-sensitive J component in TA1791 (dhuA1 hisJ5617 hisJ5620). The assays were done by the “growing cells” method (1) on cultures which had been growing at the assay temperatures for many generations and thus had the level of transport compatible with each temperature.3 The initial linear rates of uptake were determined at each temperature and are plotted in Fig. 1A. The rates in TA1014 (dhuA1) and TA1791 have then been corrected (by subtraction) for the rates due to the K-P component of transport (3), which was simultaneously assayed in TA1771 (dhuA1 hisJ5617) at each temperature. The rates in TA1791 were then expressed as a percentage of the unaltered J activity, taking the corrected values of dhuA1 as 100% (Fig. 1B).

3 TA1791 cells grown at 29° for many generations and shifted to higher temperatures, have a lower rate of uptake than TA1014 cells treated in an identical way. For example, after 30 min at 43° TA1791 has only 1% of the TA1014 activity. This demonstrates that it is the activity, rather than the synthesis of the J protein, that is temperature sensitive. We have preferred to show that transport is temperature sensitive as in Fig. 1, because both strains lose some transport activity when they undergo a sudden temperature shift. This loss is probably due to the temperature shock inflicted upon other components of transport or protein synthesis when transport is assayed in growing cells under physiological conditions; however, the loss is always larger in the temperature-sensitive mutant (TA1791) than in TA1014.
Evidence for Altered J Protein in Mutant Strain

Histidine Binding Activity Levels—Binding activity levels in the shock fluids from TA1791 (dhuA1 hisJ5617 hisJ5620) and from the control strains TA1014 (dhuA1) and TA1771 (dhuA1 hisJ5617) are given in Table II. Since the J protein from TA1791 is heat labile, cells were grown at both 37° and 30° for comparison. Strain TA1771 has no J protein activity, as previously demonstrated (3) for bacteria with mutations in the hisJ gene; the small binding activity detectable is significant and is due to other histidine-binding proteins (3, 4). The dhuA1 mutation causes elevated levels of J protein and is present in all strains. TA1791 shows the normal activity levels expected for a dhuA1 strain when grown at 30°, but it has greatly decreased activity (28% residual activity) when grown at 37°.

Chromatographic Properties—The chromatographic properties of J protein from TA1791 (J16 protein) differ markedly from those of wild type J protein isolated from either LT-2 or dhuA1 as illustrated in Fig. 3. A comparison of the elution profiles on DEAE-cellulose shows that the J16 protein from TA1791 elutes at a higher salt molarity than the wild type J protein. The elution pattern of this column is highly reproducible for different shock fluid preparations and for repacked columns. The position of the wild type protein was identical in all experiments, while the J16 protein from TA1791 was eluted at a higher salt molarity in two independent preparations. The positions of acid phenyl phosphatase activity (which is partially resolvable into two peaks) and of histidine binding activity Peak III, as well as the conductivity measurements, are highly reproducible markers. The reason for such a large difference in mobility between two proteins with the same molecular weight and isolectric point is under investigation. The J16 protein presumably differs from the wild type protein in a sequence of several amino acids, because TA1791 was obtained by reversion of a frameshift mutant with ICR-191.

Hydroxyapatite chromatography of wild type and TA1791 J proteins using gradient elution from 1 to 100 mM sodium phosphate (pH 7.0) also revealed a difference in mobility. Wild type J protein was eluted at 40 mM phosphate, whereas J16 protein was eluted at 10 mM phosphate, using the same batch of adsorbant.

Both proteins are eluted similarly on Sephadex G-100 indicating a similar molecular weight.

Dissociation Constant for Histidine—Fig. 4 shows that the J16 protein from TA1791 has a higher dissociation constant for n-histidine because of a deletion in the histidine operon; it does not grow on D-histidine. TA271 (dhuA1 hisF645) grows well on D-histidine at both 17° and 40°. Strain TA1768 (dhuA1 hisJ5617 hisF645) which has a mutation in hisJ, is unable to grow on D-histidine at either temperature, as expected from its lack of J protein. The significance of Fig. 2 is in the growth pattern of the temperature-sensitive strain, TA1789 (dhuA1 hisJ5617 hisJ5690 hisF645); this strain is able to grow at 17° (although not as well as the grandparent TA271), but it is completely unable to grow on D-histidine at 40°.

## Table II

| Strain | Genotype | Growth temperature | Histidine binding activity units per g, dry wt. cells |Histidine binding activity units per mg protein |
|--------|----------|---------------------|-----------------------------------------------------|----------------------------------------|
| TA1014 | dhuA1    | 30°                 | 2275                                                | 89                                      |
|        |          | 37°                 | 2170                                                | 82                                      |
| TA1771 | dhuA1 hisJ5617 | 30°     | 18                                                  | 2.0                                    |
|        |          | 37°                 | 11                                                  | 1.4                                    |
| TA1791 | dhuA1 hisJ5617 | 30°     | 2331                                                | 91                                      |
|        |          | 37°                 | 645                                                  | 82                                      |

## Table III

Growth Temperature—Histidine binding activity levels and J protein levels were determined in the selected strains grown at two different temperatures. TA1771 has no J protein activity; it has been previously demonstrated (3, 9) that growth on n-histidine (as a source of L-histidine for a histidine auxotroph) requires a mutation in the dhuA site and an intact J protein. This is illustrated in Fig. 2. TA831 (hisF645) is the parent strain which requires histidine because of a deletion in the histidine operon; it does not grow on D-histidine. TA271 (dhuA1 hisF645) grows well on D-histidine at both 17° and 40°. Strain TA1768 (dhuA1 hisJ5617 hisF645) which has a mutation in hisJ, is unable to grow on D-histidine at either temperature, as expected from its lack of J protein. The significance of Fig. 2 is in the growth pattern of the temperature-sensitive strain, TA1789 (dhuA1 hisJ5617 hisJ5690 hisF645); this strain is able to grow at 17° (although not as well as the grandparent TA271), but it is completely unable to grow on D-histidine at 40°.

## Fig. 3

DEAE-cellulose chromatography of J protein from the wild type and from the hisJ revertant TA1791. Shock fluids from TA1791 (dhuA1 hisJ5617 hisJ5620) cells (late exponential phase) were chromatographed successively on the same DEAE-cellulose column under identical conditions (4) with a salt gradient from 0 to 0.2 M NaCl. Frac-

## Fig. 4

Histidine binding activity units per mg protein from TA1791 (dhuA1 hisJ5620) are chromatographed successively on the same DEAE-cellulose column under identical conditions (4) with a salt gradient from 0 to 0.2 M NaCl. Fractions (15 ml) are collected. The histidine binding activity (ordinate) is estimated by the filter assay (4). The small peaks labeled III represent the histidine binding Peak III.

## Temperature Sensitivity of D-Histidine Growth—It has been demonstrated previously (3, 9) that growth on D-histidine (as a source of L-histidine for a histidine auxotroph) requires a mutation at the dhuA site and an intact J protein. This is illustrated...
histidine ($K_D = 2 \mu M$) than wild type J protein ($K_D = 0.11 \mu M$). These values were determined with the filtration assay at room temperature. A difference in affinity for histidine is also observed using the dialysis assay, which is performed at 4°C under these conditions the J protein from TA1791 has a $K_D = 2.5 \mu M$ and the wild type J protein has a $K_D = 0.14 \mu M$.

Temperature Stability—The greater heat lability of the Jts protein (from TA1791) as compared with the wild type protein is shown in Fig. 5. Binding proteins are remarkably heat stable. The activity remaining after 10 min at 100°C is about 70% for the wild type binding protein and 20% for the mutant protein. Inactivation of a mixture of the two proteins is intermediate between that of revertant and wild type proteins alone.

The instability of the Jts protein is also indicated by a faster decay during storage at 4°C than the wild type protein, which is stable for several months. We have had difficulty obtaining pure Jts protein because of this instability. The J protein from either strain is stable to quick-freezing in ethanol-Dry Ice and storage at −10°C.

Disc Gel Electrophoresis—Disc gel electrophoresis differentiates wild type from mutant J protein. The pure wild type J protein gives a single band in the low pH system (4). A preparation of Jts protein that was 80% pure showed only one major band which moves faster than the wild type protein. A mixture of the two proteins gives two bands corresponding in position to each of the single bands.

Isoelectric pH—J proteins from dhuA1 and from TA1791 have the same isoelectric pH of 5.5.

Immunology—Shock fluid from TA1791, grown either at 30°C or at 37°C, cross-reacts with antiserum prepared against the wild type J protein. Shock fluid from several hisJ mutants lacking the binding protein contained no cross-reacting material. This demonstrates that the protein produced by TA1791 is not an altogether different protein.

**Correlation between Specificity of in Vivo Histidine Transport and Isolated J Protein**

The J protein has been definitively demonstrated to be a component of high affinity histidine transport by the use of mutants. Consistent with this the binding properties of the J protein in vitro correlate well with the properties of the transport system in vivo with respect to D- and L-histidine, arginine, lysine, citrulline, ornithine, HHPA, imidazole pyruvic acid, and azaserine.

**In Vivo Specificity**

Table III shows that L-arginine inhibits L-[^3]H]histidine uptake. Essentially complete inhibition is obtained at the highest arginine concentration used, which suggests that not only the J-P permease, but also the K-P permease is inhibited by arginine. Competitors of the transport system can also be identified by the inhibition of growth on L-histidine. D-Histidine is itself a much poorer substrate of the transport system than L-histidine (9). Arginine, citrulline, lysine, and ornithine completely inhibit growth of dhuA1 hisJ revertant on D-histidine (Fig. 6). Inhibition of growth by these compounds only occurs when D-histidine is the substrate because the J protein is an obligatory step in D-histidine uptake, while L-histidine is also transported by other, parallel systems (1–3).

Histidine-requiring strains are not able to transport sufficient imidazole pyruvic acid to serve as a source of L-histidine, unless...
even in the presence of an aromatic amino acid or of an aroY mutation causes the bacteria to acquire sensitivity to azaserine, mease (WOE') (1, 2). The inhibition of growth produced by 1~1s bee11 demonstrated to be :I subst,rate for the aromatic per-
ductioll of a hisJ mutation eliminates simultaneously the ability to grow on imidazole pyruvic acid and on u-histidine. 

**TABLE III**

L-Arginine competition of L-histidine uptake

Bacteria (wild type) were added to a mixture of L-3H]histidine (0.02 μM) and L-arginine (at the indicated concentrations). The arginine was shown to contain less than 0.005% contaminating histidine using an amino acid analyzer (Beckman/Spinco model 121).

| L-Arginine μM | Rate of uptake μmol/g, dry wt/min | Control % |
|-------------|---------------------------------|-----------|
| 0           | 0.36                            | 100       |
| 10          | 0.097                           | 27        |
| 100         | 0.013                           | 4         |
| 500         | 0.0053                          | 2         |

**TABLE IV**

Specificity of the J protein

Pure J protein from either wild type or dhuA1 was used. Binding was assayed by filtration (4). All values are averages of duplicate assays. The L-3H]histidine concentration was 1 μM when the additions were in 10- and 1,000-fold excess; 0.1 μM when the additions were in 10,000-fold excess. All compounds tested, except n-histidine and p-HIPA, contained less than 0.01% contaminating histidine, either after repurification or as available commercially. L-Histidine contamination in the unlabeled n-histidine was shown to be less than 0.1% by a radioisotope dilution assay of L-3H]histidine binding by one of the other histidine-binding proteins. p-HIPA could not be checked for small histidine contamination because of technical difficulties; it is possible that p-HIPA contains as much as 0.1% n- or l-histidine.

**FIG. 6.** Inhibition of growth on n-histidine by amino acids. TA271 (dhuA1 hisP645) was grown overnight and diluted as described in the legend to Fig. 2. L-Arginine, L-lysine, L-citrulline, or DL-ornithine were added at zero time where indicated. Growth was at 37°.

Addition | % Control activity | Molar ratio (addition/histidine) |
|---------|------------------|-------------------------------|
| L-Arginine | 74 | 0 | 0 | 0 |
| L-Lysine | 84 | 34 | 13 | 13 |
| d-Lysine | " | " | 21 | 21 |
| L-Citrulline | " | 100 | 55 | 55 |
| DL-Ornithine | " | 100 | 61 | 61 |
| p-HIPA | 91 | 67 | 20 | 20 |
| n-Histidine | " | 81 | 35 | 35 |
| Azaserine | " | " | 26 | 26 |
| Imidazole pyruvic acid | " | " | 83 | 83 |

*Not assayed.*

they also contain a dhuA mutation (9). As is the case for n-histidine, growth on imidazole pyruvic acid is also completely dependent upon transport through the J protein, because introduction of a hisJ mutation eliminates simultaneously the ability to grow on imidazole pyruvic acid and on n-histidine.

We have previously demonstrated (3) that the histidine analogue HIPA is transported by the J-P permease.

Azaserine is also transported through the elevated high affinity J-P permease of the dhuA1 mutant. This inhibitory analogue has been demonstrated to be a substrate for the aromatic permease (aroP) (1, 2). The inhibition of growth produced by azaserine on the wild type is completely reversed by any of the aromatic amino acids. However, introduction of the dhuA1 mutation causes the bacteria to acquire sensitivity to azaserine, even in the presence of an aromatic amino acid or of an aroP mutation. This has been interpreted to mean that azaserine is transported through the J-P histidine permease in sufficient amount to inhibit growth, when this system is elevated. In agreement with this interpretation, it was found that introduction of a hisP mutation in the dhuA1 strain causes complete resistance to this new azaserine sensitivity, but only when an aromatic amino acid is present. In the absence of an aromatic amino acid, transport of azaserine still occurs through the aromatic permease in a dhuA hisP double mutant and causes inhibition. These data allowed us to predict that the J protein would have an affinity for azaserine, despite its lack of obvious resemblance to histidine.

**Specificity of J Protein**

The affinity of the pure J protein for a wide variety of amino acids and histidine analogues was measured by competition of the unlabelled compound with L-3H]histidine binding (Table IV). The following compounds have a significant affinity for the J protein: L-histidine, arginine, lysine, p-HIPA, azaserine, p-histidine, citrulline, and ornithine. All these compounds are known to be substrates of the high affinity histidine permease. No other natural amino acid inhibited histidine binding up to 10,000-fold excess; cysteine consistently gave a slight stimulation of histidine binding; this might indicate a sulfhydryl activation of the J protein.

The relatively high affinity of the J protein for L-arginine is in agreement with the effect of L-arginine upon transport of L- and n-histidine (Table III and Fig. 6); a 1000-fold excess of L-arginine completely inhibits L-3H]histidine-binding. A Kₜ of 10 μM for arginine can be calculated from the competition data. L-3H]Arginine was shown to bind to the J protein (data not shown);
a 1000-fold excess of unlabeled L- or D-histidine completely inhibits the binding of L-[3H]arginine. D-Arginine, however, does not inhibit L-histidine binding at 1000-fold excess.

It should be emphasized that the basic amino acids would compete more effectively with N-histidine than with L-histidine, because of the relatively poor affinity of N-histidine for the J protein. This explains the strong inhibition of N-histidine growth (Fig. 6) by citrulline and ornithine, which do not compete with L-histidine binding at 1000-fold excess.

The Kp of the D-histidine-J protein complex, measured by competition of L-[3H]histidine binding, is 500 μM. The maximal number of L-histidine-binding sites on the J protein is the same in the presence and absence of D-histidine.

**DISCUSSION**

A necessary prerequisite for a biochemical approach to a study of active transport is the isolation of a presumed transport component and its unequivocal identification as an obligatory component of the transport system under study. Numerous laboratories have recently isolated a variety of such presumed components (reviewed in Reference 13). Among these, a class of proteins called the "binding proteins," which bind a variety of small molecules and are thought to be located on the surface of active transport of these molecules. The lack of direct evidence concerning the role of binding proteins in transport has been discussed (13).

We feel that the J protein has been shown conclusively to be a component of histidine transport. We previously demonstrated (3) a direct correlation between the activity of the histidine permease and the levels of the histidine-binding protein J, by showing that (a) mutation in the attJ site causes the simultaneous elevation of the J protein and of the rate of L-histidine transport; (b) mutation in the hisJ gene causes loss of the J protein and a decrease in the rate of L-histidine transport. The identity of the J protein as the component of histidine transport is now firmly established by the finding that both are coded for by a single structural gene. The properties of a temperature-sensitive strain, TA1791, provide this evidence.

TA1791 was obtained as a revertant of a strain containing a hisJ mutation (and therefore defective in histidine transport). However, TA1791 is not a true revertant because it has not recovered the properties of a strain with an intact hisJ gene; the reversion is due to a second site mutation in the hisJ gene itself, as shown by the finding of an altered J protein and by the genetic analysis. The J protein isolated from TA1791 differs from the wild type J protein in temperature stability, affinity for histidine, chromatographic properties, and disc gel electrophoretic behavior. The production of altered J protein has been correlated with the in vivo temperature sensitivity of TA1791; both the L-histidine transport and the ability to grow on D-histidine are temperature sensitive. Both these activities require functional J protein.

The possibility that the mutant protein is a new, completely different protein is excluded. The two proteins have several properties in common, although we chose to stress the differences. The temperature-sensitive mutation maps in the same place as mutations causing loss of the J protein; both proteins bind histidine and function in the transport of L-histidine and D-histidine; they have exactly the same molecular weight and isoelectric pH; they run in the same position in some gel electrophoresis systems; they are both dependent on hisP gene product for function; the mutant protein cross-reacts with antiserum to the wild type J protein.

Many other revertant strains have been similarly obtained from a variety of hisJ-containing strains. Preliminary experiments indicate that many of these also produce J proteins with altered properties.

It should be pointed out that the temperature sensitivity of the J protein in TA1791, as assayed in vivo by histidine binding, may not necessarily account for the in vivo temperature sensitivity of growth on N-histidine. If there is an intersection between the J protein and the P protein (or any other transport protein), the temperature-sensitive mutation could have affected such in vivo interaction, rather than the binding of the substrate to the J protein.

The evidence obtained from the genetic analysis that the hisJ gene is the structural gene for the J protein agrees with the excellent correlation between the properties of the wild type transport in vivo and of the wild type J protein in vivo. We presented here information concerning the correlation in specificity for substrates and analogues.

The highest affinity of both in vivo transport and of the J protein is for L-histidine. None of the other amino acids, added at 10,000-fold excess, competed with histidine binding to the J protein except for those shown in Table IV. In fact, only those compounds which have been shown to have a physiological effect on transport inhibit histidine binding, thus demonstrating the correlation between the specificity of the J protein and of transport.

The J protein has a relatively high affinity for arginine and lysine. This is consistent with the known effects of these compounds on transport in vivo; both arginine and lysine are good competitors of L-histidine uptake and D-histidine growth. Even though the affinity of the J protein for arginine is very good, it should be emphasized that it still is considerably lower than its affinity for histidine (Kp for arginine = 10 μM; Kp for histidine = 0.1 μM). The affinity of the J protein for D-histidine, citrulline, and ornithine, and for the analogues, p-HIPA and azaserine, also correlates well with the affinity that these compounds have for the J-P permease in vivo. As a consequence of this affinity, p-histidine, p-HIPA and azaserine can enter the cell. Therefore, p-histidine can act as growth substrate, while p-HIPA and azaserine act as inhibitors. The affinity of citrulline and ornithine for the J-P permease is shown by their inhibition of growth on D-histidine.

Recently (15) a periplasmic protein which binds histidine with much poorer affinity than the J protein has been isolated and purified from S. typhimurium. This protein differs in several biochemical properties from the J protein (discussed in Reference 4), and in all likelihood it is a different protein altogether. Moreover, no genetic characterization of this protein has been presented, thus rendering it difficult to draw any conclusion concerning the relationship between this protein and either the J protein or histidine transport.

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