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A HIGH-RESOLUTION ^{1}H-NMR INVESTIGATION OF THE HISTIDINE-BINDING PROTEIN J OF *SALMONELLA TYPHIMURIUM*

SUBSTRATE-INDUCED CONFORMATIONAL CHANGES *

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High-resolution ^{1}H-NMR spectroscopy at 600 MHz has been used to investigate the conformational transitions of the histidine-binding protein J of *Salmonella typhimurium* in solution as a function of pH and of L-histidine concentration. The dissociation constant for the binding of L-histidine to histidine-binding protein J increases from 6.0×10^{-8} to 5.1×10^{-7} M in going from pH 5.57 to 8.00. The conformation of this protein as observed by ^{1}H-NMR also changes over this range of pH. However, when L-histidine is bound, the changes in conformation with pH are much smaller. Also, the pK for the single histidyl residue in histidine-binding protein J changes from 6.75 in the absence of L-histidine to 6.52 when L-histidine is bound. Earlier work in this laboratory resulted in the identification of several proton resonances believed to be at or near the L-histidine-binding site. Two of these resonances have been assigned to a tyrosine and the single histidyl residue in the histidine-binding protein J molecule.

1. Introduction

Periplasmic binding proteins have been found to be necessary for the transport of a variety of molecules and ions across bacterial cell membranes. They act as the first step in the transport process, namely, recognition and binding of a specific substrate, and then interact with other membrane-bound components to complete the transport of the substrate [1]. Genetic and biochemical evidence suggests that the high-affinity transport of L-histidine across the cytoplasmic membrane of *Salmonella typhimurium* requires the histidine-binding protein J (J protein), a periplasmic binding protein [2,3]. The earlier work of Ames and co-workers [4,5] has identified three essential genes for the high-affinity transport of L-histidine in *S. typhimurium*: *his* J (encoded for the periplasmic J protein); *his* P (encoded for P protein); and *his* Q (encoded for Q protein). Our laboratory has proposed a model for the high-affinity transport of L-histidine that views the interactions of these three proteins in a 'lock and key' type of arrangement. In this model, a stereospecific interaction occurs between J protein with L-histidine bound and the P and Q proteins in the membrane, opening a channel at the interface of the P and Q proteins [6]. Our model requires that J protein has two functional sites, one for the binding of L-histidine and the other for the interaction of J protein with the P and Q proteins. Studies of

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L-histidine transport in mutant strains of *S. typhimurium* support this part of the model [4,7]. The model also postulates that a substrate-induced conformational change which facilitates the interaction of J protein and the membrane-bound components of the transport system should occur in J protein upon the binding of L-histidine. Results obtained from experiments using fluorescent and NMR spectroscopic techniques have clearly shown that there is a substrate-induced conformational change in J protein as L-histidine is bound [8,9].

The purpose of this research is to investigate further the first step in the transport process, i.e., binding of L-histidine and the subsequent substrate-induced conformational changes in J protein. Previous work from this laboratory had used ^{1}H-NMR spectroscopy and mutants of J protein to identify resonances from protons believed to be at or near the substrate-binding site [9]. In this study, we have used selective deuteration, pH titration, and ^{1}H-NMR spectroscopy at 600 MHz to assign several of these resonances to protons in J protein and examine their behavior as L-histidine is bound.

2. Materials and methods

The methods and techniques used for the isolation and purification of J protein have been described by Ho et al. [6]. Two strains of *S. typhimurium* TA1859 (wild-type strain for J protein) and TA1859*phe*::Tn10 were used in this study. Bacteria were grown in 5-gallon carboys containing 15 l of minimal medium using glycerol as the carbon source. The medium was supplemented with 40 μg/ml of deuterated phenylalanine when growing TA1859*phe*::Tn10. The carboys were inoculated with 1 l of an overnight culture and grown to the late log phase. The cells were centrifuged at 13000 $\times$ g for 10 min, washed with 10 mM potassium phosphate buffer at pH 7.1 and stored at -80°C. J protein was isolated at room temperature as described previously [6], except that the buffer gradient for the DE-52 (Whatman) ion-exchange column was reduced in volume from 2 l per chamber to 1 l. Purified J protein was stored as a lyophilized powder at -20°C.

2.1. Determination of dissociation constant

The dissociation constant (K_D) of J protein for L-histidine was determined using an equilibrium dialysis binding assay similar to that described by Lever [10]. Triplicate 0.3-ml samples of J protein and two blank samples containing buffer were dialyzed against 250 ml of 10 mM potassium phosphate buffer at the desired pH in a beaker. After dialysis, samples were removed from the bags using a syringe and 25 μl of each sample were mixed with 3 ml of scintillation fluid for counting. The amount of L-histidine bound to J protein was determined by subtracting the number of counts found in the blank samples from those in the samples containing J protein.

2.2. Preparation of deuterated phenylalanine and histidine

Deuterated phenylalanine (L-phenylalanine-d_6) was prepared as described by Ho et al. [6]. L-Histidine deuterated at the *C*2 position, L-[2-^{2}H]histidine, was prepared by the method of Matthews et al. [11]. The 300 MHz ^{1}H-NMR spectrum of the deuterated histidine showed that the C2 proton had been fully exchanged.

2.3. NMR sample preparation

Lyophilized J protein was dissolved in ^2H_2O and 10 mM potassium phosphate buffer at pH* 7.0 to give a final protein concentration of 0.2–0.4 mM. The pH* of each sample was measured using a 4.5 mm Ingold microelectrode with a Radiometer Model 64 pH meter. All pH* values shown are the direct pH meter readings and are uncorrected for the deuterium isotope effect. Samples which were used for pH titrations also contained 0.1 M NaCl. In experiments where J protein was titrated with L-histidine, a stock solution of approx. 4.0 mM L-histidine in ^2H_2O at the same pH* as the protein solution was added to the sample in an NMR tube. pH titrations of J protein solutions were conducted by adding 2–5 μl of a 1 N NaO^{2}H or 1 N ^{2}HCl solution directly to the sample inside an NMR tube. After mixing, the pH* of the sample was measured and then checked again after the ^{1}H-NMR spectrum was recorded.

2.4. ^1H-NMR measurements

All ^{1}H-NMR spectra were obtained at 600.2 MHz on a home-built high-frequency superconducting spectrometer at Carnegie-Mellon University [12]. Spectra were obtained using NMR correlation spectroscopy with the residual $^1H^2HO$ in each sample providing an internal ^{1}H lock [13]. Data for the aromatic and aliphatic proton resonance regions of each spectrum were collected separately. In each case, the sweep width was 3600 Hz and the sweep time was 1 s. 1000 scans were usually accumulated for each spectra region. The proton chemical shifts are referenced to the residual water proton signal which resonates 4.88 ppm downfield from 2,2-dimethyl-2-silapentane-5-sulfate (DSS) at 22°C. A positive sign for a chemical shift indicates that a resonance is downfield from $^1H^2HO$ and the accuracy of a chemical shift measurement is ± 0.01 ppm (based on repeated measurement of a single resonance). The temperature inside the probe was 22°C.

2.5. Calculation of pH titration parameters

The pH titrations of the histidine resonance in J protein were obtained by fitting the ^{1}H chemical shift (δ) and pH parameter to the ^{1}H-NMR titration equation [14,15]:

$$\delta_{obsd} = \frac{\delta^+ [H^+]^n + \delta^o K^n}{K^n + [H^+]^n} \quad (1)$$

where $[H^+]$ is the concentration of H^+, K the H^+ dissociation constant for the histidine residue, n a titration coefficient, δ_{obsd} the observed chemical shift, and δ^+ and δ^o the chemical shifts for the protonated and unprotonated forms of the histidyl residue, respectively. The data were fitted using a nonlinear least-squares analysis with K, δ^+, δ^o and n as the variables in eq. 1.

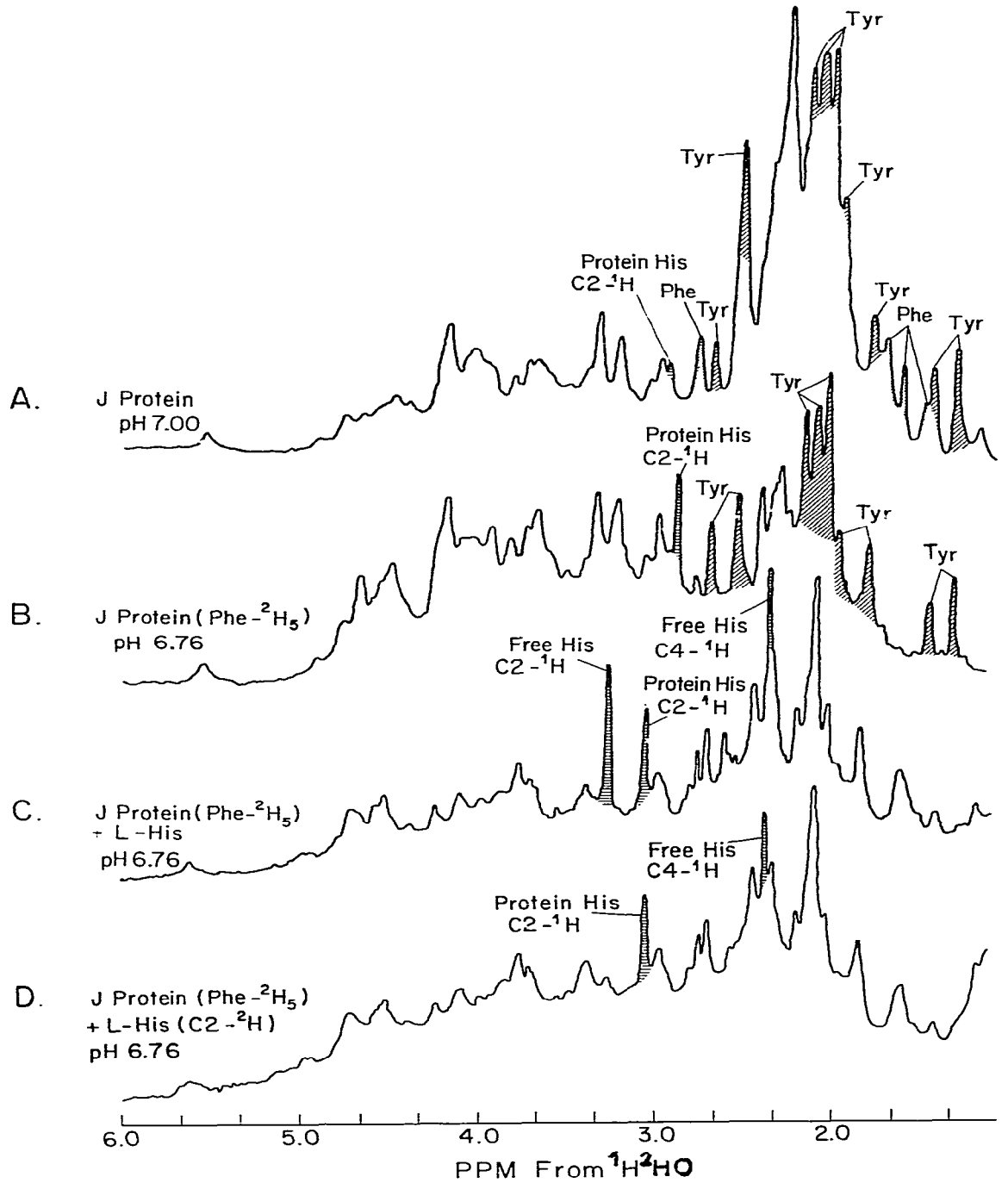
3. Results

3.1. Isolation of J protein and incorporation of deuterated phenylalanine

The incorporation of deuterated phenylalanine into J protein was accomplished by using a phenylalanine-requiring auxotroph [TA1859(phe$^-$)] of *S. typhimurium* as described in section 2. The level of incorporation was estimated by a comparison of the ^{1}H-NMR spectrum of J protein containing fully protonated amino acids and that containing deuterated phenylalanine. J protein contains 12 phenylalanines [9,16–18] which represent over half of the total aromatic amino acid residues in the protein molecule. The regions of spectrum investigated were 1.0–6.0 ppm upfield and downfield from the residual $^1H^2HO$ signal. The integrated area of the aliphatic region of the spectrum, 0.5–6.0 ppm upfield from $^1H^2HO$, was used as a reference because this area is not affected by the substitution of deuterated phenylalanines for normal phenylalanines. The integrated areas of the aromatic proton resonance region of the spectrum for the deuterated phenylalanine protein and normal J protein were then compared in order to determine the amount of deuterium incorporation. The bacterial cultures underwent a minimum of four doublings in the presence of deuterated phenylalanine. In all the preparations used in this work, the level of deuterated phenylalanine incorporation varied between 85 and 90%. The K_D for L-histidine binding by the deuterated phenylalanine-containing protein was found to be identical within experimental errors to that obtained for the normal J protein.

3.2. Assignment of ^1H resonances at or near the substrate binding site

Proton resonances of J protein were assigned using two experimental methods: (i) specific substitution with deuterated amino acids; and (ii) ^{1}H-NMR pH titration. Fig. 1 summarizes assignments of the proton resonances of aromatic amino acids using specific deuterations. Fig. 1A shows the 600 MHz ^{1}H-NMR spectrum of the aromatic proton resonance region for the fully protonated J protein. The same spectral region for the J protein molecule containing deuterated phenylalanine is shown in fig. 1B. A comparison between these two spectra clearly indicates that the proton resonances of J protein at 2.70, 1.75, 1.60 and 1.50 ppm from $^1H^2HO$ originate from protons of phenylalanine residues. These assignments are in per-


A.
J Protein
pH 7.00
Tyr
Tyr
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Protein His
C2-^{1}H
Phe
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Phe
Tyr
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B.
J Protein (Phe-$^2H_5$)
pH 6.76
Protein His
C2-^{1}H
Tyr
Tyr
Tyr
Free His
C4-^{1}H
Free His
C2-^{1}H
Protein His
C2-^{1}H
C.
J Protein (Phe-$^2H_5$)
+ L-His
pH 6.76
Free His
C4-^{1}H
Protein His
C2-^{1}H
D.
J Protein (Phe-$^2H_5$)
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PPM From $^1H^2HO$

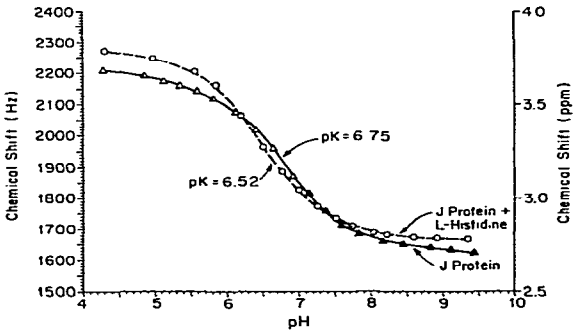

Fig. 2. 600 MHz ^{1}H-NMR titration of the C2 proton of the histidine residue of J protein as a function of pH in 0.1 NaCl at 22°C. The pK for titration of the resonance without substrate L-histidine bound is 6.75 ± 0.05 and 6.52 ± 0.05 when J protein contains bound L-histidine. The titration coefficient for the ^{1}H-NMR pH titration is about 1 with or without L-histidine bound to J protein.

fect agreement with the assignments of phenylalanine proton resonances of J protein previously reported by our laboratory [6]. The assignments of the proton resonances of tyrosyl residues are also summarized in fig. 1A following previous results [6]. The tyrosine proton resonance at 1.38 ppm from ^{1}H^{2}HO is of special interest, since this resonance was first suggested by Manuck and Ho [9] to originate from a proton at or near the substrate binding site.

The assignment of the C2 proton of the single histidyl residue in J protein shown in fig. 1B was accomplished by a ^{1}H-NMR pH titration. J protein containing deuterated phenylalanine was used for the titration because its aromatic spectral region is greatly simplified compared to that of normal J protein. The aromatic proton resonances were observed at 600 MHz and the pH of the protein solution was varied from pH 4.3 to 9.3 in approx. 0.2 pH unit steps.

The results of this ^{1}H-NMR titration indicated that two aromatic proton resonances in the spectra of J protein with substrate show the spectral behavior characteristic for the C2 proton of a histidine residue, namely, a pK value around neutral pH and a total titration shift, $\delta^+ - \delta^\circ$, of approx. 1 ppm [14]. We have assigned these two resonances to the C2 proton of free L-histidine and to the C2 proton of the L-histidine of J protein, respectively, using two experimental methods. In one experiment, when adding free L-histidine to a J protein solution already saturated with substrate, we have observed an increase in the intensity of the resonances at 3.2 and 2.45 ppm from ^{1}H^{2}HO. Thus, we have assigned these resonances to the C2 and C4 protons of the free L-histidine, respectively (fig. 1C). The assignment of the C2 proton of free L-histidine was further confirmed by titrating J protein with L-histidine deuterated at the C2 position. As shown in fig. 1D, the C2 proton resonance corresponding to the free substrate is missing from the ^{1}H-NMR spectrum. Apart from this difference, the spectrum in fig. 1D is identical to that in fig. 1C. This identity indicates that the resonances of the C2 and C4 protons of the L-histidine bound to J protein are not among the proton resonances that can be readily resolved in the ^{1}H-NMR spectra. This fact may be due to an increase in the linewidth of these resonances resulting from the binding of L-histidine to the protein molecule. Fig. 2 presents the ^{1}H-NMR titration of the C2 proton resonance of the histidyl residue in J protein as a function of pH in the absence and presence of L-histidine. The corresponding ^{1}H-NMR pH titration parameters are summarized in the legend to fig. 2. These parameters were calculated using eq. 1 and a nonlinear least-squares program. Under the same experimental conditions, the pK value for the free L-histidine was found to be 6.66 ± 0.03.

The C2 proton resonance from the histidyl re-

Fig. 1. 600 MHz ^{1}H-NMR spectra of J protein in 0.01 M potassium phosphate buffer at 22°C. The aromatic proton resonance region shown here is from 1.0 to 6.0 ppm downfield from the residual ^{1}H^{2}HO signal. Spectrum A is the ^{1}H-NMR spectrum of fully protonated J protein. In spectrum B, deuterated phenylalanine has been incorporated into the protein. In spectrum C, L-histidine at a mole ratio of 1.5 L-histidines per J protein molecule has been added to J protein containing deuterated phenylalanine. To produce spectrum D, L-histidine deuterated at the C2 position was added in the same ratio as L-histidine in spectrum C to J protein containing deuterated phenylalanine.

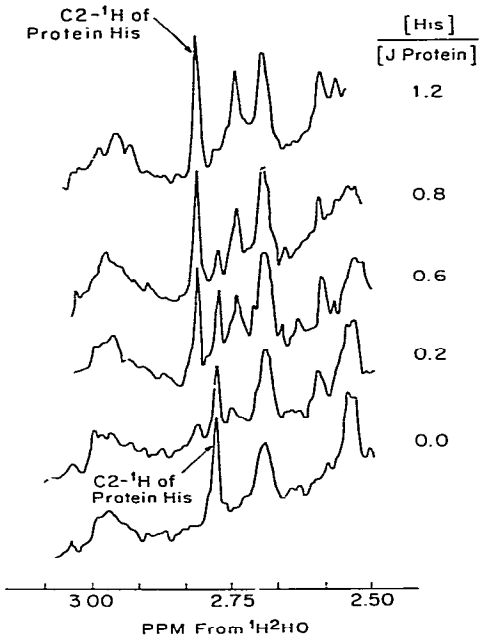

Fig. 3. ^{1}H-NMR spectra of J protein containing deuterated phenylalanine as a function of L-histidine concentration at pH 8.00. The region shown here contains the C2 proton resonance for the protein histidine residue. The addition of substrate L-histidine was performed as described in section 2 and the ratio of J protein with histidine bound to free J protein was calculated using Eq. 4 of ref. 9.

sidue in J protein is one of those (at +3.30 ppm) suggested as being at or near the substrate-binding site [9]. Our present ^{1}H-NMR results indicate that the direction and the magnitude of the change in the chemical shift of this resonance upon the addition of substrate L-histidine are pH dependent (fig. 2). Upon the addition of substrates, at high and low pH, the resonance shifts downfield whereas at a pH of 6.5 it shifts upfield. We have confirmed these findings by titrating the J protein solutions with L-histidine at pH 5.57, 6.76 and 8.00 (see, for example, fig. 3). In each case, the magnitude and direction of movement of the protein histidyl C2 resonance were those predicted by the titration curves in fig. 2.

The present ^{1}H-NMR results on the change in conformation of the histidyl residue of the J protein upon substrate binding allow us to estimate the lifetime of the substrate bound to the protein. For example, as illustrated in fig. 3, at intermediate levels of substrate saturations, two C2 proton resonances of the J protein histidine residue are observed corresponding to the free and complexed states. The difference in chemical shift

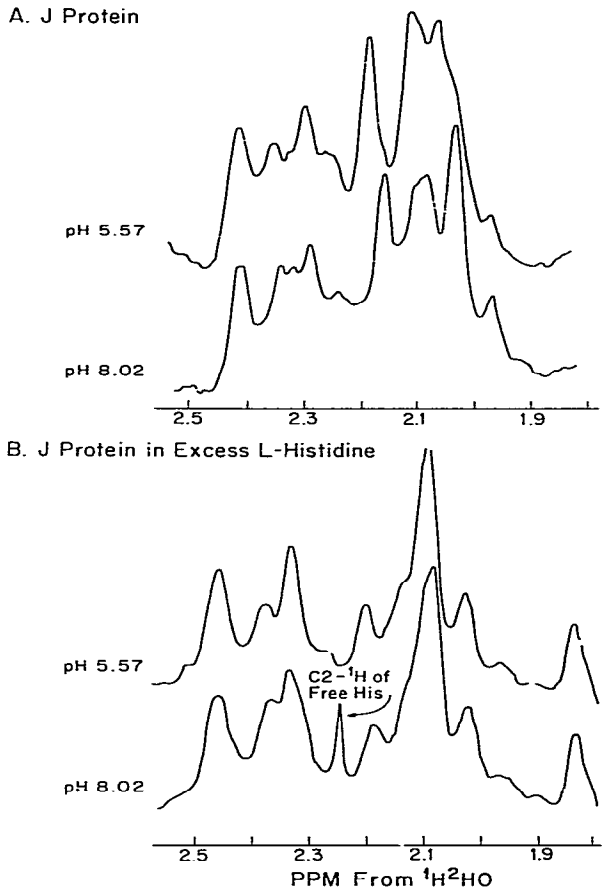

Fig. 4. 600 MHz ^{1}H-NMR spectra of the aromatic proton resonances of J protein containing deuterated phenylalanine. The spectral region shown here is between +1.83 and +2.50 ppm. In fig. 5A, the J protein sample is free of bound L-histidine. In fig. 5B, L-histidine was added to the J protein samples at a mole ratio of 1.2 L-histidines to 1 J protein. In both panels A and B, the pH of the sample for the lower spectrum was 8.02 and that for the upper spectrum was 5.57.

between these two resonances is, at pH 8.00, approx. 30 Hz. Therefore, under these experimental conditions, the lifetime of the substrate in the bound state (τ_b) should be longer than approx. 5.5 ms. This corresponds to a dissociation rate constant for the complexed J proton k_{-1} of less than 180 s^{-1}. This range is in good agreement with that previously estimated by Manuck and Ho [9], namely, $60 < k_{-1} < 280\ s^{-1}$.

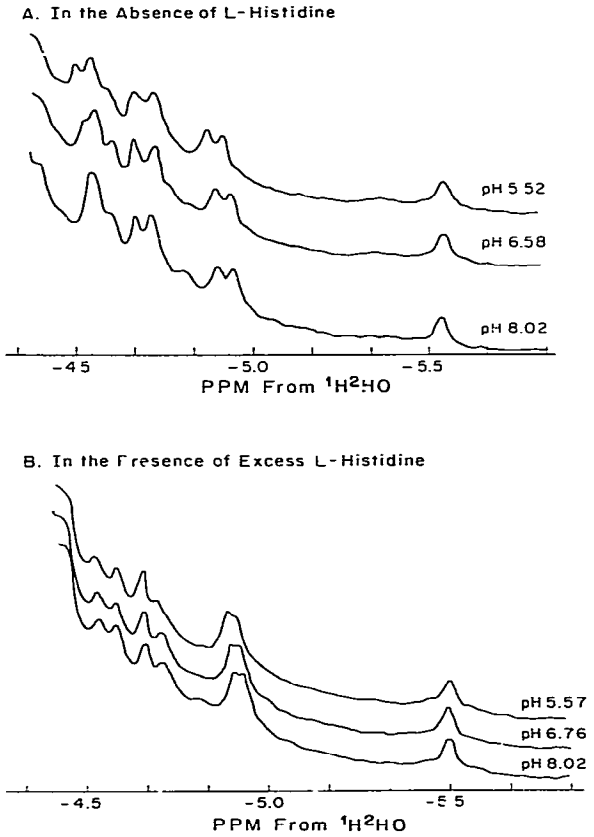

Fig. 5. pH dependence of the chemical shift position for the ring-current shifted protein resonances in J protein at 600 MHz. The pH for the entire series of experiments was varied from pH 5.75 to 8.02 in 0.5 pH unit steps. The region shown in the figure is from −4.3 to −5.9 ppm upfield from $^1H^2HO$. Panel A shows the resonances for J protein in the absence of L-histidine bound. In panel B, L-histidine was added to the J protein solution in a slight excess of that needed to bind one histidine per J protein molecule.

The ^{1}H-NMR pH titration of J protein also reveals one other resonance whose chemical shift is pH dependent. Fig. 5A illustrates the chemical shift change as a function of pH for the ring-current shifted proton resonance at approx. −4.45 to −4.52 ppm. This peak is the third resonance which has been suggested as being at or near the substrate-binding site. We have not identified this resonance.

There are also other regions of the spectrum which show reproducible and significant rearrangements as the pH is varied. Fig. 4A shows an expanded region of the ^{1}H-NMR spectrum of J protein which is +1.8 to +2.3 ppm downfield from $^1H^2HO$. It can be seen that there is a change in both the chemical shift and the intensity of the resonances as the pH is changed from 5.57 to 8.02. In the region of the J protein's NMR spectrum which arises from aliphatic protons, two areas show significant changes as the pH is varied. Fig. 5A illustrates the pH dependence of the chemical shifts for ring-current shifted proton resonances.

3.3. Binding of L-histidine to J protein

Fig. 1C and D shows ^{1}H-NMR spectra of J protein with bound L-histidine. By comparing the spectra given in fig. 1A and B with those in fig. 1C and D, there are significant changes in the proton resonances of J protein upon the addition of substrate. This suggests that there could be significant substrate-induced conformational changes over different parts of the J protein molecule. The environments of the tyrosine resonances shown in fig. 1B from +1.0 to +1.5, +2.0 to +2.3 and +2.5 to +2.8 ppm change appreciably as L-histidine is added to J protein (fig. 1C). For the histidyl residue of J protein, the pK value decreases from 6.75 to 6.52 (fig. 2) and there is a small change in the pH titration coefficient upon the binding of L-histidine (results not shown). There are, however, areas in the spectra which do not change appreciably. In the aromatic region, the tyrosine peak at +1.82 ppm and the region of the spectrum from +2.24 to +2.50 ppm do not show significant alterations upon the addition of L-histidine.

It is also interesting to note that pH-dependent

changes in the ^{1}H-NMR spectrum of J protein are much smaller when L-histidine is bound. Fig. 4B illustrates the same region of the ^{1}H-NMR spectrum shown in fig. 4A except in this case, L-histidine has been added to the J protein solution. It can be seen that between pH 5.57 and 8.02, the changes in the spectrum are much smaller than those observed in the absence of bound substrate. Fig. 5B shows the ring-current shifted proton resonance region of the NMR spectrum at several pH values in the presence of L-histidine. In contrast to the data shown in fig. 5A, the changes in the spectrum as the pH is varied are relatively small.

3.4. pH dependence of L-histidine binding to J protein

The data in fig. 6 illustrate the results of experiments performed to measure the dissociation constant for L-histidine at three pH values. It can be seen that the K_D varies over almost an order of magnitude from pH 5.57 to 8.00. Extrapolation of the data to the abscissa results in a determination of approx. 1 for the number of L-histidine-binding sites for each J protein molecule.

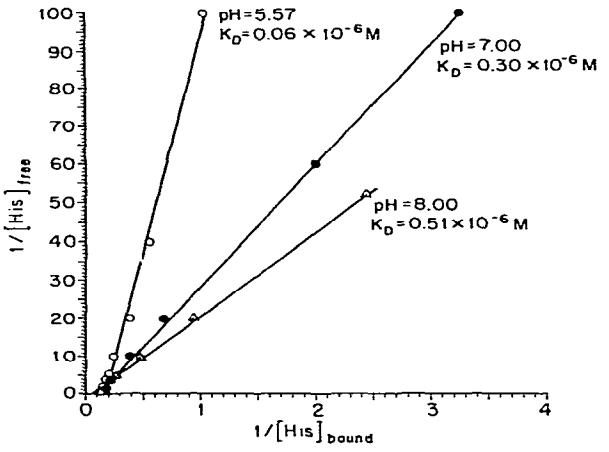

Fig. 6. Double-reciprocal plot of the binding of tritiated L-histidine (NET-197) to J protein. The specific activity of the radioactive L-histidine was 3300 cpm/μmol. The J protein concentration was 10 μM and the concentration of L-histidine was varied from 0.01 to 5.00 μM. The average error in each experiment was less than 10%.

4. Discussion

The results presented in this paper clearly indicate the value of the specific deuteration method in assigning the proton resonances in the NMR spectra of proteins. We have studied the aromatic region of J protein's NMR spectrum because we can quantitatively deuterate and incorporate phenylalanine or tyrosine [6] into the protein, thereby reducing the total number of resonances observed by ^{1}H-NMR by one-half. The aliphatic region of the spectrum is significantly more complicated and contains approx. 10-times more amino acid residues. Thus, it is more difficult to observe and assign individual amino acid protons. The data presented here and those in ref. 6 have resulted in the assignments presented in fig. 1.

Upon binding of substrate, the ^{1}H-NMR spectrum of J protein shows significant changes, especially in the aromatic proton resonance region (fig. 1B and C). For the single histidyl residue in the protein, the pK value and the chemical shifts in protonated and unprotonated forms are altered by substrate binding (fig. 2). Also, the environments of a number of tyrosyl residues change so that the aromatic region of the ^{1}H-NMR spectrum of J protein with L-histidine bound is different from that of the protein molecule without L-histidine bound (fig. 1B and C). Subtler changes also occur in the aliphatic region of the ^{1}H-NMR spectrum. A ring-current shifted proton resonance at −4.47 ppm, whose chemical shift is pH dependent when J protein does not have bound substrate, is no longer affected by changes in pH when the protein is ligated. There are a number of other rearrangements that occur in the aliphatic proton resonance region of the spectrum from −0.5 to −5.0 ppm (data not shown), but we have not as yet assigned any of these resonances to specific amino acids.

Alterations in the ^{1}H-NMR spectrum of J protein upon the binding of L-histidine could be due to the binding process itself or be the result of a substrate-induced conformational change. Manuck and Ho [9] used two mutant strains of *S. typhimurium* with altered J proteins to identify proton resonances at the binding site and protein-protein interaction sites. Figs. 1–3 illustrate the results of our experiments designed to assign the resonances

at +3.30, +1.37 and −4.47 ppm suggested by Manuck and Ho [9] as being at or near the substrate-binding site. We have assigned the resonances at +1.37 and +3.30 ppm to a tyrosyl residue and the C2 proton of the single histidyl residue in J protein, respectively. It was not possible to identify the C4 proton resonance for the histidine because it apparently overlaps with other aromatic proton resonances. The chemical shift of the third resonance at −4.47 ppm was found to titrate as a function of pH. The empirical chemical shift of this resonance suggests that it may be a leucyl, isoleucyl, or valyl residue which is being influenced by the histidyl residue on J protein. The data then imply that a tyrosine, the single histidine in J protein, and a ring-current shifted aliphatic proton group may be at or near the substrate-binding site. It is interesting to note that histidine-binding protein J which is responsible for the high-affinity transport of L-histidine has its single histidyl either at or near the substrate-binding site.

Recently, Higgins et al. [17] have shown that the previously defined *his* Q gene consists of two separate cistrons, *his* Q and *his* M. Their present evidence suggests that the M, P, and Q proteins are all associated with the membrane. The model proposed by Ho et al. [6] can be readily modified to take into account this new component. The M protein can be inserted into the membrane along with the P and Q proteins to form the channel complex. It should be mentioned that the models proposed by Ho et al. [6] and Higgins et al. [17] both emphasize that there is a substrate-induced conformational change in J protein. This is then followed by the appropriate interactions between J protein and membrane-bound components to translocate L-histidine across the cytoplasmic membrane.

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References

1 D.B. Wilson and J.B. Smith, in: Bacterial transport, ed. B.P. Rosen (Marcel Dekker, New York, 1978) p. 495.
2 G.F.-L. Ames and J.E. Lever, Proc. Natl. Acad. Sci. U.S.A. 66 (1970) 1096.
3 G.F.-L. Ames and J.E. Lever, J. Biol. Chem. 247 (1972) 4309.
4 G.F.-L. Ames and E.N. Spudich, Proc. Natl. Acad. Sci. U.S.A. 73 (1976) 1877.
5 G.F.-L. Ames and K. Nikaido, Proc. Natl. Acad. Sci. U.S.A. 75 (1978) 5447.
6 C. Ho, Y.-H. Giza, S. Takahashi, K.E. Ugen, P.F. Cottam and S.R. Dowd, J. Supramol. Struct. 13 (1980) 131.
7 S.G. Kustu and G.F.-L. Ames, J. Biol. Chem. 249 (1974) 697.
8 D.E. Robertson, P.A. Kroon and C. Ho, Biochemistry 16 (1977) 1443.
9 B.A. Manuck and C. Ho, Biochemistry 18 (1979) 566.
10 J.E. Lever, J. Biol. Chem. 247 (1972) 4317.
11 H.R. Matthews, K.S. Matthews and S.J. Opella, Biochim. Biophys. Acta 497 (1977) 1.
12 A.A. Bothner-By and J. Dadok, in: NMR and biochemistry, eds. S.J. Opella and P. Lu (Marcel Dekker, New York, 1979) p. 169.
13 J. Dadok and R.F. Sprecher, J. Magn. Resonance 13 (1974) 243.
14 J.L. Markley, Acc. Chem. Res. 8 (1975) 70.
15 I.M. Russu, N.T. Ho and C. Ho, Biochemistry 21 (1982) 5031.
16 C.F. Higgins and G.F.-L. Ames, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 6038.
17 C.F. Higgins, P.P. Hoag, K. Nikaido, F.I. Ardeshir, G. Garcia and G.F.-L. Ames, Nature 298 (1982) 723.
18 R.W. Hogg, J. Biol. Chem. 356 (1981) 1935.