University of Groningen

Escherichia coli Phosphoenolpyruvate Dependent Phosphotransferase System. NMR Studies of the Conformation of HPr and P-HPr and the Mechanism of Energy Coupling

Dooijewaard, G.; Roossien, F.F.; Robillard, G.T.

Published in:
Biochemistry

DOI:
[10.1021/bi00581a014](https://doi.org/10.1021/bi00581a014)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1979

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Dooijewaard, G., Roossien, F. F., & Robillard, G. T. (1979). Escherichia coli Phosphoenolpyruvate Dependent Phosphotransferase System. NMR Studies of the Conformation of HPr and P-HPr and the Mechanism of Energy Coupling. *Biochemistry*, 18(14), 2996-2999. <https://doi.org/10.1021/bi00581a014>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

The polysaccharide could also originate in the membrane where it may have a specific role in the binding of P-HPr to membrane-bound E_{II} . This would correlate with its apparent activation effect which we find in the phosphotransferase activity measurements. (3) If one assumes that the α 1-6 glucan is a form of sugar storage not previously detected, one could suggest that the polysaccharide may function as a mechanism for controlling the activity of the PTS. By considering that the PTS appears to be involved in regulating a number of other systems (adenylate cyclase, transport of non-PTS sugars (Saier, 1977)), it is possible that the polysaccharide has a more general role in the control of these processes. (4) The polysaccharide could be an intermediate product of the sugar transport process itself. Its discovery, then, would suggest that the PTS might transport sugar in the form of long polymers. In this respect it is tempting to speculate whether the structure of the polymer, being an α 1-6 helix, has a special function. α 1-6 helices should be much longer than the usual α 1-4 amylose helices which are involved in the storage of sugar. These various possibilities are currently under investigation.

Recent experiments isolating HPr from *Salmonella typhimurium* grown and harvested under a variety of conditions consistently result in an HPr preparation which contains the α 1-6 glucan homopolymer.

Acknowledgments

We wish to gratefully acknowledge the expert technical assistance of R. Ten Hoeve-Duurkens and the generosity of the Gist en Spiritus factory, Delft, The Netherlands, for the large-scale fermentation of *E. coli*. The gas chromatographic and mass spectral analyses have been performed in the organic chemistry department at the University of Utrecht. We are indebted to Drs. J. F. G. Vliegthart and J. P. Kamerling for these analyses. The generosity of Dr. P. Postma in supplying the *Salmonella typhimurium* pts mutants is

gratefully acknowledged. Lastly, we wish to acknowledge Dr. R. Kaptein and K. Dijkstra for their technical assistance with the NMR spectrometer.

References

- Anderson, B., Weigel, N., Kundig, W., & Roseman, S. (1971) *J. Biol. Chem.* **246**, 7023-7033.
- Beyreuther, K., Raufuss, H., Schrecker, O., & Hengstenberg, W. (1977) *Eur. J. Biochem.* **75**, 275-286.
- Cirillo, V. P., & Razin, S. (1973) *J. Bacteriol.* **113**, 212-217.
- Fiske, C. H., & SubbaRow, Y. (1925) *J. Biol. Chem.* **66**, 375-381.
- Greenwood, C. T. (1970) in *The Carbohydrates* (Pigmon, W., & Horton, D., Eds.) Vol. IIB, pp 471-513, Academic Press, New York.
- Jennings, H. J., & Smith, I. C. P. (1973) *J. Am. Chem. Soc.* **95**, 606-608.
- Kundig, W., & Roseman, S. (1971a) *J. Biol. Chem.* **246**, 1393-1406.
- Kundig, W., & Roseman, S. (1971b), *J. Biol. Chem.* **246**, 1407-1418.
- Kundig, W., Kundig, F. D., Anderson, B. F., & Roseman, S. (1966) *J. Biol. Chem.* **241**, 3243-3246.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275.
- Postma, P. W. (1976) *FEBS Lett.* **61**, 49-53.
- Postma, P. W., & Roseman, S. (1976) *Biochim. Biophys. Acta* **457**, 213-257.
- Robillard, G. T., Dooijewaard, G., & Lolkema, J. (1979) *Biochemistry* **18** (first of three papers in this issue).
- Roseman, S. (1969) *J. Gen. Physiol.* **54**, 138s-180s.
- Saier, M. H., Jr. (1977) *Bacteriol. Rev.* **41**, 856-871.
- Sutherland, I. W. (1977) in *Surface Carbohydrates of the Prokaryotic Cell* (Sutherland, I., Ed.) pp 27-96, Academic Press, New York.
- Winzler, R. J. (1955) *Methods Biochem. Anal.* **2**, 279-311.

Escherichia coli Phosphoenolpyruvate Dependent Phosphotransferase System. NMR Studies of the Conformation of HPr and P-HPr and the Mechanism of Energy Coupling[†]

G. Dooijewaard, F. F. Roossien, and G. T. Robillard*

ABSTRACT: ^1H and ^{31}P nuclear magnetic resonance investigations of the phosphoprotein intermediate P-HPr and the parent molecule HPr of the *E. coli* phosphoenolpyruvate dependent phosphotransferase system (PTS) show that HPr can exist in two conformations. These conformations influence

the protonation state of the reactive histidine residue, thereby determining the reaction pathway in the phosphoryl group transfer step. A general mechanism is proposed for the energy-coupling process in the PTS.

The transport of sugars by the bacterial phosphoenolpyruvate dependent phosphotransferase system is coupled to the energy source, phosphoenolpyruvate, via a series of phosphoryl group transfer steps involving phosphoenzyme intermediates (Roseman, 1969; Postma & Roseman, 1976; Hengstenberg,

1977).

[†] From the Department of Physical Chemistry, University of Groningen, Paddepoel Groningen, The Netherlands. Received January 22, 1979. This research and the 360-MHz NMR facility at the University of Groningen have been supported by the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

¹ Abbreviations used: PEP, phosphoenolpyruvate; PTS, phosphoenolpyruvate dependent phosphotransferase system; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate; DTT, dithiothreitol; P (as in P-histidine), phospho.

Depending on the system under investigation, either P-HPr or P-F_{III} serves as the phosphoryl group donor at the membrane-bound E_{II} level.

Since purification procedures for all proteins of the PTS have been developed (Anderson et al., 1971; Kundig & Roseman, 1971; Dooijewaard et al., 1979; Robillard et al., 1979; Hays et al., 1973; Schrecker & Hengstenberg, 1971) and the number of components is limited, we are now in a position to ask what are the molecular details of energy coupling? What mechanism drives the phosphoryl group transfer process? What physical events describe sugar transport?

Nuclear magnetic resonance is capable of probing the structure of macromolecules and furnishing data concerning the environment of various groups, their ionization state, and changes in these parameters. We have applied this technique to the phosphoprotein intermediate, P-HPr, and its parent molecule, HPr. The information obtained has provided a mechanism for the formation and breakdown of the P-HPr intermediate. This mechanism may be generally applicable to all the phosphoprotein intermediates in the energy-coupling process.

Materials and Methods

HPr was purified according to the procedure presented in the previous publication of this series (Dooijewaard et al., 1979).

E_I. At the time that this research was performed, the hydrophobic interaction chromatography procedure for the purification of E_I (Robillard et al., 1979) had not been completed; therefore, the E_I used in this research was partially purified in the following manner. After suspending 100 g of *Escherichia coli* cells (wet weight) in 500 mL of 20 mM K₂HPO₄, 1 mM DTT, 1 mM EDTA, pH 7.6, they were ruptured by passage through a French press at 10 000 psi, 5 °C, and the suspension was centrifuged at 48 000g for 30 min at 5 °C. The supernatant was brought to 0.33% protamine sulfate by dropwise addition of a 2% protamine sulfate stock solution, with stirring. The suspension was stirred for 30 min and then centrifuged at 48 000g for 30 min. The supernatant was brought to 0.27 M KCl, loaded on a DEAE-23 cellulose column (5 × 60 cm), and eluted with a linear gradient of 0.27 M KCl to 0.47 M KCl in 10 mM Tris-HCl buffer containing 1 mM DTT and 1 mM EDTA, pH 7.6. The total volume of the gradient was 6 L. The peak of E_I activity was pooled, concentrated on an Amicon ultrafiltration apparatus equipped with a UM 20 membrane, and lyophilized.

Phosphoenolpyruvate (potassium salt) was purchased from Sigma. All other chemicals were reagent grade.

NMR spectra were recorded at 360 MHz for proton and 145.7 MHz for phosphorus on a Bruker HX 360 spectrometer operating in either the Fourier or correlation mode. The temperature of the sample was regulated by a constant temperature accessory to ±1 °C. The intensity of the HOD resonance was decreased during data acquisition in the Fourier mode by selective saturation at the HOD frequency. All proton spectra were recorded at 37 °C. The phosphorus spectra were recorded at 25 °C. The stated pH values of the samples are the values measured with a combination glass electrode. They are not corrected for the presence of D₂O. The chemical shift parameters are reported relative to DSS for proton and concentrated H₃PO₄ for the phosphorus NMR spectra. Proton spectra were recorded in 5-mm cylindrical microcells (Wilmaad). Phosphorus spectra were recorded by using 10-mm tubes with a microcell insert (Wilmaad).

FIGURE 1: ¹H NMR spectra of the aromatic absorption region of *E. coli* HPr in 50 mM sodium phosphate buffer.

FIGURE 2: (1) pH 6 spectrum and (2) pH 5 spectrum from Figure 1. [(1)-(2)] Computer subtraction of spectrum 2 from spectrum 1.

Results

¹H NMR Studies on the Histidine Residues of Native HPr.

Figure 1 shows the aromatic absorption region of the ¹H NMR spectrum of HPr recorded at several pH values. The two lowest field resonances shifting belong to the C-2 protons of the imidazole rings of the two histidines which this protein contains. In addition, there are two resonances between -6.8 and -7.5 ppm which also shift upfield with increasing pH; they belong to the C-4 protons of the two histidines. The remainder of the resonances arise from the protons of the aromatic amino acids (unpublished data), some of which will be discussed in more detail later. Since the C-4 proton resonances overlap with resonances from aromatic protons, the pH-dependent chemical shifts were clarified by difference spectra as shown in Figure 2. The pH-dependent titration curves, derived in this manner for the C-2 and C-4 protons, are presented in Figure 3. The resonances of the imidazole protons titrating with a pK of 5.6 were arbitrarily assigned to His A (the amino acid sequence of *E. coli* HPr is unknown). Those titrating with a pK of 6.0 were assigned to His B. The titration curves drawn through the points are theoretical curves for a single protonation of the imidazole rings with a Hill coefficient of 1.

The most noteworthy feature of the curves in Figure 3 is the abnormally small change in chemical shift which the C-4

FIGURE 3: pH dependence of the histidine resonances in HPr as determined from spectra, some of which are shown in Figure 1. Circles represent the C-2 proton resonances; triangles represent the C-4 proton resonances. Open symbols are assigned to His A, the histidine which becomes phosphorylated. Closed symbols are assigned to His B. The pH was determined as described under Materials and Methods.

FIGURE 4: ^1H NMR spectra of the aromatic proton resonance absorption region of (1) HPr at pH 8.4 in the absence of PEP and E_1 . (2) P-HPr prepared in situ as described under Results. The sample in the NMR tube contained 5 mg/mL HPr, 5 mM MgCl_2 , 5 mM PEP, 50 mM sodium phosphate buffer, and 1 mg/mL protein from a partially purified E_1 preparation (see Materials and Methods). The pH of the sample was 8.4. At 35 $^\circ\text{C}$, all HPr was converted to P-HPr within 5 min. [(1)-(2)] A computer subtraction of spectrum 2 from spectrum 1.

proton resonance of His A experiences upon deprotonation, as contrasted with the normal chemical shift range for the C-2 proton resonance of the same histidine. This will be treated after the following section.

NMR Studies on the Histidine Residues of P-HPr. Since, below pH 8.0, the half-time of the rate of hydrolysis of pure P-HPr, free in solution, becomes equal to or higher than the accumulation time needed for a single NMR spectrum (15 min for ^1H NMR; 30–60 min for ^{31}P NMR), these studies were made feasible by the enzymatic phosphorylation of HPr in situ. Spectra of P-HPr were collected under conditions where the rate of formation of P-HPr was at least ten times higher than its rate of hydrolysis. A time course of the spectra thus obtained (not shown) indicates that 5 mM PEP is sufficient to keep 0.5 mM HPr in the phosphorylated form for at least 2 h. The subsequent addition of 5 mM PEP prolonged the steady-state period of P-HPr for at least another hour, despite the fact that 5 mM pyruvate had already been formed.

FIGURE 5: pH dependence of the chemical shifts of the histidine resonances of P-HPr under conditions listed in the legend to Figure 4. Circles are the C-2 proton resonances; triangles represent the C-4 proton resonances. The open symbols are the resonances assigned to His A, the histidine which is phosphorylated in this sample. The closed symbols are assigned to His B. The dashed lines are taken directly from the titration curves presented in Figure 3.

FIGURE 6: ^{31}P NMR spectra of P-HPr at several pH values. The sample contained 3 mg of HPr in 0.5 mL of 20 mM Tris-HCl buffer at pH 7.8. Phosphorylation was achieved by addition of 25 μL of 0.2 M PEP, 25 μL of 0.1 M MgCl_2 , 25 μL of a partially purified preparation of E_1 for a final protein concentration of 1 mg/mL. (Spectrum A) pH 7.8; (spectrum B) pH 11.8; (spectrum C) pH 12.8; (spectrum D) pH 9.0. The spectra were taken in the sequence A, B, C, and D. Thus, the sample at pH 9 in spectrum D had already been denatured by treatment at pH 12.8. The assignments of the resonances are discussed in the text.

^1H NMR. Figure 4 shows the aromatic absorption region of the NMR spectra of HPr and P-HPr, and their difference spectrum, recorded at pH 8.4. In P-HPr, the C-2 proton of His A is shifted downfield and its C-4 proton upfield, indicating that His A is the residue carrying the phosphoryl group. Further, there is a small shift of two Phe protons (peak X) from -7.24 to -7.28 ppm; the remainder of the resonances is essentially unaffected. The same type of titration studies, as described above for HPr, was carried out with P-HPr. Figure 5 presents the titration curves. For comparison, the curves obtained with HPr in Figure 3 are also drawn. The pK value of His A increases from 5.6 to 7.8 upon phosphorylation, while that of His B is not affected ($\text{pK} = 6.0$). Data below pH 6.0 could not be collected since the rate of hydrolysis of P-HPr was too high even though P-HPr was generated, in situ, as described above.

^{31}P NMR. Figure 6 shows ^{31}P NMR spectra of P-HPr, recorded at several pH values in the presence of excess PEP and catalytic amounts of E_1 . The resonance at low field

FIGURE 7: pH dependence of the resonances in the ^{31}P NMR spectra of P-HPr. (Open circles) Inorganic phosphate; (open triangles) PEP; (closed circles) P-HPr. The dashed line in the P-HPr titration curve represents the chemical shift position of the resonance once the sample has been raised to pH 12.8, a spectrum collected, and then the pH lowered to pH 11 and subsequently to pH 9 for additional spectra.

belongs to inorganic phosphate, a product of the hydrolysis of P-HPr, and that at 0.6 ppm to PEP. The upfield resonance was assigned to P-His A of P-HPr since, in time-dependent studies (not shown), the signal appeared after the addition of PEP and disappeared by the time all the PEP had been consumed. The titration curves of inorganic phosphate, PEP, and P-HPr obtained from these measurements are presented in Figure 7. The titration of P-HPr with a pK of 7.8 reflects the deprotonation of the phosphorylated imidazole ring of His A, consistent with the pK measured in Figure 5 by proton NMR. The curves appropriate to inorganic phosphate and PEP agree with published data. Above pH 11.8, P-HPr was irreversibly denatured. This process was accompanied by a shift from 3.22 to 5.4 ppm in the ^{31}P NMR spectrum (Figure 6, spectra C and D) as well as major changes in the ^1H NMR spectrum (not shown). Subsequent decreases of the pH to 9.0 did not restore the original spectrum.

Structural Changes Reported by Aromatic and Aliphatic Proton Resonances. In Figure 2 we see that the C-4 proton resonance of His A is the only histidine resonance occurring in the midst of the bulk of the aromatic resonances. However, the changes in the aromatic region which occur as a function of pH are rather large (see Figures 1 and 2). They do not correlate with only the shift of the histidine C-4 proton. If one compares the intensities of the positive and negative peaks at approximately -7.35 ppm in the difference spectrum, Figure 2, relative to the other peaks in the spectrum, it is apparent that more than one proton at this resonance position must be shifting. These data suggest that one or more protons of a phenylalanine ring is responding to the protonation state of the molecule. In addition, the difference spectrum of HPr minus P-HPr, Figure 4, indicates that an aromatic proton resonance (peak X in the difference spectrum) also senses the phosphorylation of the molecule. Peak X, at -7.24 ppm in native HPr, shifts downfield 15 Hz in the phosphorylated species.

The changes in HPr structure during pH titration or phosphorylation, as reported by the resonances of aromatic protons, are confirmed by shifting resonances from aliphatic protons in the region between 0 and -1.5 ppm. Figure 8 presents the high-field region of resolution enhanced proton NMR spectra of HPr and P-HPr. A considerable number of resonances in the region 0 to -1.5 ppm shift in the HPr spectra recorded at different pH values. Many of the shifting peaks overlap and, even with resolution enhancement, it is impossible to follow the titration of all resonances. Three resonances marked by dotted lines are easy to see at all pH values. We draw attention to them because they are sensitive to the

FIGURE 8: Resolution enhanced proton NMR spectra of the aliphatic proton resonance region of HPr and P-HPr. P-HPr was prepared by using the sample which generated the pH 8.1 HPr spectrum, with the subsequent addition of Mg^{2+} , PEP, and E_1 as described in the text.

FIGURE 9: Plot of the chemical shifts vs. pH for the resonances marked with dashed lines in Figure 8.

difference in structure between HPr and P-HPr. As shown in Figure 9, these three peaks shift with an apparent transition midpoint between pH 5.4 and 5.6. When Mg^{2+} , PEP, and E_1 are added to the pH 8.1 HPr sample, P-HPr is formed and these three resonances shift back toward their position in protonated HPr. P-HPr spectra at different pH values (data not shown) show that these resonances now titrate with a transition midpoint of pH 7.6–7.8. In addition, the extent of the shift for each resonance is only 80–90% of the shift observed in HPr. The changes observed in the conversion of HPr to P-HPr are all reversible. The interpretation of these data will be presented below.

Discussion

Evaluation of Structural Data. When a histidine ring is protonated, its positive charge is delocalized over the ring through resonance stabilization. Consequently, the electron density at the C-2 and C-4 atoms is lowered and their proton resonances shift to lower fields. The effect on the C-2 proton resonance is, generally, larger because of its position between the two nitrogen atoms. Structural information can be extracted from the chemical shift and pK values of the histidines

Table I^a

	chemical shifts				ref
	C-2 protons		C-4 protons		
	δ^+	δ^0	δ^+	δ^0	
His	-8.61	-7.66	-7.37	-6.93	<i>b</i>
1-P-His	-8.46	-7.71	-7.30	-6.83	<i>b</i>
3-P-His	-8.46	-7.78	-7.30	-7.04	<i>b</i>
His A (HPr)	-8.62	-7.88	-7.43	-7.33	
P-His A (P-HPr)	-8.69	-8.00	-7.38	-7.03	
His B ((P)HPr)	-8.78	-7.88	-7.10	-6.72	

^a δ^+ and δ^0 represent the chemical shifts in the protonated and neutral species. ^b Gassner et al. (1977).

Table II

	pK	ref
imidazole	7.0	<i>a</i>
P-imidazole	7.0	<i>a</i>
His	6.0	<i>b</i>
3-P-His	6.4	<i>c</i>
1-P-His	7.0	<i>b</i>
α -N-acetyl-His	6.9	<i>c</i>
His A (HPr)	5.6	
P-His A (P-HPr)	7.8	
His B ((P)HPr)	6.0	

^a Jencks & Gilchrist (1965). ^b Gassner et al. (1977).

^c Hullquist et al. (1966).

under certain circumstances. The chemical shift values for His A and His B of HPr and P-HPr are listed in Table I together with similar parameters published for some model compounds. The pK values are listed in Table II. The chemical shift values of the protonated and neutral forms, δ^+ and δ^0 , respectively, of His B both in HPr and P-HPr (the histidine which does not become phosphorylated) are shifted 0.2 ppm downfield for the C-2 resonance and 0.2 ppm upfield for the C-4 resonance compared with the resonance positions of the free amino acids. The titration range, $\delta^+ - \delta^0$, for both the C-2 and C-4 proton resonances of His B, 0.9 and 0.4 ppm, respectively, is similar to that of free histidine.

The one striking feature of His A, the histidine which becomes phosphorylated, is the abnormal chemical shift of -7.33 ppm for δ^0 of the C-4 proton resonance in HPr and, in conjunction with this, the very small chemical shift titration range of this resonance. The $\delta^+ - \delta^0$ value of only 0.1 ppm instead of 0.4 ppm suggests that, on deprotonation of His A, the normal change in chemical shift position of approximately 0.4 ppm which should occur is negated by another local effect in the protein occurring in the same pH range. For instance, if a phenylalanine were to move toward the histidine ring, its ring current could deshield the C-4 proton, depending on the relative orientation of the two rings, and generate a downfield shift partially compensating the normal upfield shift which occurs on deprotonation. Since the difference spectrum in Figure 2 shows that aromatic protons change their chemical shift position in this pH range, the involvement of an aromatic amino acid in a local conformational change is reasonable. In P-HPr, the C-4 proton of His A titrates normally over the entire pH range. The presence of the phosphoryl group appears to prevent the pH-dependent conformational change found in HPr. This could be caused by a steric effect, the phosphoryl group blocking the movement of the aromatic ring, or it could arise as a result of a conformational change which occurred in the process of phosphorylating HPr itself.

Table II shows that phosphorylation of the imidazole ring as such does not change its pK value. The values for imidazole

and P-imidazole are equal to 7.0 and those of histidine and 3-P-histidine are equal to about 6. The difference in pK value between imidazole and histidine is caused by the repulsion of the proton at the N-1 ring atom exerted by the positive charge of the protonated amino group of histidine (Hultquist et al., 1966; Jencks & Gilchrist, 1965). In keeping with this explanation, it has disappeared in α -N-acetylhistidine (pK = 6.9). The negatively charged phosphoryl group in 1-P-histidine also restores the pK value of the imidazole ring to 7, probably because it is close enough to the protonated amino group to compensate for its repulsion. Thus, the pK values of histidine, 1-P-histidine, and 3-P-histidine are expected to be about 7 in a random coil protein. In a folded protein, however, their pK values may differ appreciably as a result of electrostatic forces exerted by charged groups on nearby residues or as a result of changes in the polarity of the environment. As shown in Figure 5 and Table II, phosphorylation of His A results in a shift in pK of 2.2 pH units. Since phosphorylation of the imidazole ring, as such, should not change its pK, the different pK of His A in HPr (5.6) and P-HPr (7.8) must arise from different local conformations around His A in the two proteins. Again an aromatic amino acid reports this conformational change as may be concluded from the downfield shifting phenylalanine, Figure 4, upon phosphorylation of His A.

The shift of the aliphatic resonances in response to the state of protonation and phosphorylation of HPr (see Figures 8 and 9) may be reporting the charge state of His A, by assuming the protons concerned are in the direct vicinity of this histidine. On the other hand, if these protons are more distant from His A, their resonances are probably reporting a conformational change which occurs as a result of (de)protonation of (P) His A.

Implication of the Different Conformations for the Mechanism of Phosphorylation. On the basis of the results presented in this paper, HPr exists in a minimum of two conformations. Since the changes observed are limited [(i) titration behavior of the His A proton; (ii) shift of the aromatic proton resonances; (iii) shifting methyl resonances], we suggest that the conformational changes are restricted to a small area of the protein. In fact, it is sufficient to assume two different local conformations around His A, one for the deprotonated His A in HPr (conformation I) and one for protonated P-His A in P-HPr (conformation II). Conformation II equals, more or less, the conformation around the protonated His A in HPr. At physiological pH, HPr will be in conformation I with its active His A deprotonated (pK = 5.6) and P-HPr in conformation II, with P-His A protonated (pK = 7.8). Conformation I makes the deprotonated His A, with its free electron pair on the imidazole nitrogen, an excellent nucleophile for the attack on the phosphoryl group of P-E_I. In conformation II, the protonated imidazole ring of P-His A becomes an attractive leaving group, a requirement for transfer of the phosphoryl group to E_{II} or F_{III}. Owing to this conformation change in the protein, both reactions are favored at physiological pH as shown in Scheme I.

Comparison of E. coli HPr with S. aureus HPr. A study similar to that described in this paper was carried out by Gassner et al. (1977) for HPr from *Staphylococcus aureus*. Since the latter protein differs from the *E. coli* HPr in molecular weight (7700) and amino acid composition (among other changes, it contains only one histidine and mainly tyrosine instead of phenylalanine), it is interesting to compare the structural data found for both proteins. The only histidine in *S. aureus* HPr obviously fulfills the same function as His A in the *E. coli* HPr, since it is phosphorylated by PE_I from

Scheme I

S. aureus. Its pK changes from 6.0 in HPr to 8.3 in P-HPr, indicating a similar function for the pK alteration in both proteins. Moreover, evidence was found (Maurer et al., 1977) for a conformational change in *S. aureus* HPr on deprotonation of its histidine, since a simultaneous shift in the position of the aromatic proton resonances of a tyrosine was detected. The most striking similarity between the two proteins, however, is the abnormally small shift which the C-4 proton resonances of the reactive histidines in both HPr's experience upon titration. As in the case of the *E. coli* HPr, after phosphorylation occurs, the C-4 proton resonance in *S. aureus* P-HPr assumes a normal titration behavior.

Despite these similarities, the two HPr's do not substitute for each other in heterologous systems (Hengstenberg, 1977). This should not be surprising in light of the different amino acid compositions of the two proteins, but it is also possible that the larger HPr from *E. coli* (9500 daltons) fulfills a special function missing in *S. aureus* HPr.

One possible reason for the inability of the two proteins to substitute for one another is simply that they might carry their phosphoryl groups on different positions of their respective imidazole rings. Our ^{31}P NMR measurements show, however, that the phosphohistidine resonance of both *E. coli* and *S. aureus* P-HPr, when denatured at high pH, have the same chemical shift of approximately 5.4 ppm indicating that they, most likely, carry the phosphoryl group on the same position of the imidazole ring. On the basis of the ^{31}P NMR model compound studies of Gassner et al. (1977), one could conclude that the imidazole is phosphorylated at the N-1 nitrogen.

Gassner et al. (1977) have also prepared P-HPr chemically by reacting HPr and phosphoamidate. They found a pK of 6.9 for the phosphohistidine of P-HPr (chemical) vs. a pK of 8.3 for the phosphohistidine of P-HPr (enzymatic). They conclude, from this difference in pK , that the chemically prepared intermediate is phosphorylated at the N-3 position vs. the N-1 position in the enzymatically prepared intermediate. This is the reason which they also give for the inability of P-HPr (chemical) to function in their assay system. Their interpretation is questionable, however, in light of the analysis of Jencks & Gilchrist (1965) showing that phosphorylation does not change the pK of the imidazole ring. The pK changes observed in phosphohistidine model compounds are due to interactions between the phosphate and free ionized α -amino group (Hultquist et al., 1966). These should be much smaller or nonexistent in peptides where the free α -amino group is missing. The low pK of the histidine in P-HPr (chemical) could be a signal that the conformational change reported

Scheme II

above for the enzymatically phosphorylated protein failed to occur in the chemically phosphorylated species due to the incorrect positioning of the phosphoryl group. The absence of the conformational change and the corresponding low pK of the histidine in P-HPr (chemical) would then explain the low specific activity of this preparation.

Conclusion

The known phosphoprotein intermediates of the phototransferase system, P- E_I , P-HPr, and P- F_{III} , all carry the phosphoryl group on a histidine. It may, therefore, be reasonable to postulate Scheme II as a general mechanism for phosphoryl group transfer in the PTS. The acceptor would be in the deprotonated form and the donor in the protonated form. The acceptor in the present step becomes the donor in the following step. Owing to the differences in pK values between the donor and acceptor proteins, both reactions are favored at physiological pH and the energy accumulated in the energy-rich N-P bond is very efficiently conserved upon phosphoryl group transfer.

Acknowledgments

We wish to gratefully acknowledge the expert technical assistance of R. Ten Hoeve-Duurkens who is responsible for the continuous purification of HPr and K. Dijkstra and Dr. R. Kaptein for their help with the NMR spectrometer.

References

- Anderson, B., Weigel, N., Kundig, W., & Roseman, S. (1971) *J. Biol. Chem.* **246**, 7023-7033.
- Dooijewaard, G., Roossien, F. F., & Robillard, G. T. (1979) *Biochemistry* **18** (second of three papers in this issue).
- Gassner, M., Stehlik, D., Schrecker, O., Hengstenberg, W., Maurer, W., & Ruterjans, H. (1977) *Eur. J. Biochem.* **75**, 287-296.
- Hays, J. B., Simoni, R. D., & Roseman, S. (1973) *J. Biol. Chem.* **248**, 941-956.
- Hengstenberg, W. (1977) *Curr. Top. Microbiol. Immunol.* **77**, 98-126.
- Hultquist, D. E., Moyer, R. W., & Boyer, P. D. (1966) *Biochemistry* **5**, 322-331.
- Jencks, W. P., & Gilchrist, M. (1965) *J. Am. Chem. Soc.* **87**, 3199-3209.
- Kundig, W., & Roseman, S. (1971) *J. Biol. Chem.* **246**, 1407-1418.
- Maurer, W., Ruterjans, H., Schrecker, O., Hengstenberg, W., Gassner, M., & Stehlik, D. (1977) *Eur. J. Biochem.* **75**, 297-301.
- Postma, P. W., & Roseman, S. (1976) *Biochim. Biophys. Acta* **457**, 213-257.
- Robillard, G. T., Dooijewaard, G., & Lolkema, J. (1979) *Biochemistry* **18** (first of three papers in this issue).
- Roseman, S. (1969) *J. Gen. Physiol.* **54**, 138s-180s.
- Schrecker, O., & Hengstenberg, W. (1971) *FEBS Lett.* **13**, 209-212.