CONFORMATIONAL DYNAMICS OF TWO HISTIDINE-BINDING PROTEINS OF *SALMONELLA TYPHIMURIUM*

R. SUZANNE ZUKIN,* MARGARET F. KLOS,‡ AND RHODA ELISON HIRSCH‡
*Departments of *Neuroscience, *Biochemistry, and ‡Medicine, Albert Einstein College of Medicine, Bronx, New York 10461*

ABSTRACT The *Salmonella typhimurium* periplasmic histidine-binding J-protein is one of four proteins encoded by the histidine transport operon. Mutant J-protein *hisJ5625* binds L-histidine, but does not transport it. The tertiary structure and conformational dynamics of native and mutant J-protein have been compared using steady state fluorescence, fluorescence polarization, and fluorescence energy transfer measurements. The two proteins have different three-dimensional structures and exhibit different responses to histidine binding. Ligand-induced conformational changes were demonstrated in both J-proteins using fluorescence energy transfer (distant reporter method) between the single tryptophan residue per mole of protein and a fluorescein-labeled methionine residue. However, the conformational change of the mutant protein is qualitatively and quantitatively different from that of the wild-type protein. Moreover, the microenvironment of the tryptophan and its distance from the labeled methionine (44Å for the wild type, 60Å for the mutant J-protein) are different in the two proteins. In conclusion, these results indicate that the specific conformational change induced in the wild type J-protein is a necessary requirement for the transport of L-histidine.

INTRODUCTION

The periplasmic histidine-binding J-protein for *Salmonella typhimurium* functions as the primary receptor in histidine transport in this organism (Ames and Lever, 1970) and may serve as a chemoreceptor as well. This protein is one of four proteins encoded by the histidine transport operon, all of which are required for high-affinity histidine transport (Higgins et al., 1982). Genetic evidence has demonstrated that the ligand-activated J-protein binds directly to the membrane-bound P or permease protein (Ames and Spudich, 1976) as a first step to transfer histidine through the plasma membrane. The question arises as to the mechanisms by which the J-protein is activated by its ligand. Previous studies provided evidence that binding of L-histidine to the J-protein perturbs the environment of specific aromatic amino acid residues of the receptor (Robertson et al., 1977). The possibility of a delocalized ligand-induced conformational change was never distinguished from that of direct interaction between the substrate and these residues. Sequencing of the J-protein (Hogg, 1982) and of the gene that codes for it (Higgins and Ames, 1982; Higgins et al., 1982) have established that the J-protein contains one tryptophan residue. Thus, fluorescence analyses of the tryptophan and of a covalently attached dye, 5-iodoacetamidofluorescein, can be used in the "distant reporter group" method (Zukin et al., 1977 and 1979; Zukin, 1979) to document and elucidate the details of a ligand-induced conformational change.

The mutant J-protein *hisJ5625* isolated from *Salmonella* TA300 (Kustu and Ames, 1974) has an altered J-P interaction and thus provides an ideal system in which to test the physiological significance of such a conformational change. The mutant protein can bind L-histidine, but cannot transport it, despite the presence of a normal P component. The amino acid sequence of the mutant J-protein has been deduced from its gene sequence (Higgins and Ames, 1981); it is a single polypeptide chain and has a cysteine substituted for arginine at residue 176 (Noel et al., 1979). By analogy to several other periplasmic binding proteins (Zukin et al., 1977 and 1979; Zukin, 1979), it is predicted that under normal conditions, a ligand-induced conformational change in the wild-type J-protein is an obligatory first step enabling it to interact with the P-protein. Thus, the spectral changes associated with histidine binding to wild-type J are predicted to differ significantly from those associated with binding to the mutant protein. We present evidence that a conformational change does occur upon the binding of L-histidine to the wild-type J-protein. The ligand-activation mechanism and tertiary structure of this protein are contrasted with those of the mutationally-altered receptor.

Please send all correspondence to Dr. R. Suzanne Zukin, Department of Neuroscience, Albert Einstein College of Medicine, Forch.-Rm. 354, 1300 Morris Park Avenue, Bronx, NY 10461.

BIOPHYS. J. © Biophysical Society · 0006-3495/86/06/1229/7 $1.00
Volume 49 June 1986 1229-1235

MATERIALS AND METHODS

The histidine binding receptor J-protein (isolated from wild-type *S. typhimurium*) and the correlate mutant receptor *hisJ5625* (isolated from *S. typhimurium* TA300) were obtained in the purified form from G. Ames, University of California. Protein concentrations were determined by the procedure of Lowry et al. (1951). Binding activity was determined using the equilibrium dialysis assay of Lever (1972). The dye, 5-iodoacetamido-fluorescein,[1] was experimentally attached to the purified receptor by described procedures (Zukin et al., 1977) in 50 mM potassium phosphate buffer, pH 6.8 for 24 h. Covalent modification was shown by comigration of fluorescence at 520 nm with the single protein band during $NaDodSO_4$ polyacrylamide gel electrophoresis. Modification proceeded to a fixed value of 1.2 mol dye/mol protein after which it leveled off. Amino acid analyses of the native and mutant J-proteins and their fluorescein-labeled derivatives were determined using an updated Beckman Model 120 C amino acid analyzer (Takahashi et al., 1981). Samples to be analyzed were hydrolyzed 24 h. Values were normalized to the known values for alanine, aspartate, and glutamate (Hogg, 1981). Duplicate determinations were performed. In general, the composition determined by amino acid analysis agreed closely with that determined by sequencing of the protein (Hogg, 1981). Accurate values for cysteine, however, were not determined. The effect of L-histidine on the intrinsic fluorescence of the J-protein and the effect of L-histidine on the fluorescein fluorescence of the 5-AF-J-protein were examined. Steady-state fluorescence measurements were performed on a fluorescence spectrophotomer (model 650-10S; Perkin-Elmer Corp., Instrument Div., Norwalk, CT) at ambient temperature. Scanning emission and excitation spectra were recorded using excitation and emission slit widths of 8 nm. Steady-state polarization measurements were made by using the standard polarization accessory for the instrument and were normalized for a constant grating transmission (Zukin et al., 1979). Energy transfer measurements were calculated from the fluorescence excitation spectra of fluorescein bound to the protein (Zukin et al., 1977). The quantum yields of the J-protein and mutant J-protein tryptophans were determined by comparison of their fluorescent spectra with that of quinine bisulfate according to

$$\frac{\theta_2}{\theta_1} = \frac{\Sigma F_2(\lambda)}{\Sigma F_1(\lambda)} * \frac{A_1(\lambda_{ex})}{A_2(\lambda_{ex})} * \frac{I_1^0(\lambda_{ex})}{I_2^0(\lambda_{ex})}, \quad (1)$$

where $F(\lambda)$ is the integrated area of the fluorescence emission spectrum, $A(\lambda_{ex})$ is the absorbance at the excitation wavelength (measured in a double beam UV/VIS spectrophotometer), and $I^0(\lambda_{ex})$ is the intensity of the lamp measured at the excitation wavelength of the sample (2) and the standard (1, quinine bisulfate, $\theta_1 = 0.55$) (Teale and Weber, 1957).

RESULTS

Effect of Histidine on the Intrinsic Tryptophan and Attached Reporter Group Fluorescence of the Native J-Protein

The intrinsic steady-state fluorescence of the purified histidine J binding protein was examined in the presence and absence of saturating histidine (Fig. 1 *a* and *b*). Histidine caused a 30% enhancement and a 3 nm red shift of the tryptophan emission spectrum. In contrast, no such changes are observed in the control upon addition of L-lysine, an amino acid that shows weak affinity for the protein (data not shown) (Ames and Lever, 1972). The

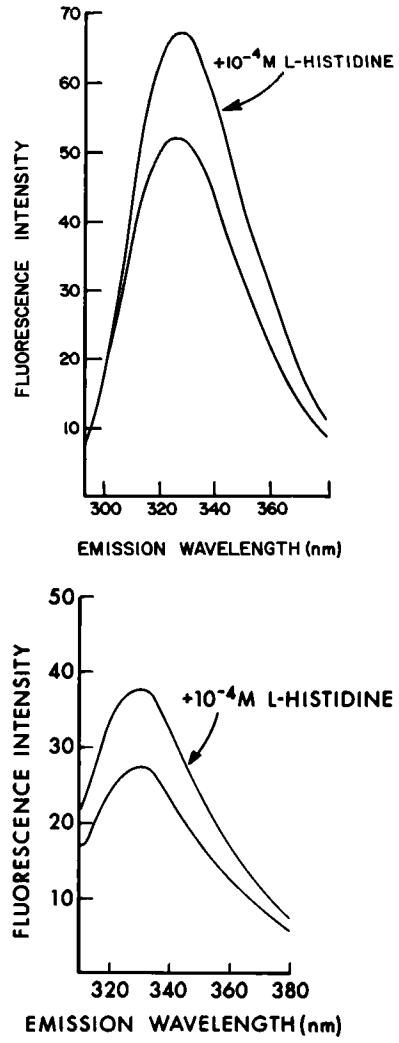

FIGURE 1 Fluorescence emission spectra (uncorrected) of the *S. typhimurium* histidine J-protein in the presence of absence of 10^{-4} L-histidine. Maximal spectral changes were observed upon addition of 10^{-5} M L-histidine. Addition of increasing amounts of L-histidine up to a final concentration of 10^{-4} L-histidine resulted in no further detectable changes in the protein fluorescence spectrum, here and in Figs. 2, 3, and 4. Spectra were recorded on a Perkin-Elmer Hitachi Model 650-10S fluorescence spectrometer at ambient temperature with an excitation wavelength of (*A*) 280 nm or (*B*) 296 nm. J-protein (70 μg/ml) was in 50 mM potassium phosphate buffer, pH 6.8.

tryptophan content of this protein was shown to be one residue per mol of protein by sequencing of both the protein itself (Hogg, 1980) and of the gene that codes for it (Higgins and Ames, 1981). By comparison with model fluorescence studies of proteins (Burstein et al., 1973; Konev, 1967), the changes in the fluorescence spectrum indicate movement of the tryptophan to a more polar environment, when histidine binds to the J-protein.

To differentiate between two possible mechanisms, (*a*) a direct interaction between the ligand histidine and the J-protein tryptophan residue with no conformational change in the receptor and (*b*) a ligand-induced conformational change, the J-protein was modified with an addi-

[1] *Abbreviations used in this paper*: 5-AF, 5-acetamidofluorescein; $NaDodSO_4$, sodium dodecyl sulfate.

tional chromophore. The purified protein was reacted with 5-iodoacetamidofluorescein, a sulfhydryl reagent with an attached fluorescent dye. Covalent modification was achieved, as shown by comigration of fluorescence at 520 nm with a single protein band during $NaDodSO_4$-polyacrylamide gel electrophoresis. Measurement of the absorbance at 490 nm of the protein in 8 M urea showed 1.2 fluorescent groups per molecule of protein, as calculated from the molar extinction coefficient of the free dye in urea (Zukin et al., 1977). Amino acid analyses of the protein before and after modification suggested that a single methionine residue was modified by this reagent (Table I). The values of lysine, methionine, and methionine sulfone in the unmodified performic acid-treated protein, were found to be in good agreement with those expected from the known sequence. It is well established that the iodoacetamide moiety of the 5-IAF dye can label only a small number of amino acids: cysteine, methionine, histidine, and lysine. In our amino acid determinations, there is no substantial difference in the number of histidine residues in the modified vs. the unmodified wild-type or mutant protein. Although there is a difference of 0.5 lysine residues in the case of the wild-type protein and 1.6 residues in the lysine content of the mutant protein, these values represent small differences between two large numbers (e.g., 21.6 vs. 20.0 for the mutant); thus, our data do not indicate a significant change in the lysine content for either protein. By contrast, in the case of methionine, a difference of 0.84 residues compared with a total of three residues is highly significant. The dye-modified J-protein exhibited full histidine-binding activity. As the fluorescein residue is a large bulky molecule, this result provides evidence that the site of modification is distant from the ligand binding site.

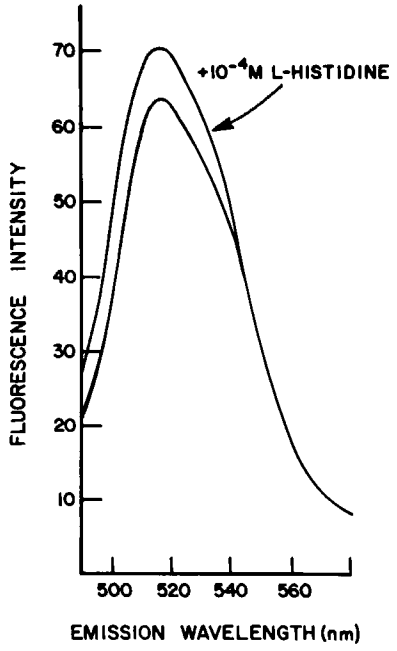

FIGURE 2 Fluorescence emission spectra (uncorrected) of the 5-acetamidofluorescin-labeled J-protein in the presence and absence of 10^{-4} M L-histidine. Spectra were recorded at ambient temperature with an excitation wavelength of 460 nm. The 5-AF-J-protein (70 μg/ml) was in 50 mM KPO_4 buffer, pH 6.8.

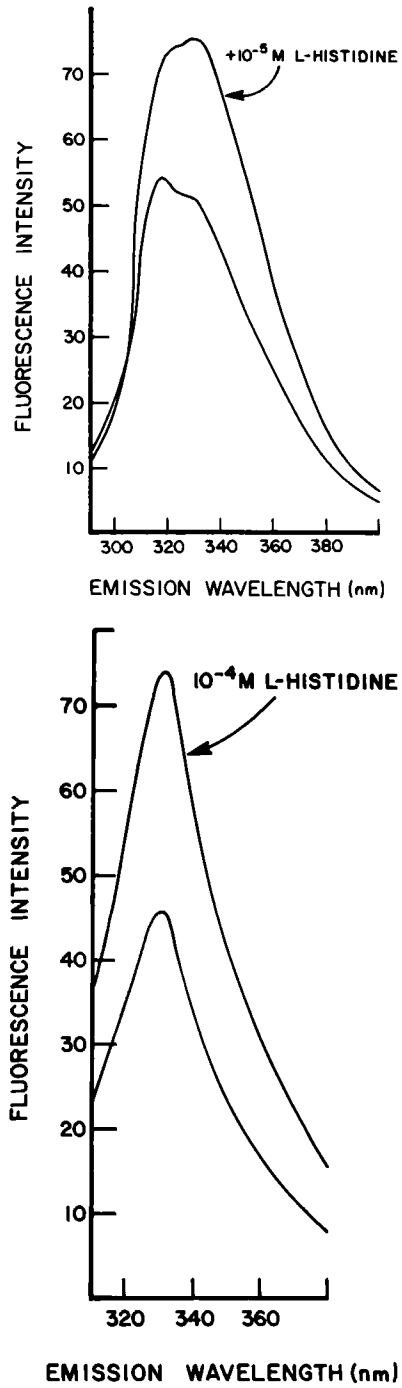

FIGURE 3 Fluorescence emission spectra (uncorrected) of the *S. typhimurium hisJ5625* mutant J-protein in the presence of and absence of (*A*) 10^{-5} M and (*B*) 10^{-4} M L-histidine. Maximal changes are found with 10^{-5} M L-histidine. Spectra were recorded at ambient temperature with an excitation wavelength of (*A*) 280 nm or (*B*) 296 nm. The mutant J-protein (70 μg/ml) was in 50 mM KPO_4 buffer, pH 6.8.

The fluorescence emission spectrum of the reporter group of the protein showed a 14% enhancement with essentially no shift in wavelength, upon binding of L-histidine at saturating levels (Fig. 2). By comparison with model systems (Hartig et al., 1977*a*), one possible interpretation is that the fluorescein is shifted to a more hydrophobic environment.

Effect of Histidine on the Tryptophan and Reporter Group Fluorescence of the Mutant J Protein

The steady-state fluorescence emission spectrum (λ_{exc} = 280 nm) of the mutant J-protein *hisJ5625* is shown in Fig. 3 *a*. This spectrum exhibits two peaks at 317 and 332 nm and is thus different from that of the native protein (Fig. 1 *a*). Excitation of a solution of the mutant protein by light at 296 nM (a wavelength at which tryptophan, but not tyrosine, is excited) (Eisinger, 1969) results in a single peak at 332 nm (Fig. 3 *b*). Separate control experiments in which the wavelength of excitation was varied from 270 to 300 nm showed that the peaks observed in these spectra do not correspond to Raman bands. Thus, the 317 nm and 332 nm peaks can most probably be assigned to tyrosine and tryptophan fluorescence, respectively. There are eight tyrosine residues in the mutant J-protein (Noel et al., 1979; Hogg, 1981). Addition of saturating L-histidine caused a 57% enhancement of the tryptophan emission at 332 nm, with essentially no change in the wavelength maximum (Fig. 3 *b*). Thus, the change in the tryptophan fluorescence is qualitatively similar to, but of approximately twofold greater magnitude, than that observed for the native J-protein.

To determine whether the mutant J-protein also undergoes a ligand-induced conformational change, the dye 5-IAF was also experimentally attached to the purified protein. Modification proceeded to a fixed value of 1.1 dye/mol protein. Amino acid analyses again revealed that a single methionine residue had been labeled (Table I). The reporter-modified protein retained full histidine-binding activity.

The effect of L-histidine on the reporter group fluorescence of the 5-AF-mutant J-protein was examined (Fig. 4). Binding of histidine (10^{-4} M) resulted in a 32% enhancement of the fluorescein fluorescence, with no detectable shift in wavelength maximum. Again, by comparison with model systems (Hartig et al., 1977*a*), one possible interpretation is that the fluorescein is shifted to a more hydrophobic environment; the extent of enhancement was again approximately twofold greater than that for the native protein.

Distance between the Reporter Groups

To test the hypothesis that the wild-type and mutant J-protein undergo markedly different conformational changes upon histidine binding, fluorescence energy transfer was used as a measure of interatomic distances. In the case of each protein, the distance from the single tryptophan residue to the experimentally attached fluorescein was calculated using the theory of Förster (1966). The Förster equation (Eq. 2) relates the distance, R, between two fluorophores that undergo excitation transfer to the transfer efficiency E.

$$\frac{9\ (\ln 10)\ Q_D K^2}{128\ \pi^5 N n^4 \tau_D R^6} J_{\tilde{\nu}} = E. \qquad (2)$$

$J_{\tilde{\nu}}$ is the spectral overlap integral between the corrected donor emission and the acceptor absorption, Q_D is the quantum yield of the donor in the absence of the acceptor, n is the index of refraction of the intervening medium, and K^2 is the orientation factor of the donor and acceptor

TABLE I
AMINO-ACID ANALYSES OF THE WILD-TYPE AND MUTANT HISTIDINE J-PROTEINS OF *S. TYPHIMURIUM* AND THE CORRESPONDING ACETAMIDOFLUORESCEIN-LABELED ANALOGUES

Amino acid	Wild-type J-protein		5-AF-Wild-type J-protein	Mutant J-protein		5-AF Mutant J-protein
	Determined by amino acid analysis	Determined by sequencing*	Determined by amino acid analysis	Determined by amino acid analysis	Determined by sequencing	Determined by amino acid analysis
Lysine	21.6	24	22.1	21.6	24	20.0
Histidine	1.4	1	0.7	1.3	1	0.8
Arginine	8.4	9	8.5	7.9	8	6.7
Aspartate	24	24	24	27	24	24.0
Cys/2	—§	1	—§	—§	3	—§
Methionine	3.3 ± 0.15	3	2.46 ± 0.08	3.05 ± 0.57	3	1.9
Methionine sulfone‡	2.8		2.45 ± 0.15			

*From Hogg (1981).
‡Value reported is for the performic acid-treated protein.
§Not determined.

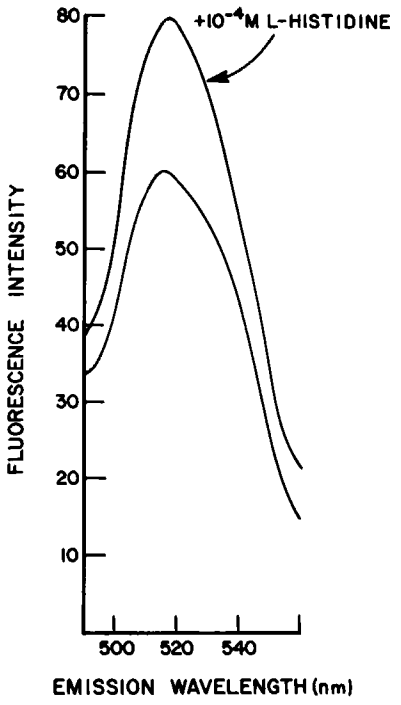

FIGURE 4 Fluorescence emission spectra (uncorrected) of the 5-AF-labeled-mutant J-protein in the presence and absence of 10^{-4} M L-histidine. Spectra were recorded at ambient temperature with an excitation wavelength of 460 nm. The 5-AF-mutant protein (70 μg/ml) was in 50 mM KPO_4 buffer, pH 6.8.

transition moments (Forster, 1966). This theory has been tested in well-defined model systems (Latt et al., 1965; Stryer and Haughland, 1967) and has been used to deduce distance in various biological systems.

The measured polarizations for tryptophan in the modified native and mutant J-protein are 0.21 and 0.27, respectively (Table II). P_0 for immobilized tryptophan is 0.3 (Weber, 1966). Thus, the tryptophan of the J-proteins exhibits some freedom of motion. The measured polarization values for 5-AF bound to the native and to the mutant J-protein are 0.06 and 0.04, respectively; these values are close to the polarization value observed for the free dye in water, 0.02 (Hartig et al., 1977*a*). The theoretical value of fluorescence polarization for this dye randomly immobilized on a protein of $M_r \approx 30$ kD with a 2:1 axial ratio for a prolate ellipsoid (in analogy to other periplasmic binding proteins characterized thus far [Pardee, 1966; Quiocho et al., 1977; Hartig et al., 1977*b*]) is 0.34 (Zukin et al., 1977*a*). Thus, the reporter group has considerable independent freedom of motion. Therefore, a K^2 value of 2/3 was used in all distance calculations.

The energy transfer efficiencies from the donor tryptophan to the acceptor 5-AF were determined from the corrected excitation spectra of 5-AF bound to each protein. These were 15% for the wild-type protein and 5% for the mutant protein. Using the Förster equation (Eq. 2), and assuming the usual values for the other terms (Wu and Stryer, 1972), a measured quantum yield of 0.73, an efficiency of 15%, and a K^2 of 2/3 give an interatomic distance of 44 ± 4 Å for the fluorophores in the wild-type J-protein. In the presence of L-histidine (10^{-5} M), the efficiency decreases to 5%, and the distance between the dyes lengthens to 60 ± 6 Å. In the case of the mutationally altered receptor, using a quantum yield of 0.75, a distance of 60 ± 6Å is calculated for the fluorophores in the absence of ligand and 70 ± 7 Å, in the presence of ligand. Together, these data indicate that the wild-type and mutant J-proteins have significantly different three-dimensional structures both in the ligand-bound and ligand-free states.

Histidine Effects on Tryptophan and Fluorescein Polarization

Table II summarizes the effects of ligand binding on fluorophore mobilities in the two proteins. Addition of L-histidine (10^{-5} M) to the wild-type J-protein resulted in a modest (24%) decrease in the observed polarization of the tryptophan residue and a modest (17%) increase in that for the attached acetamidofluorescein. These data indicate that binding of ligand to the normal receptor causes an increase in molecular freedom for the tryptophan (17% decrease in P), but no significant change in that for the fluorescein label.

DISCUSSION

The present study provides evidence from steady-state fluorescence measurements, fluorescence energy transfer, and polarization measurements that the native histidine J-protein of *S. typhimurium* undergoes a ligand-induced conformational change upon binding of L-histidine. By fluorescence energy transfer measurements, we have determined that the single tryptophan residue and the single acetamidofluorescein moiety of the 5-IAF-modified J-protein are 44 ± 4 Å apart. Since (*a*) the histidine molecule is only 10 Å in length, (*b*) only one molecule of ligand binds per molecule of receptor, and (*c*) its binding perturbs the microenvironments of both the tryptophan and fluorescein fluorophores, it is clear that the changes introduced at a minimum of one of these sites are the result of an indirect

TABLE II
POLARIZATION OF FLUORESCENCE VALUES TRYPTOPHAN AND 5-ACETAMIDOFLUORESCEIN IN THE MODIFIED WILD-TYPE AND MUTANT HISTIDINE J-PROTEINS

	Tryptophan*	5-AF‡
5-AF – J-protein	0.21	0.06
5-AF – J-protein and 10^{-5}M L-his	0.16	0.07
% change	−24%	+17%
5-AF – mutant J-protein	0.27	0.04
5-AF – mutant J-protein and 10^{-5}M L-his	0.23	0.04
% change	−17%	0%

*Excitation wavelength, 290 nm; emission wavelength, 330 nm.
‡Excitation wavelength, 490 nm; emission wavelength, 520 nm.

effect. Hence, there must be a ligand-induced conformational change that is propagated a minimum of 30 Å through the receptor molecule.

Several findings of this study independently corroborate the occurrence of a ligand-induced conformational change in the J-protein. First, L-histidine binding to the receptor results in a significant increase in the distance between the fluorophores. Secondly, labeling of a methionine residue in the J-protein with the large, bulky fluorescein molecule results in no detectable loss of binding activity. If ligand binding involved direct participation of this residue, a substantial loss of activity would be expected upon its chemical modification. Thirdly, addition of L-histidine results in an increase in tryptophan mobility. Direct interaction of ligand with this residue would be expected to decrease its molecular freedom. Since we began writing this paper, another report (Post et al., 1984) has been published concerning spectroscopic studies of the J-protein. That study found no change in the intrinsic protein fluorescence spectrum upon addition of substrate. However, their earlier study (Robertson et al., 1977) does show spectra which are in agreement with our results, that the intrinsic fluorescence of the J-protein increases in the presence of L-histidine. It is possible that the difference between the study by Post et al. (1984) and the present study is due to (*a*) use of a different strain and (*b*) a rather different sensitivity setting giving rise to considerable noise in their spectrum.

The present study also provides evidence that the wild-type and mutant J-proteins differ in their three-dimensional structures. For example, the intrinsic protein fluorescence emission spectra of the two proteins are markedly different. In the case where the excitation wavelength was 280 nm, the mutant protein exhibited a biphasic fluorescence emission apparently assignable to tryptophan and tyrosine fluorescence as discussed above. This result is in contrast to that observed for the normal J-protein which exhibits a monophasic spectrum characteristic of tryptophan fluorescence. In addition, the inter-fluorophore distance is 44 ± 4 Å in the native J-protein and 60 ± 6 Å in the mutant protein in the absence of 10^{-5} M L-histidine. Polarization measurements indicate that the wild-type J tryptophan has more mobility than does the mutant protein tryptophan, whereas 5-AF has less mobility in the wild-type than in the mutant protein.

Finally, this study demonstrates that the two proteins undergo conformational changes that differ qualitatively and quantitatively. Hence, the enhancement of tryptophan fluorescence upon ligand binding is greater for the mutant than for the wild-type J-protein. This finding most likely indicates that when histidine binds to the mutant protein, this tryptophan is shifted farther away from the hydrophobic core of the protein than is the tryptophan of the wild-type protein. In contrast, the distance between tryptophan and the attached fluorescein changed relatively more in the case of the wild-type J-protein than for the mutant protein. In the case of fluorescence polarization determinations, the changes in mobilities of the two chromophores differed in magnitude and direction.

The distant reporter group method for estimating molecular distance utilizing either steady-state fluorescence or excited-state fluorescence lifetimes requires placing a chromophore at a position too distant to allow direct interactions with the ligand. Any combination of intrinsic or experimentally attached fluorophores can serve equally well. The approach requires only that a minimum distance be established between a pair of fluorophores and is therefore less demanding than mapping positions on protein surfaces by fluorescence energy transfer measurements. If that minimum is far greater than the size of the ligand (in this case, 43 or 60 Å vs. 10 Å), the chance for an erroneous conclusion is very remote.

The above distance calculation makes a number of assumptions, many of which are based on experimental facts and have been summarized previously (Zukin et al., 1977*a*). It has been assumed that tryptophan and fluorescein each has considerable independent freedom of motion. This assumption is based on several observations. First, polarization measurements for the protein-bound fluorescein residues are close to the values for the corresponding free molecules in solution. Secondly, more detailed determinations of K^2 for this pair have been made in several analogous protein systems (Zukin et al., 1977; Zukin, 1979; and Zukin et al., 1979); in each case the approximation of $K^2 = 2/3$ has been shown to be a good one. In general, the parameters used to calculate R_0 enter into the distance calculation only by their sixth root, so that uncertainties in these have little effect on the distance calculation. In particular, there is little uncertainty in the donor quantum yield or the spectral overlap integral (Wu and Stryer, 1972) as these are based on direct measurements. Thus, the various potential errors in estimation of the interdye distances will not reduce the minimum values by >10%.

Genetic studies of the histidine transport operon have suggested that the P, Q, and M proteins exist in a multicomponent complex in the cytoplasmic membrane, and the the soluble J-protein serves as the primary receptor in histidine transport, directly interacting with membrane-bound P-protein (Ames and Spudich, 1976; Ames and Nikaido, 1978). The same three membrane components, P, Q, and M, function in the arginine transport system, but the lysine-arginine-ornithine binding protein (LAO) (Kustu et al., 1979) replaces the J-protein as the periplasmic component. The LAO and J-proteins have been shown to share substantial regions of sequence homology and are thought to compete for the same site on the P-protein during their respective transport events (Higgins and Ames, 1982). Thus, the finding of a ligand-induced conformational change in the J-protein is of particular interest because such a mechanism could result in the creation of a combining site on J enabling it to bind to the P-protein.

Several other bacterial receptor systems have been found to undergo analogous mechanisms (Zukin et al., 1977 and 1979; Zukin, 1979). The mutant J-protein *hisJ5625* binds L-histidine normally, but has an altered J/P interaction. Thus, it has provided an ideal system in which to test the physiological significance of the ligand-induced conformational change in the wild-type protein. The present study has shown that the mutant J-protein undergoes a molecular rearrangement that differs qualitatively and quantitatively from that of the wild-type protein. Thus, the specific conformational change induced in the wild-type J-protein appears to be a necessary requirement for the transport of L-histidine.

We thank Dr. Giovanna Ferro-Luzzi Ames (Department of Biochemistry, University of California at Berkeley for the purified samples of wild-type *S. typhimurium* J-protein and the mutant *hisJ5625* protein and for helpful discussions. We also wish to thank Dr. Howard M. Steinman (Department of Biochemistry, Albert Einstein College of Medicine) for the amino acid analyses. M. F. Klos was an undergraduate biology major at the College of Mount Saint Vincent under the direction of Dr. R. E. Hirsch (who at the time was a faculty member of the Department of Biology, Manhattan College/College of Mount Saint Vincent as well as a faculty member of the Department of Medicine, Albert Einstein College of Medicine), when these experiments were performed.

This work was supported in part by National Science Foundation grant no. BNS-8308634 (to R. S. Zukin), Cottrell College Science Grant (Research Corp.) no. C 1281 (to R. E. Hirsch), National Institutes of Health grant no. HL-21026 (to R. E. Hirsch), J. Fred Weintz Investigatorship (New York Heart Association) (to R. E. Hirsch).

Received for publication 15 July 1985 and in final form 25 November 1985.

REFERENCES

Ames, G. F.-L., and J. E. Lever. 1970. Components of histidine transport: histidine binding proteins and his P protein. *Proc. Natl. Acad. Sci. USA.* 66:1096–1103.

Ames, G. F.-L., and J. E. Lever. 1972. The histidine-binding protein J is a component of histidine transport. *J. Biol. Chem.* 247:4309–4316.

Ames, G. F.-L., and K. Nikaido. 1978. Identification of a membrane protein as histidine transport component in *S. typhimurium. Proc. Natl. Acad. Sci. USA.* 75:5447–5451.

Ames, G. F.-L., and E. Spudich. 1976. Protein-protein interaction in transport: periplasmic histidine-binding protein J interacts with P protein. *Proc. Natl. Acad. Sci. USA.* 73:1877–1881.

Burstein, E. A., N. S. Vadenkima, and M. N. Ivkova. 1973. Fluorescence and the location of tryptophan residues in protein molecules. *Photochem. Photobiol.* 18:263–279.

Eisinger, J. 1969. Intramolecular energy transfer in adrenocorticotropin. *Biochemistry.* 8:3902–3908.

Foster, T. 1966. *In* Modern Quantum Chemistry, Instanbul Lectures. O. Sinanoglu, edtior. Section III-B, Garcia Academic Press, Inc., New York. 93–137.

Hartig, P. R., N. Bertrand, and K. Sauer. 1977*a*. 5-Iodoacetamidofluorescein-labeled chloroplast coupling factor 1: conformational dynamics and labeling-site characterization. *Biochemistry.* 16:4275–4285.

Hartig, P. R., R. S. Zukin, and D. E. Koshland, Jr. 1977*b*. CD and fluorescence evidence for conformational changes in galactose and ribose receptors of *S. typhimurium. Biophys. J.* 17:204–208.

Higgins, C. F., and G. F.-L. Ames. 1982. Two periplasmic transport proteins which interact with a common membrane receptor show extensive homology: complete nucleotide sequences. *Proc. Natl. Acad. Sci. USA.* 78:6038–6042.

Higgins, C. F., P. D. Haag, K. Nikaido, F. Ardeshir, G. Garcia, and G. F.-L. Ames. 1982. Complete nucleotide sequence and identification of membrane components of the histidine transport operon of *S. typhimurium. Nature* (*Lond.*). 298:723–727.

Hogg, R. 1981. The amino acid sequence of the histidine binding protein of *Salmonella typhimurium. J. Biol. Chem.* 256:1935–1939.

Konev, S. 1967. Fluorescence and phosphorescence of proteins and nucleic acids. Plenum Publishing Corp., New York.

Kustu, S. G., and G. F.-L. Ames. 1974. The histidine binding protein J, a histidine transport component, has two different functional sites. *J. Biol. Chem.* 249:6976–6983.

Kustu, S. G., N. C. McFarland, S. Hui, B. Esmond, and G. F.-L. Ames. 1979. Nitrogen control is *S. typhimurium*: co-regulation of synthesis of glutamine synthetase and amino acid transport systems. *J. Bacteriol.* 138:218–234.

Latt, S. A., H. T. Cheung, and E. R. Blout. 1965. Energy transfer. A system with relatively fixed donor acceptor separation. *J. Am. Chem. Soc.* 87:995–1003.

Lever, J. E. 1972. Quantitative assay of the binding of small molecules to protein: comparison of dialysis and membrane filter assays. *Anal. Biochem.* 56:73–83.

Lowry, O. H., A. L. Rosebrough, A. L. Far, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193:265–275.

Noel, D., K. Nikaido, and G. F.-L. Ames. 1979. A single amino acid substitution in a histidine-transport protein drastically alters its mobility on SDS polyacrylamide gel electrophoresis. *Biochemistry.* 18:4159–4165.

Pardee, A. B. 1966. Purifications and properties of a sulfate-binding protein from *Salmonella typhimurium. J. Biol. Chem.* 241:5886–5892.

Post, J. F. M., P. F. Cottam, V. Simlaceanu, and C. Ho. 1984. Fluorine-19 NMR study of 5-fluorotryptophan-labeled histidine-binding protein-J of *Salmonella typhimurium. J. Mol. Biol.* 179:729–743.

Quiocho, F. A., G. L. Gilliland, and G. N. Phillips, Jr. 1977. The 2.8 Å resolution structure of the L-arabinose-binding protein from *Escherichia coli. J. Biol. Chem.* 252:5142–5149.

Robertson, D. E., P. A. Kroon, and C. Ho. 1977. NMR and fluorescence studies of substrate induced conformational changes of histidine-binding protein J of *Salmonella typhimurium. Biochemistry.* 16:1443–1450.

Stryer, L., and R. P. Haughland. 1967. Energy transfer: a spectroscopic ruler. *Proc. Natl. Acad. Sci. USA.* 58:719–726.

Takahashi, S., R. S. Zukin, and H. M. Steinman. 1981. γ-Glutamyl transpeptidase from WI-38 fibroblasts: purification and active site modification studies. *Arch. Biochem. Biophys.* 207:87–95.

Teale, F. W. J., and G. Weber. 1957. Ultraviolet fluorescence of the aromatic amino acids. *Biochem. J.* 65:476–482.

Weber, G. 1966. *In* Fluorescence and Phosphorescence Analysis. D. M. Hercules. Wiley Interscience Press, New York. 217–240.

Wu, C. W., and L. Stryer. 1972. Proximity relationships is rhodopsin. *Proc. Natl. Acad. Sci. USA.* 64:1104–1108.

Zukin, R. S. 1979. Evidence for a conformational change in *Escherichia coli* maltose receptor by excited-state fluorescence lifetime data. *Biochemistry.* 18:2139–2145.

Zukin, R. S., P. R. Hartig, and D. E. Koshland, Jr. 1977. Use of a distant reporter group as evidence for a conformational change on a sensory receptor. *Proc. Natl. Acad. Sci. USA.* 74:1932–1936.

Zukin, R. S., P. R. Hartig, and D. E. Koshland, Jr. 1979. Effect of an induced conformational change on the physical properties of two receptor molecules. *Biochemistry.* 18:5599–5605.