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A Proton Nuclear Magnetic Resonance Investigation of Histidine-Binding Protein J of Salmonella typhimurium: A Model for Transport of L-Histidine Across Cytoplasmic Membrane

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Genetic evidence suggests that the high-affinity L-histidine transport in Salmonella typhimurium requires the participation of a periplasmic binding protein (histidine-binding protein J) and two other proteins (P and Q proteins). The histidine-binding protein J binds L-histidine as the first step in the high-affinity active transport of this amino acid across the cytoplasmic membrane. High-resolution proton nuclear magnetic resonance spectroscopy at 600 MHz is used to investigate the conformations of this protein in the absence and presence of substrate. Previous nuclear magnetic resonance results reported by this laboratory have shown that there are extensive spectral changes in this protein upon the addition of L-histidine. When resonances from individual amino acid residues of a protein can be resolved in the proton nuclear magnetic resonance spectrum, a great deal of detailed information about substrate-induced structural changes can be obtained. In order to gain a deeper insight into the nature of these structural changes, deuterated phenylalanine or tyrosine has been incorporated into the bacteria. Proton nuclear magnetic resonance spectra of selectively deuterated histidine-binding protein J were obtained and compared to the normal protein. Several of the proton resonances have been assigned to the various aromatic amino acid residues of this protein. A model for the high-affinity transport of L-histidine across the cytoplasmic membrane of S typhimurium is proposed. This model, which is a version of the pore model, assumes that both P and Q proteins are membrane-bound and that the interface between these two proteins forms the channel for the passage of substrate. The histidine-binding protein J serves as the "key" for the opening of the channel for the passage of L-histidine. In the absence of substrate, this channel or gate is closed owing to a lack of appropriate interactions among these three proteins. The channel can be opened upon receiving a specific signal from the "key"; namely, the substrate-induced conformational changes in the histidine-binding protein J molecule. This model is consistent with available experimental evidence for the high-affinity transport of L-histidine across the cytoplasmic membrane of S typhimurium.

Key words: ^{1}H NMR, periplasmic binding protein, histidine-binding protein J, membrane transport, pore model, transport of L-histidine, Salmonella typhimurium, substrate-induced conformational changes, deuterated amino acids, partially deuterated protein

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On the basis of genetic studies, Ames and co-workers [1–5] have proposed that the high-affinity L-histidine transport in Salmonella typhimurium LT2 requires the participation of three proteins; namely, histidine-binding protein J (J protein, a periplasmic histidine binding protein coded by the *His* J gene), P protein (a membrane-bound protein coded by the *His* P gene), and Q protein (a protein coded by the *His* Q gene; the nature and location of the Q protein are not known). They [2, 3, 5] have further proposed that the J protein has two functional sites: one site responsible for the binding of the substrate (L-histidine) and the other site responsible for the interaction with the membrane-bound components (presumably P and Q proteins). It is believed that the J protein acts as the first step (the recognition step) in the high-affinity L-histidine transport. On binding L-histidine in the periplasmic space, J protein must undergo specific substrate-induced conformational changes that allow it to deliver the substrate to the cytoplasmic membrane for the translocation process. However, the molecular basis for the transport of L-histidine in S typhimurium is not understood.

Preliminary ^{1}H and ^{19}F nuclear magnetic resonance (NMR) studies reported by this laboratory [6] have provided strong evidence for a substrate-induced conformational change of the J protein molecule upon binding L-histidine. By comparing ^{1}H NMR spectra of three J proteins obtained from TA1859 (a strain producing wild-type J protein), TA301 (a mutant strain containing J protein with altered L-histidine binding), and TA300 (a mutant strain containing J protein with altered J-P protein interaction), we have obtained preliminary evidence to suggest that several proton resonances are affected by mutations that alter L-histidine binding and J–P protein interaction [7].

The present study is an extension of our earlier ^{1}H NMR studies. It was undertaken to characterize the conformation of J protein in solution and better understand the substrate-induced conformational changes in J protein. The value of the ^{1}H NMR results will be greatly enhanced if we can resolve various overlapping resonances and assign individual resonances to specific amino acid residues. In general, resolution can be improved by obtaining ^{1}H NMR spectra with a higher frequency NMR spectrometer (ie, resolution is directly proportional to the resonance frequency) and using partially deuterated protein to simplify the ^{1}H NMR spectrum. The assignment of proton resonances to specific types of amino acids can be obtained by comparing the spectra of normal and selectively deuterated proteins. A general discussion of this approach is given elsewhere [8].

MATERIALS AND METHODS

Bacterial Strains

S typhimurium TA1859(*dhu*A1) was supplied by Dr. G. F.-L. Ames. NK337 (*leu*-am515 *su*19 [P22 Tc10::Tn10 *c2ts*29 12^{-} *am*N11 *13*$^{-}$*am*H101 *int*3 *sie*A44 *mntts ant*$^{-}$]), DB7004 (*leu*am515 *su*19) and phage DB1416 (P22 *c2am*08 *13*$^{-}$ *am*H101 *8*$^{-}$*am*H202 *5*$^{-}$*am* N114 *mnt*-1) were the gifts of Dr. D. Botstein. Tc10 phage are P22 (Tn10 *c2ts*29 *12*$^{-}$*am*N11 *13*$^{-}$*am*H101 *int*3). Phage lysates were prepared from NK337 by induction following temperature shift from 27° to 39°C, and the titer was optimized by addition of tail preparation made from infection of DB7004 with phage DB1416 (D. Botstein, personal communication). TA1859 (*phe*$^{-}$) and TA1589 (*phe*$^{-}$ *tyr*$^{-}$) were derived by integration of the translocatable genetic element Tn10 (which confers tetracycline resistance) into the chromosome of TA1859 via infection with phage P22Tc10 [9, 10]. Cultures were grown to log phase in L broth [11], concentrated to a density of 5×10^{9} cells/ml, and in-

fected with P22Tc10. One-tenth of one milliliter of the culture was spread on green indicator plates [12] containing 25 μg tetracycline per ml. Tetracycline resistant colonies arising at 37°C were replicated to minimal glucose and green plates, both containing 25 μg tetracycline per ml. Auxotrophs were selected and their nutritional requirements confirmed by usual procedures.

Growth of Bacteria

Bacteria were grown in 5-gal carboys containing 15 liters of medium aerated through two fritted filter tubes. The minimal medium used was based on the medium A of Willis et al [13] and contained per liter: 18 g Na_2HPO_4, 9 g KH_2PO_4, 5 g NH_4Cl, 1.1 g Na_2SO_4, 0.1 g $MgSO_4$, and 30 g glycerol. This medium was supplemented with 20 μg/ml of L-phenylalanine or L-phenylalanine and L-tyrosine when growing strains [TA1859(*phe*$^-$) or TA1859(*phe*$^-$*tyr*$^-$), respectively] requiring one or two specific amino acids, or with 40 μg/ml of deuterated D,L-phenylalanine or D,L-tyrosine when preparing deuterated J protein. Carboys were inoculated with 1 liter of culture grown overnight in the same medium, and growth was followed to late log phase. The cells were harvested by centrifugation at 13,000g for 10 min, washed once with 10 mM potassium phosphate buffer, pH 7.6, and stored frozen at −80°C until needed.

Preparation of J Proteins

J protein was purified using modifications of the method of Noel et al [14]. All steps were carried out at 0–4°C. Three hundred to three hundred fifty grams of cell paste were thawed and resuspended in 213 ml of 10 mM potassium phosphate buffer, pH 7.6, per 100 g of cell paste. Cells were sonicated in 2 batches with a Sonifier Cell Disrupter (Heat Systems Co.) at the highest setting with the standard probe. Sonication was carried out for 10-min intervals until the absorbance of an aliquot diluted 1:20 with 1% Triton X-100 read 0.8–1.0 optical density units at 550 nm in a Zeiss PMQ II spectrophotometer. The sonicate was then centrifuged for 90 min at 13,000g. The supernatant was brought to 50% saturation with ammonium sulfate (Ultra Pure, Schwarz/Mann), stirred for 30 min, and centrifuged for 90 min at 13,000g. The supernatant was brought to 85% saturation with ammonium sulfate, stirred for 30 min, and centrifuged for 40 min at 13,000g. The pellets were resuspended in 5 mM Tris-HCl, pH 8.3, to a final volume of 25 ml and dialyzed overnight against 4 liters of 5 mM Tris-HCl, pH 8.3.

The dialyzed ammonium sulfate fraction was applied to a 2.0 × 84 cm DE52 (Whatman) anion exchange column equilibrated with 5 mM Tris-HCl, pH 8.3. After the column was washed with 300 ml of the same buffer, the binding activity was eluted with a gradient prepared with 2 liters of 5 mM Tris-HCl, pH 8.3, in the mixing chamber and 2 liters of 5 mM Tris-HCl, pH 8.3, plus 5.84 g NaCl in the reservoir. Fractions containing the highest binding activity were pooled and lyophilized.

The lyophilized material was dissolved in 2 ml of 10 mM sodium phosphate buffer, pH 7.0, and applied to a 2.4 × 48 cm column of Sephadex G-75 (Pharmacia) equilibrated with the same buffer. This buffer was used to elute the activity, and the peak fractions were pooled and lyophilized. The lyophilized material was resuspended in 1–2 ml of H_2O and applied to a 1.5 × 120 cm column of P-2 Bio-Gel (Bio-Rad) in H_2O. The activity was eluted with H_2O, and the peak tubes were pooled, lyophilized, and stored at −20°C. The yield of J protein was about 50 mg from 300 g of wet cell paste. The same procedures were used to purify J protein containing deuterated phenylalanine or tyrosine.

Preparation of Deuterated Tyrosine and Phenylalanine

A modification of the procedures of Griffiths et al [15] and of Matthews et al [16] was used to prepare D,L-tyrosine-d_5 and D,L-phenylalanine-d_6. L-tyrosine and L-phenylalanine were purchased from Sigma; D_2SO_4 (99% D content), from Stohler Isotope; and D_2O (99.8% content), from Bio-Rad.

Preparation of D,L-tyrosine-d_5. L-tyrosine (20 g) was treated with D_2O at room temperature overnight and then lyophilized. To a 250 ml round-bottom flask fitted with a 10 cm length of heavy-walled tubing was added the tyrosine, followed by D_2O (100 g) and D_2SO_4 (50 g, 27 ml). The flask was sealed, then heated to 185°C in a bomb (Parr shaker) with shaking for 48 h. The cooled solution was poured into D_2O (50 ml), and the aqueous solution was neutralized with concentrated NH_4OH (29%) to pH 4.5. The solution was cooled to 4°C overnight, and the dark brown solid was collected and purified by dissolving in hot 1 N HCl, extracting with charcoal, and precipitating out with NH_4OH. The dried D,L-tyrosine (11.5 g, 57% yield) showed a 95% recovery on amino acid analysis. The 250 MHz ^1H NMR spectrum of a solution of deuterated tyrosine in 1 N DCl (9.6 mg/5 ml) showed only very low intensity peaks in the aromatic region; no peak assignable to the α-proton was present.

Preparation of D,L-phenylalanine-d_6. In a similar fashion, D,L-phenylalanine-d_6 (10.3 g, 51% yield) was prepared from L-phenylalanine (20 g) and 50% D_2SO_4 in D_2O at 185°C. The recovery of D,L-phenylalanine on amino acid analysis was 95.5%. The 250 MHz ^1H NMR spectrum of a solution of deuterated phenylalanine in 1 N DCl (7.6 mg/ml) showed less than 5% of the intensity of the undeuterated form in the aromatic region; no peak assignable to the α-proton was present.

Determination of the Dissociation Constant of J Protein for L-Histidine

The dissociation constant (K_D) of J protein for L-histidine was determined using the dialysis assay described by Lever [17].

^1H NMR Measurements

Lyophilized protein was dissolved in 10 mM sodium phosphate buffer in D_2O at pH* 6.5 to give a final concentration of 0.3–0.4 mM. Concentrations of J protein were determined using an extinction coefficient of 0.71 for a 0.1% protein solution in a 1-cm curvette at 280 nm [17]. The protein solution was then transferred to a standard 5 mm NMR sample tube, and the pH was measured directly inside the tube with a 4.5 mm Ingold electrode (model 6030-01) on a Radiometer model 26 pH meter. The pH* value is given as the direct pH meter reading uncorrected for the deuterium isotope effect. When a titration of J protein with L-histidine was required, aliquots of a stock solution of 3.9 mM L-histidine (in the same buffer and at the same pH) were added to the protein solution in the NMR sample tube.

^1H NMR spectra were obtained on an ultra-high frequency spectrometer located at Carnegie-Mellon University. This instrument operates at 600.2 MHz for ^1H resonances [18]. An internal lock was provided by the residual HDO in each sample. The signal-to-noise ratio was improved by NMR correlation spectroscopy [19]. The sweep width was 4,865 Hz, with a sweep time of 1 s/scan. Usually, 500–1,000 scans were taken for each region. The proton chemical shifts are referred to the residual water proton signal (HDO) in each sample, which resonates at 4.83 parts per million (ppm) downfield from the proton reso-

nance of 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) at 27°C. A positive sign indicates that a resonance is downfield from HDO.* The accuracy of the chemical shift measurements is better than ±0.01 ppm.

RESULTS

Improvement of the Resolution of ^{1}H NMR Spectra

Our previous ^{1}H NMR spectra were obtained at 250 MHz [6, 7]. With the availability of a 600 MHz NMR spectrometer in Pittsburgh, we have obtained the ^{1}H NMR spectra of J protein using this ultra-high frequency instrument. Figures 1 and 2 give a comparison of the aromatic and ring-current shifted ^{1}H resonance regions of J protein at 250 and 600 MHz. It is obvious that the spectral resolution is greatly improved at 600 MHz as compared to that at 250 MHz. At 600 MHz, we can now begin to monitor a number of individual resonances as a function of the experimental conditions.

Assignments of Aromatic Proton Resonances

By incorporating deuterated phenylalanine or tyrosine into S typhimurium TA1859-(*phe*$^-$*tyr*$^-$) and TA1859(*phe*$^-$), we can obtain partially deuterated J proteins. By comparing the 600 MHz ^{1}H NMR spectrum of normal J protein with the spectra of selectively deuterated J proteins, we can assign those proton resonances due to the phenylalanines and tyrosines in normal J protein, as shown in Figure 3. Similar studies have also been used to make spectral assignments of J protein in the presence of excess L-histidine (results not shown).

According to the amino acid analysis of J protein [7], there are 1 histidine, 1–2 tryptophans, 8 tyrosines, and 12 phenylalanines per molecule. (The amino acid sequence of this protein recently reported by Hogg and Isihara [20] indicates that there is only one tryptophan residue per J protein.) Thus, there are a large number of aromatic proton resonances, together with numerous resonances due to protons on the α-carbon atoms, unexchangeable NH, and backbone *H*-bonded protons, over the spectral region from 1 to 6 ppm downfield from HDO. The spectral assignments as given in Figures 3 and 4 are based on a comparison of normal and partially deuterated J proteins. We believe that the spectral assignments of the major peaks are reasonable. However, there are a few resonances, especially shoulder peaks and the resonances due to the histidyl residues (from the protein and the substrate), which are less definitive at this stage. Another factor that contributes to our difficulty in making a complete spectral assignment is the incomplete replacement of all tyrosines or phenylalanines in partially deuterated J proteins. We estimate that the substitution of normal tyrosine or phenylalanine by its deutered analog is about 75–80%. A detailed analysis of our 600 MHz ^{1}H NMR spectra of J protein will be published elsewhere.

*In conforming with the recommendation for the presentation of NMR data for publication in chemical journals proposed by the International Union of Pure and Applied Chemistry (No. 38, August 1974), we have adopted the IUPAC convention; namely, the chemical shift scale is defined as positive in the low-field (or high-frequency) direction. This convention is different from that used by this laboratory [6, 7]. Previously, we had used the negative sign to indicate that the chemical shift of a given resonance is downfield from the resonance of a standard, such as the proton resonance of the residual water (HDO) signal [6, 7]. Hence, this change in the sign of the chemical shift scale should be noted when referring to earlier publications reported by this laboratory [6, 7].

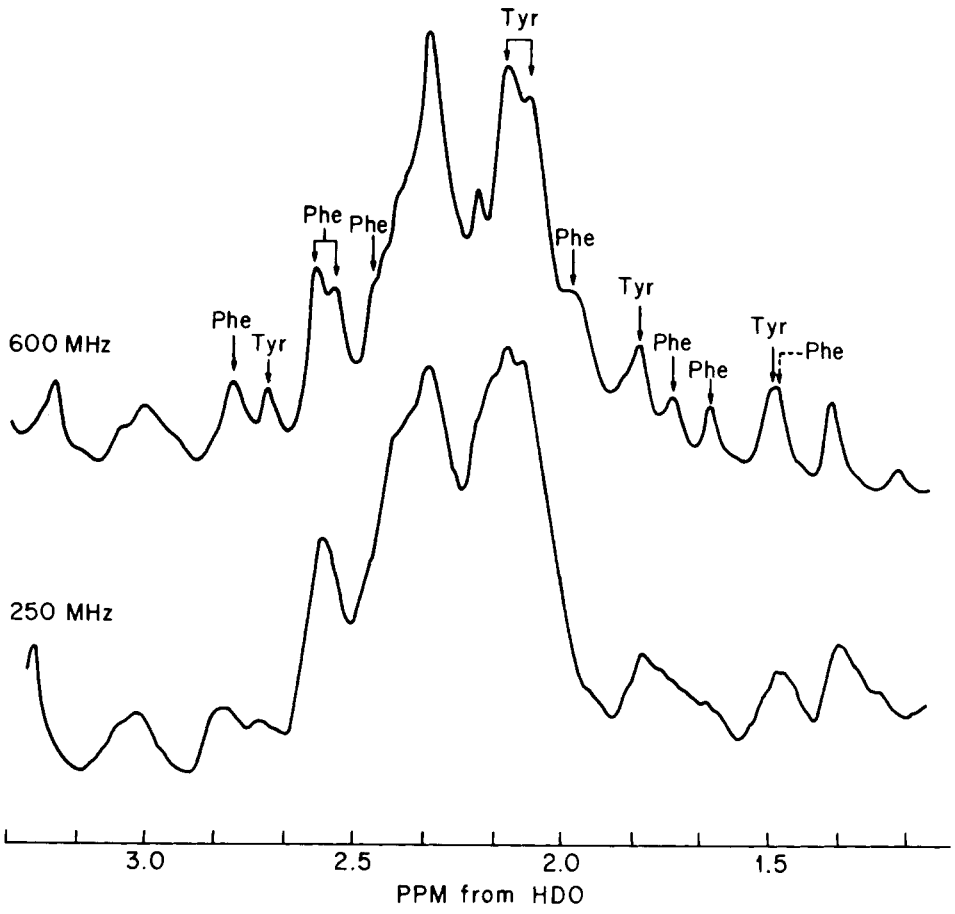

Fig. 1. A comparison of the aromatic proton resonance region for the 250 MHz and the 600 MHz ^{1}H NMR spectra of J protein from S typhimurium TA1859 in 0.01 M Phosphate buffer at pH* 6.5 and 27°C.

We have determined the K_D values of the two partially deuterated J proteins for the binding of L-histidine. The K_D values in 10 mM phosphate at pH 7.0 and 10°C are as follows: 0.23 μM for J protein; 0.38 μM for J protein containing deuterated phenylalanines; and 0.18 μM for J protein containing deuterated tyrosines. There is no significant difference among the binding constants of these three J proteins. This suggests that the structural and functional properties of J proteins containing deuterated phenylalanine and tyrosine are not altered and allows us to use deuterated analogs to assign the proton resonances of these amino acids in J protein.

Substrate-Induced Conformational Changes of J Protein

Figure 4 shows the 600 MHZ ^{1}H NMR spectra of J protein in the absence and presence of L-histidine over the region from 1 to 6 ppm downfield from HDO. It is obvious that there are extensive spectral changes in the J protein molecule upon the addition of substrate. This suggests that a number of amino acid residues undergo substrate-induced conformational changes.

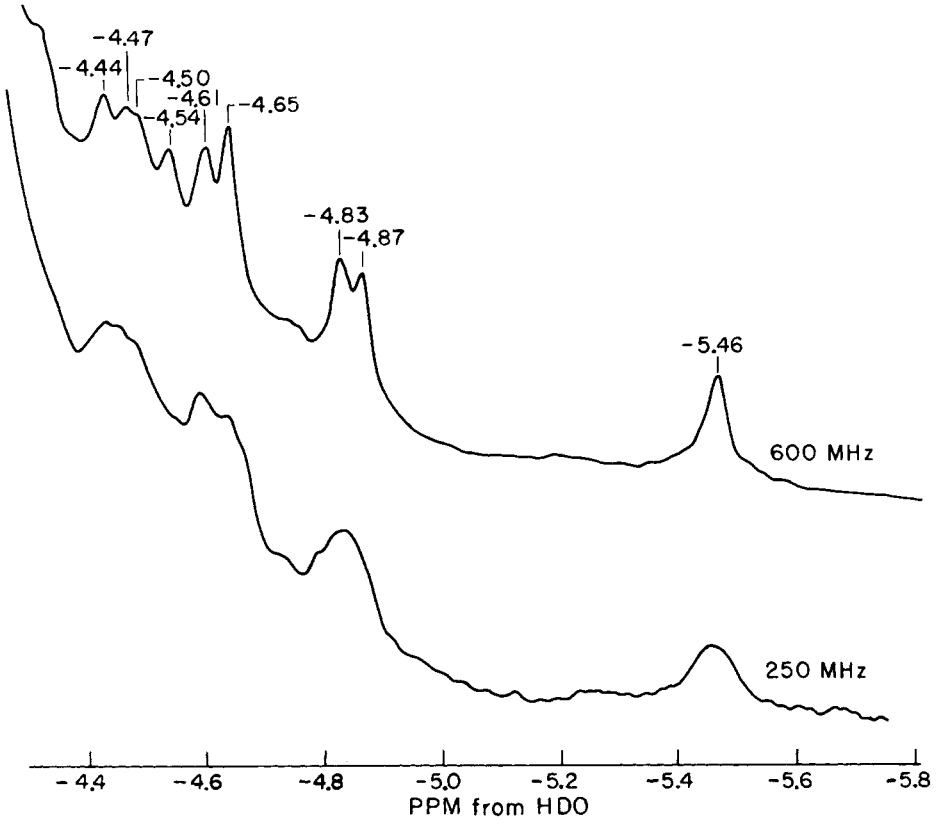

Fig. 2. A comparison of the ring-current shifted proton resonance region for the 250 MHz and the 600 MHz ^{1}H NMR spectra of J protein from S typhimurium TA1859 in 0.01 M phosphate buffer at pH* 6.5 and 27°C.

In order to gain a deeper insight into the nature of substrate-induced conformational changes of J protein, we have carried out a titration of J protein and two partially deuterated J proteins as a function of L-histidine concentration. Figures 5 and 6 give representative spectra of our titration results. A detailed description of our ^{1}H NMR titration studies of J proteins will be presented elsewhere. A major difference between the present study and that reported by Manuck and Ho [7] is the different NMR time scale in this study at 600 MHz compared to that in the earlier study at 250 MHz. At lower frequency, we could observe both fast and slow exchange rates for certain resonances of J protein upon binding of L-histidine. We had estimated both lower and upper limits for the off-rate constant for the binding of L-histidine to J protein and the range of the off-rate constants (k_{-1}) was found to be $60 < k_{-1} < 280\ s^{-1}$ [7]. At 600 MHz, we can only observe slow exchange between the bound and free species; therefore, we can only estimate the upper limit for the k_{-1} value. The experimental values for k_{-1} obtained at 600 MHz are consistent with the range of k_{-1} values obtained at 250 MHz. Using the higher resolving power at 600 MHz, we can now follow the variation of several resonances known to be affected by the mutations at the substrate binding and protein-protein interaction sites in greater detail.

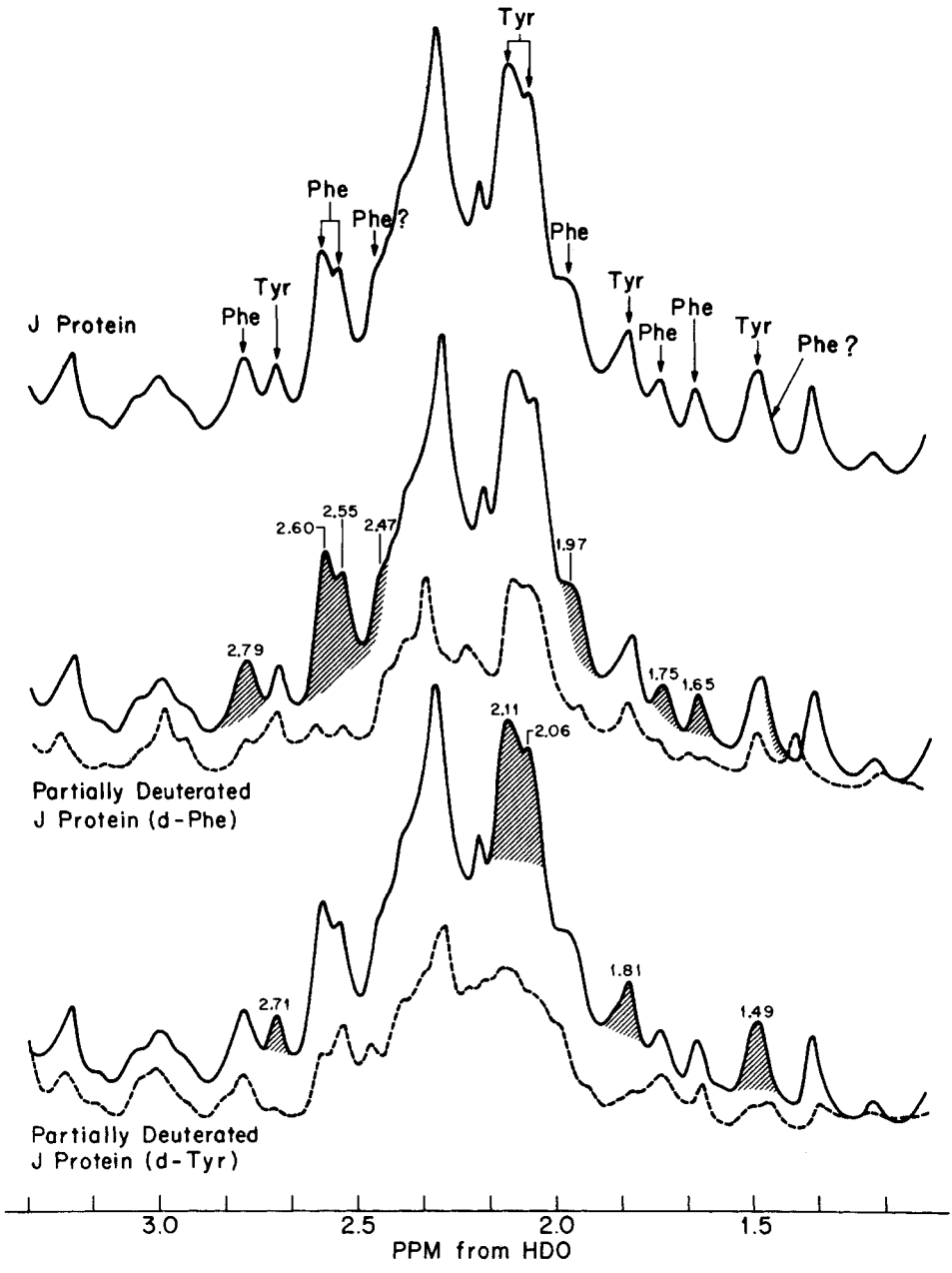

Fig. 3. The assignments of aromatic amino acid residues of J protein from S typhimurium TA1859 (*phe*$^-$) and TA1859 (*phe*$^-$ *tyr*$^-$) based on the 600 MHz ^{1}H NMR spectra of partially deuterated J proteins in 0.01 M phosphate buffer at pH* 6.5 and 27°C. [J protein] = 3.3×10^{-4} M.

Conformation of J Protein in Solution

Figure 7 gives the 600 MHz aromatic proton resonance spectra of J protein in the absence and presence of 7.5 M urea. Most globular proteins investigated would be unfolded into the random coil conformation in the presence of 7–8 M urea [21]. The ^{1}H

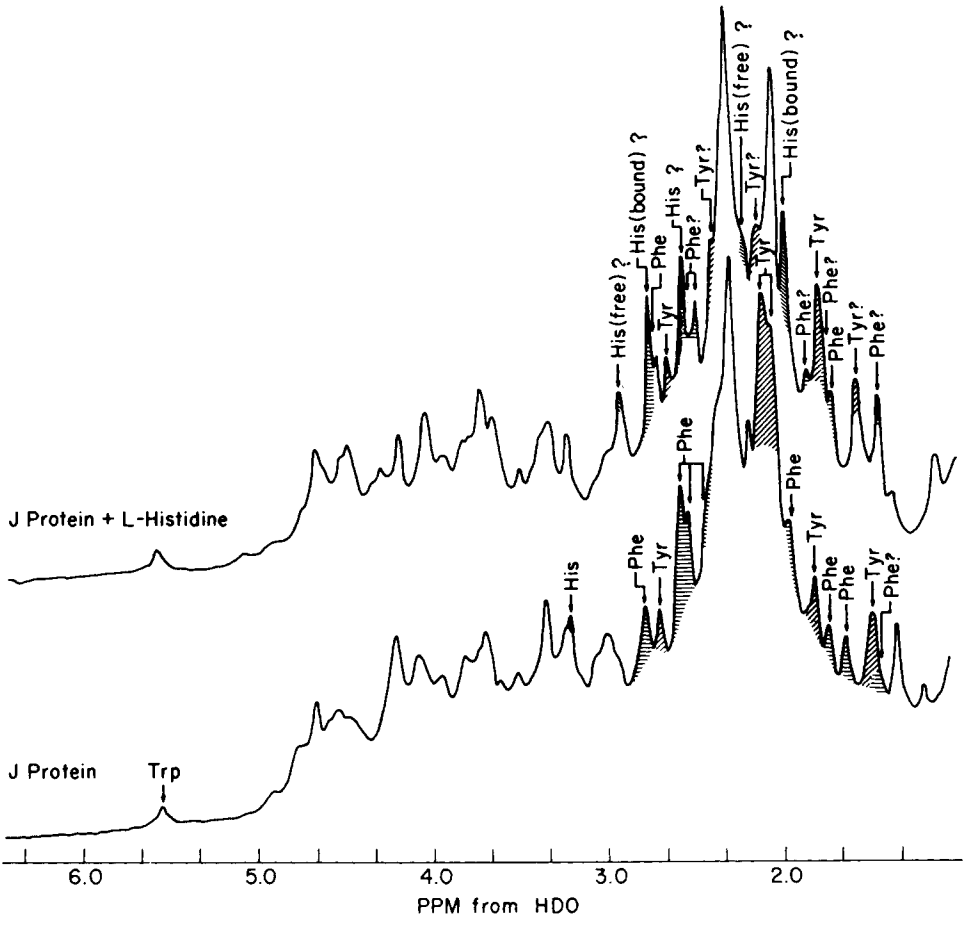

Fig. 4. The effects of L-histidine on the aromatic proton resonance region from 1.0 to 6.5 ppm downfield from HDO in the 600 MHz ^{1}H NMR spectra of J protein from S typhimurium TA1859 (*phe*$^-$) in 0.1 M phosphate buffer at pH* 6.5 and 27°C. The ? means that the spectral assignment is tentative.

NMR spectrum of a fully denatured or unfolded protein is very sharp and should be the arithmetical sum of individual amino acid residues of that protein [22]. The spectrum of J protein in 7.5 M urea is distinctly different from that of its constituent amino acids, as shown in Figure 7. This suggests that J protein retains a significant amount of its secondary and tertiary structure in the presence of 7.5 M urea.

DISCUSSION

The experimental results shown in Figure 4 clearly indicate that there are extensive changes in the spectrum of the J protein molecule upon binding L-histidine. Data obtained at 600 MHz confirm a major conclusion reached in our earlier studies [6, 7] – that the substrate-induced conformational changes are not limited to the substrate binding site but propagate to other regions of the molecule. In addition, the higher resolving power at 600 MHz has enabled us to extend and clarify some of the findings reported at 250

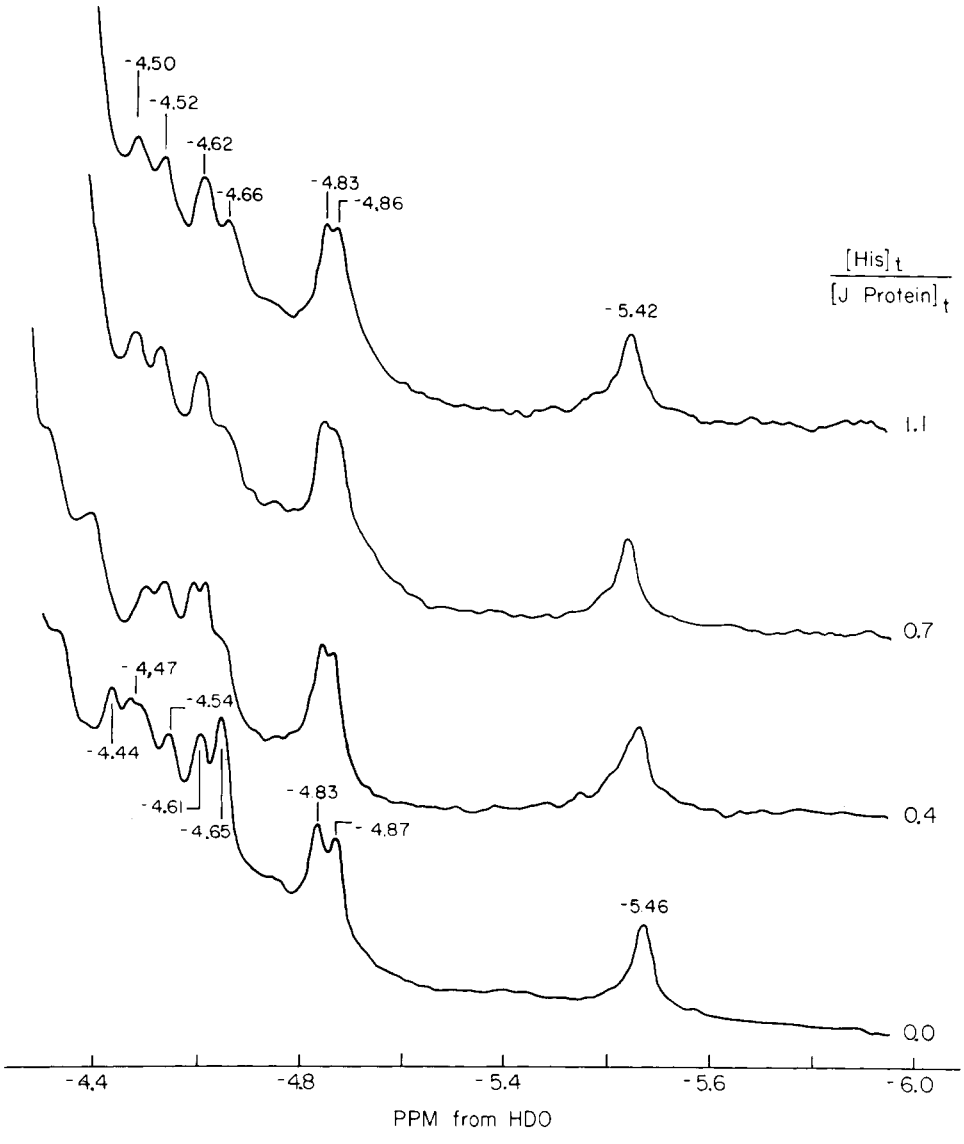

Fig. 5. The effects of L-histidine on the ring-current shifted proton resonances in the 600 MHz ^{1}H NMR spectra of J protein from S typhimurium TA1859 (*phe*$^-$) in 0.01 M phosphate buffer at pH* 6.5 and 27°C.

MHz [7]. By comparing the 250 MHz ^{1}H NMR spectra of normal J protein from TA1859 with J protein from TA300 (containing a mutation affecting J–P interaction) and J protein from TA301 (mutation affected substrate-binding site), we previously identified several proton resonances arising from or near substrate binding sites (such as –5.47, 1.37, and 3.30 ppm from HDO) and from protein-protein interacting sites (such as –4.61, 1.51, and 3.75 ppm from HDO). One of these resonances, the peak at –4.61 ppm was observed not to be affected by the binding of L-histidine at 250 MHz, while the other resonances mentioned are affected by the addition of substrate [7]. At 600 MHz, the resonance at –4.61 ppm is

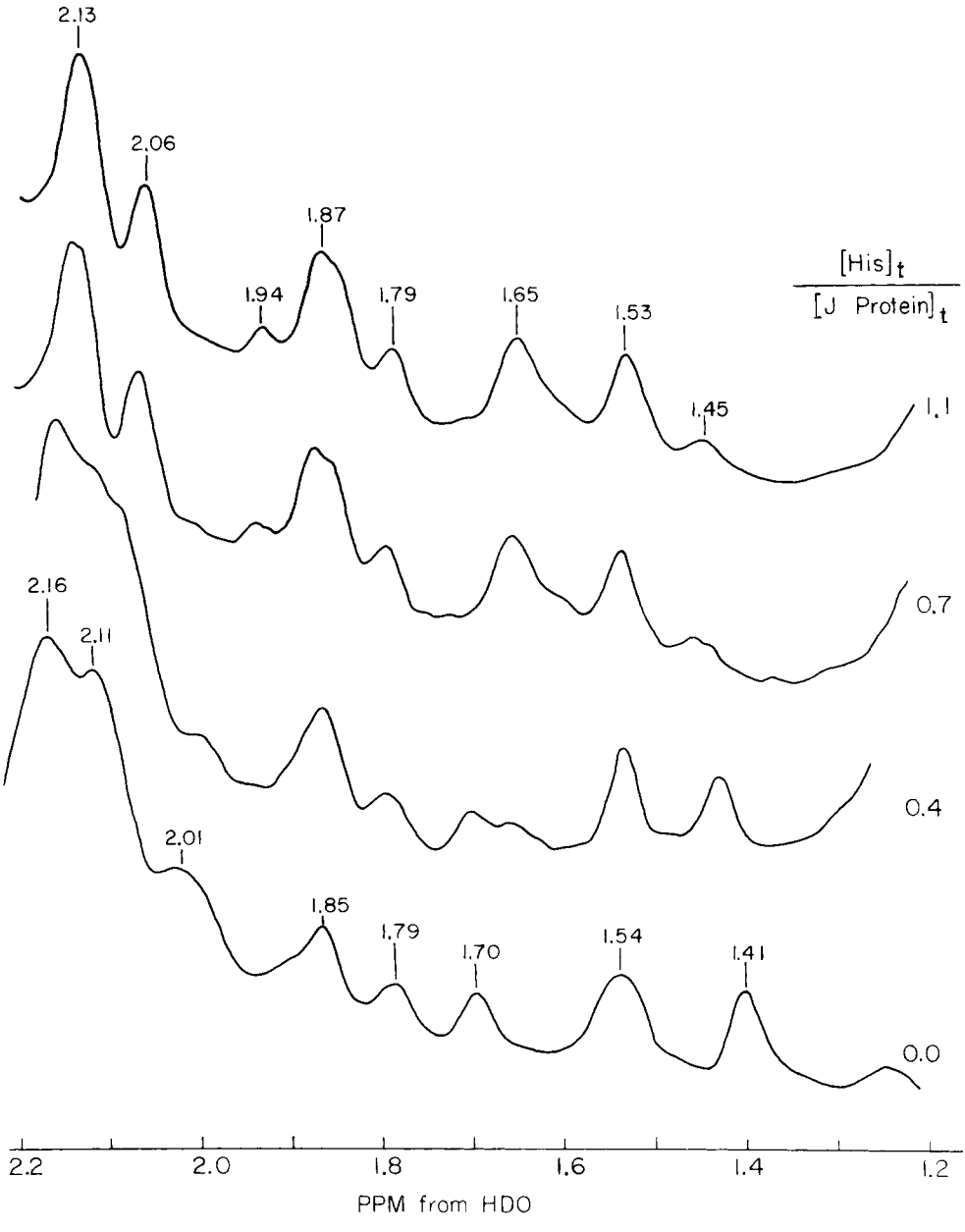

Fig. 6. The effects of L-histidine on the aromatic proton resonance region from 1.2 to 2.2 ppm downfield from HDO in the 600 MHz ^{1}H NMR spectra of J protein from S typhimurium TA1859 (*phe*$^-$) in 0.01 M phosphate buffer at pH* 6.5 and 27°C.

clearly altered upon binding of L-histidine (Fig. 5). The inability to observe a change in the resonance at −4.61 ppm at 250 MHz is due to the overlap of this resonance with another one at ∿−4.70 ppm. At higher frequency, these two resonances can be resolved. Hence, it is much easier to monitor their changes as a function of experimental conditions. The

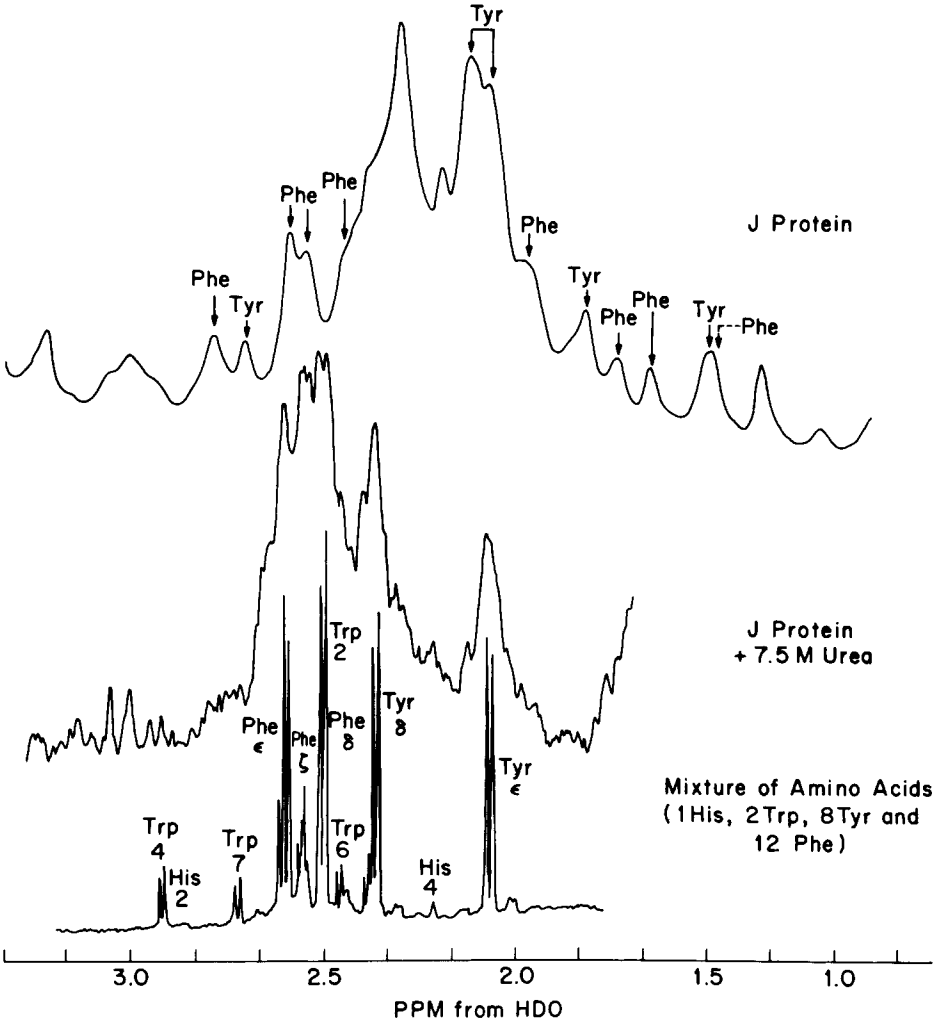

Fig. 7. A comparison of the aromatic proton resonance region for the 600 MHz ^{1}H NMR spectra of J protein from S typhimurium TA1859 in the absence and presence of 7.5 M deuterate urea, and a mixture of appropriate amino acids in 0.01 M phosphate buffer at pH* 6.5 and 27°C. The number or Greek letter under each amino acid in the bottom spectrum refers to the position of a proton in a given amino acid.

present results suggest that substrate-induced conformational changes can affect not only the conformations of those amino acid residues at the substrate-binding site but also those residues at the protein-protein interaction site.

A possible working model for the translocation of L-histidine across the cytoplasmic membrane, involving J, P, and Q proteins, is given in Figure 8. This model is a version of the pore model for transport suggested by a number of investigators [eg, 3, 23, 24]. In particular, it is an extension of the suggestion made by Ames and Spudich [3] based on genetic evidence that J protein must interact with a membrane-bound component, the P protein, for the high-affinity transport of L-histidine across the cytoplasmic membrane of S

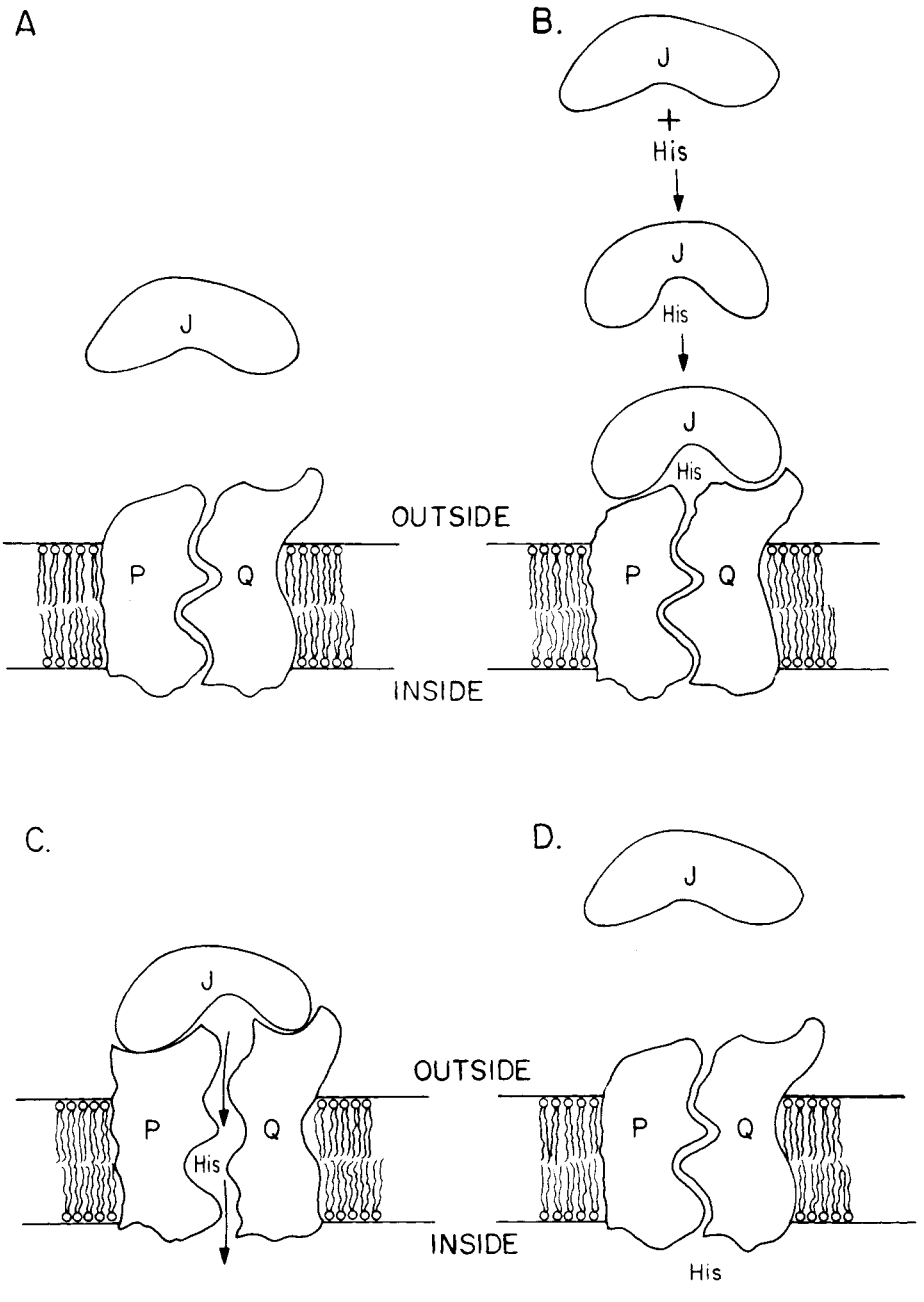

Fig. 8. A schematic model for the high-affinity transport of L-histidine across the cytoplasmic membrane of S typhimurium. A: the conformations of J, P, and Q proteins in the absence of L-histidine; B: the substrate-induced conformational changes in J protein and the interaction among J protein-*His* complex, P and Q proteins. C: further conformational rearrangements among J, P, and Q proteins to permit the translocation of L-histidine across the cytoplasmic membrane. D: the conformations of J, P, and Q proteins return to their initial states as shown in A.

typhimurium. Our model assumes that the intersubunit spacing between P and Q proteins forms the pore or channel for the passage of substrate. The "key" (J protein with bound L-histidine) can fit into a complex "lock" (P and Q proteins), which then allows specific passengers to pass through the gate into the cytoplasm. The opening of the gate or channel at a particular time for a given substrate depends on the stereospecific interaction between a particular periplasmic binding protein and its corresponding membrane-bound receptors. In Figure 8, we assume that both P and Q proteins are membrane-bound. Ames and Nikaido [5] have reported that P protein is membrane-bound. Q protein has been shown genetically to be an essential component of the high-affinity L-histidine system in S typhimurium [5], but its nature and location are not known. Our model is consistent with the genetic and biochemical evidence of Ames and co-workers [2, 3, 5], which requires the participation of both P and Q proteins. Second, the fact that J protein is not fully denatured in the presence of 7.5 M urea, as shown in Figure 7, suggests that this molecule is quite compact – ie, with extensive noncovalent interactions (hydrophobic, electrostatic, and/or hydrogen bonding). This structural feature of J protein agrees with the "lock" and "key" type of arrangement of our model. It is also consistent with the structure of a binding protein, L-arabinose-binding protein, from Escherichia coli, whose crystal structure has been determined at 2.8 Å resolution [25]. This protein molecule is ellipsoidal in shape (axial ratio = 2:1), with an overall dimension of 70 × 35 × 35 Å, and consists of two distinct globular domains. Third, the model fits nicely with our earlier and present NMR data, as well as with biochemical evidence for the substrate-conformational changes in J protein [6, 7]. In particular, the substrate-induced conformational changes are not localized to the substrate binding site, but can be propagated to the protein-protein interaction site. These structural features fit nicely with the proposed "lock" and "key" type of arrangement for the proposed L-histidine transport as shown in Figure 8. Fourth, it can account for the multi-functional roles of membrane-bound receptors, such as P protein [26]. The conformational changes induced by a given substrate in a periplasmic binding protein (such as J protein) can determine which gate- or pore-opening will occur in order to permit passage of a specific substrate. It is known that J protein binds L-histidine, L-arginine, L-lysine, and D-histidine [27]. The obseved substrate-induced conformational changes in J protein in the presence of each of these amino acids are different [6]; therefore, the interaction of J protein with its membrane-bound receptors in these four cases should be different.

The present model assumes that, after the interaction of J protein with the P and Q proteins, two major types of conformational rearrangements are needed for the translocation of L-histidine: A conformational change in the J protein molecule, which allows the release of L-histidine from J protein; and the actual translocation of L-histidine across the membrane. Either or both steps would require metabolic energy. A recent discussion of the energetic requirements of active transport in gram-negative bacteria is given elsewhere [28]. It should be mentioned that the present model, as shown in Figure 8 for active transport in shock-sensitive bacterial membranes, is independent of whether Q protein is membrane-bound. If Q protein proves not to be membrane-bound or the partner for P protein, our model needs to be modified somewhat. In other words, the channel between P and Q proteins could be formed by two P proteins or a P protein with another membrane-bound protein. However, in order to form a channel for the passage of substrate, the two proteins ought to be complementary, which from a stereochemical point of view means that they ought to be non-identical proteins. In view of the genetic

evidence requiring J, P, and Q for the high-affinity transport of L-histidine across the cytoplasmic membrane of S typhimurium, we believe that our model presented in Figure 8 can serve as a good starting point for further investigation of membrane-transport phenomena.

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