Escherichia coli Phosphoenolpyruvate Dependent Phosphotransferase System. Copurification of HPr and α1-6 Glucan†

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

ABSTRACT: A rapid, high-yield procedure has been developed for the purification of HPr from the Escherichia coli phosphoenolpyruvate dependent phosphotransferase system. During this procedure, the protein copurifies with a 2500-dalton homopolysaccharide which we have identified as α1-6 glucan. The results of steady-state kinetic measurements of the phosphotransferase activity demonstrate that the polysaccharide works as an activator of the phosphotransferase system probably at the level of the HPr·P·E1 complex or the P-HPr·E1·complex.

The phosphoenolpyruvate dependent hexose transport system is, relative to the number of macromolecules involved, a rather simple, energy-dependent, transport system. As characterized by Roseman and co-workers (Roseman, 1969) the system is composed of one soluble carrier protein, HPr, one soluble enzyme, E2, and a membrane-bound complex, E3. It catalyzes the following reactions.

\[
\text{PEP}^+ + \text{HPr} \rightarrow \text{P-HPr} + \text{pyruvate} \\
\text{P-HPr} + \text{hexose (out)} \rightarrow \text{hexose-P (in)} + \text{HPr}
\]

Variations on this basic system have been found in Escherichia coli and other microorganisms (Saier, 1977; Postma & Roseman, 1976; Cirillo & Razin, 1973), but the elements stated above are common to all these systems. The apparent simplicity of this system makes it an attractive candidate for detailed physical-chemical studies of the transport process. Many physical techniques, however, demand large quantities of material for measurement. For this reason, we have focused our attention initially on developing high yield, rapid purification procedures for HPr and E2 (Robillard et al., 1979). During the course of the isolation, losses in HPr were encountered which were larger than expected for a single purification step, as judged by PTS activity measurements. Closer investigation has shown that these apparent losses result from the decrease in "specific activity" of HPr upon removal of its phosphate acceptor and a crude cell extract of the ptsH mutant, SB2226, as the source of E2 and E3 or pure E3 (see Robillard et al., 1979) and a partially purified E3 preparation (Postma, 1976) from the ptsHI mutant SB2950. The assay procedure used has been described previously (Kundig & Roseman, 1971b). Methyl α-D-[U-14C]glucopyranoside (180 mCi/mmol) was purchased from The Radiochemical Centre, Amersham. Phosphoenolpyruvate (monocyclohexylammonium salt) was purchased from Sigma. All other chemicals were analytical grade reagents.

Sugar Determination. The presence of sugar was detected by using an orcinol-sulfuric acid method (Winzler, 1955). The concentration of sugar was determined by comparison with a standard solution of glucose analyzed by the same procedure.

Inorganic phosphate was detected by using the ammonium molybdate procedure of Fiske & SubbaRow (1925).

Protein was determined by the method of Lowry et al. (1951).

NMR spectra were obtained on a Bruker HX 360-MHz spectrometer operating in the Fourier transform mode. All spectra were recorded at 305 K. Proton spectra at 360 MHz were recorded on samples in 5-mm cylindrical microcells (Wilmad) by using a pulse repetition rate of 1.7 s. 13C natural abundance NMR spectra were collected at 90.5 MHz in the quadrature detection mode by using 10-mm tubes containing a microcell insert (Wilmad). The chemical shift relative to Me₄Si was determined by using a sealed capillary containing Me₄Si. Decoupled spectra were collected with broadband noise decoupling (5 W) and a pulse repetition rate of 35 s. Proton coupled spectra were recorded by using gated decoupling between data acquisition to preserve the nuclear Overhauser enhancement. 31P NMR spectra were collected at 145.7 MHz on the same samples employed for 13C NMR spectra. No proton decoupling was used. The pulse repetition rate was 30 s. All spectra were collected by using a D lock on the D₂O signal of the solvent.

HPr Isolation. HPr was isolated by a simplification of the procedure of Anderson et al. (1971); the purification from unheated crude cell extract. The following modifications were made: (1) the DEAE-cellulose column was eluted with a 6-L linear gradient of 0-100 mM KC1 in 10 mM Tris-Cl buffer, pH 7.6; (2) concentrating and desalting steps were performed on an Amicon ultrafiltration apparatus equipped with a UM 2 membrane; (3) the CM-23 and Sephadex G-10 columns.

†Abbreviations used: PTS, phosphotransferase system; PEP, phosphoenolpyruvate; Mg,Si, tetramethylsilane; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate; αMglc, methyl α-D-glucopyranoside; NaDodSO₄, sodium dodecyl sulfate; ptsH, ptsI, mutant lacking HPr, E2, and HPr plus E3, respectively; Sephadex, registered trademark of Pharmacia Fine Chemicals.
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FIGURE 1: DEAE-cellulose chromatography of the protamine sulfate treated crude cell extract with (A) batchwise elution as described (Anderson et al., 1971) and (B) gradient elution which uses a linear gradient from 0 to 100 mM KCl (6 L total). Absorbance (230 nm) (●●●); HPr concentration (○○○); sugar concentration (ΔΔΔ); KCl concentration (---). Measurements were made as described under Materials and Methods.

Scheme I

100 g of E. coli K235

French press centrifuge 48000g

Protamine sulfate centrifuge 48000g

DEAE-cellulose

Ultrafiltration

Sephadex G-75

Ultrafiltration

were omitted. The isolation procedure is summarized in Scheme I. Further data are presented under Results.

Results

Isolation of HPr. Figure 1 shows the elution profile of the supernatant of the protamine sulfate step in Scheme I on DEAE-cellulose under two conditions. On batchwise elution (Figure 1A) as used by Anderson et al. (1971), HPr elutes after the major absorption peaks in a broad zone apparently consisting of several overlapping peaks. Gradient elution (Figure 1B), which results in a more highly resolved chromatogram, shows two peaks of HPr concentration eluting at 45 and 60 mM KCl, respectively. The HPr-containing fractions in Figure 1A were pooled as a single peak, those in Figure 1B as two separate peaks. The three preparations of partially purified HPr were concentrated by ultrafiltration and chromatographed on Sephadex G-75. The elution patterns showed a single peak of HPr at a molecular weight of 9500 irrespective of the HPr peak chromatographed. Moreover, the two preparations from Figure 1B, chromatographed either separately or recombined into one preparation, gave identical profiles of absorption and HPr concentration, showing that, on Sephadex G-75, there is no difference between the various HPr peaks from DEAE-cellulose. Some of these results are presented in Figure 2. The preparation from the batchwise DEAE elution is not yet pure (see Figure 2A); the absorption profile is asymmetric and much broader than the HPr peak. With the recombined preparation from the gradient DEAE elution, however, the symmetric profiles of the 230-nm absorption and HPr concentration clearly coincide (see Figure 2B), suggesting that the protein is pure. This is confirmed by polyacrylamide gel electrophoresis of the pooled peak which shows a single band on pH 9, pH 4, and NaDodSO₄ gels (see Figure 2B). The yields of HPr by this procedure will be treated, in detail, further on.

Copurification of a Polysaccharide. The two HPr peaks from the gradient DEAE elution, Figure 1B, do not result from two types of HPr with differing charge or size. The purified
peaks are identical in these properties as shown on subsequent Sephadex chromatography and polyacrylamide gels. The two peaks could signal, however, a specific binding of some molecule to a fraction of HPr altering, in turn, the total charge on that HPr population and, thus, its elution point from the DEAE column. When the concentration of sugar in the fractions from the gradient DEAE elution was determined, we found that the earliest eluting HPr peak overlapped with a broad sugar peak (see Figure 1B). However, in the subsequent Sephadex chromatography, Figure 2B, no sugar was found. The sugar had been destroyed as a result of a small amount of bacterial growth during the 48-h ultrafiltration step between the two columns. When the HPr pool from the gradient DEAE elution was made 1 mM in NaN₃, before the concentration step, bacterial growth was inhibited. The elution profile of the subsequent Sephadex G-75 chromatography, Figure 4, shows that a sugar peak elutes directly after an asymmetric HPr peak. When the sugar peak was pooled and rechromatographed over the same Sephadex column, its elution point was unchanged. This isolation procedure yields 50 pmol of sugar per 100 g of E. coli wet cell paste.

Characterization of the Polysaccharide. (1) Chemical Composition. Coupled gas chromatography and mass spectral analysis showed that the sugar polymer was composed, exclusively, of D-glucose units. Inorganic phosphate analysis was unable to detect phosphate in the polymer above 0.1 mol of Pi/mol of glucose. Similar negative results were obtained with 31P NMR measurements.

(2) Molecular Weight. The point of elution of the sugar peak from Sephadex G-75 has been used to approximate its molecular weight. It is equivalent to the point of elution of dextran polymers with a molecular weight of 2500. This corresponds to a polysaccharide 15 glucose units in length. Figure 5 presents the natural abundance 13C NMR spectra of the polysaccharide in Tris buffer. The decoupled spectrum, Figure 5A, shows that all units are in the same configuration, either α or β, resulting in a single resonance for each carbon atom. If both α and β anomers were present, two sets of resonances would be observed with relative intensities reflecting the relative populations of the anomers. In the proton coupled spectrum, Figure 5B, the peak at approximately 68 ppm which splits into a triplet is assigned to the glucose 6 carbon (see Figure 6, inset). The splitting arises from coupling to the two methylene protons. The resonance of the 6 carbon in glucose monomers or polymers with 1-4 glycosidic linkages occurs at higher fields, approximately 62 ppm (Jennings & Smith, 1973). The shift downfield is caused by formation of a glycosidic bond with the 6 carbon. The resonance at 96 ppm, which splits into a doublet in the coupled spectrum, is assigned to the 1 carbon. In an α-1-4 linkage, this resonance occurs downfield of 100 ppm, while in an α-1-6 linkage it occurs upfield of 100 ppm (Jennings & Smith, 1973).

The simplicity of the 1H NMR spectrum of this polysaccharide also indicates that all units are in the same configuration. The position of the anomeric proton resonance at -5.4 ppm is consistent with an α-anomer configuration. Thus, the isolated polymer is a homopolymer of D-glucose constructed entirely from α anomers via 1-6 glycosidic linkages.

Conformation of the Polysaccharide. Further studies of the proton NMR spectra reveal details of the configuration of the polymer. An intensity of seven protons is present in Figure 6, one proton each in resonances 1, 4, and 5 and two protons each in resonances 2 and 3. The numbers above the peaks indicate which protons in the structural formula resonate at the various spectral positions. These assignments have been made via selective decoupling experiments as shown in Figure
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FIGURE 7: The 360-MHz 1H NMR spectra of α1-6 glucan during selective decoupling. The frequency of irradiation is marked by an arrow in each spectrum.

FIGURE 8: Measurements of the rate of αMglc phosphorylation (activity) as a function of HPr concentration for HPr from the various stages of the isolation procedure. Crude cell extract (O-O); DEAE pool ( ―); Sephadex G-75 pool (Δ-Δ); Sephadex G-75 pool recombined with a saturating amount of α1-6 glucan just before assaying (▲-▲). The activities, expressed in arbitrary units, were determined in the presence of a fixed amount of E1 and EII, by using 50 μL of the crude cell extract of the mutant SB2226, containing 500 μg of protein. The activities were obtained from time-dependent plots and represent the initial rate of phosphorylation in the steady state. The concentration of HPr in the Sephadex G-75 pool was determined by Lowry's method; the concentration of HPr in the crude cell extract and the DEAE pool was computed as described in the Discussion.

7. In this figure, only one small coupling (±3 Hz) is present, between protons 1 and 4, indicating that they are in an axial-equatorial arrangement. The remainder of the couplings are large (10 Hz) corresponding to axial-axial couplings as shown in Figure 6, inset. For proton 2 to be axial relative to proton 3' requires that the glycosidic linkage have the same configuration as the α1-4 linkage of amylose (Greenwood, 1970). Thus, it is possible for this α1-6 glucan to form the same type of helical structures found for the amylose helix. However, the parameters of the helix will be different.

Role of α1-6 Glucan in the PTS. Since α1-6 glucan copurifies with HPr and the elution patterns from the DEAE-cellulose and Sephadex G-75 columns could be interpreted as indicating a binding between these two molecules, we have tested whether α1-6 glucan effects the PEP-dependent phosphotransferase activity. The phosphotransferase activity was measured as a function of HPr concentration at fixed concentrations of E1 and EII. Figure 8 presents the results of these measurements for HPr taken from various stages in the purification procedure. All plots show saturation behavior as expected, HPr being a substrate of the PTS (Postema & Roseman, 1976). The shape of the curve, however, differs markedly, depending on whether HPr is in a stage of isolation where the preparation is rich in α1-6 glucan or not. HPr in the sugar-containing preparations yields curves approximating a hyperbola; the pure HPr yields a sigmoidal shaped curve. Recombination of pure HPr and α1-6 glucan just before assaying the phosphotransferase activity restores the hyperbola (see Discussion). The same results are obtained whether the PTS activity is measured by using a ptsH mutant cell extract as the source of E1 and EII or pure E1 and a partially purified EII membrane fraction. The interpretation of these results is presented below.

Discussion

Relationship between Phosphotransferase Activity and the Concentration of the PTS Components: Theoretical Treatment. The relative concentration of the PTS components (HPr, E1, and EII) can be estimated from the rate of sugar phosphorylation of the PTS if conditions are found where this rate is approximately proportional to the concentration of the component under investigation. Since this rate generally will be a function of all three PTS components, measurements should be made at fixed concentrations of the two components not under investigation. Figure 8, presenting plots of phosphotransferase activity against HPr concentration, at fixed concentrations of E1 and EII, shows that, although the above condition is fulfilled, the shape of the plots may vary considerably. This observation, by itself, implies that another component should be involved. To obtain more insight into this phenomenon, a detailed theoretical treatment of the shape of the curves in Figure 8 will be given here. This will be done on the basis of the tentative model in Scheme II, which, of course, is a simplification of the PTS but suffices to explain the complications encountered in Figure 8.

In Scheme II, the following assumptions are made.

1) The reaction PEP + αMglc = Ppyr + αMglc-6-P is catalyzed by a combination of three components (HPr, E1, and EII) which can be added separately to the reaction mixture. Generally HPr and E1 are used in pure form; EII is added as a membrane fraction. The overall conversion is followed by measuring the αMglc-6-P concentration as a function of time. Since, in our experiments, the slope of these plots was constant for at least the first 10% of the αMglc conversion, it gives the initial rate of phosphorylation in the steady state, as plotted on the ordinate in Figure 8. This rate equals

\[ v = \frac{d(\alpha\text{Mglc-6-P})}{dt} = k_4(E_{II}\text{P-HPr}) \]

The reactions

\[ \text{HPr} + \text{PEP} = \text{P-HPr} + \text{Pyr} \]

and

\[ \text{P-HPr} + \alpha\text{Mglc} = \text{HPr} + \alpha\text{Mglc-6-P} \]

catalyzed by E1 and EII, respectively, operate according to...
ping-pong mechanisms. However, if one of these, or both, would operate according to an ordered mechanism, the basic principles of the model in Scheme II would remain the same by substituting PEP:ET for EIP, PEP:ETI1HPr for EIP:ETI1HPr, aMglc:ET1 for ETI1, and aMglc:ET1I1HPr for ETI1:ETI1HPr.

(3) The conversions of ET to ETI and ETI1 to ETI1 are fast compared with the other steps in Scheme II, implying that

\[ e_1 = (E_1P) + (E_1P:ET_1HPr) \]  
\[ e_{I1} = (E_{I1}) + (E_{I1}:P:ET_1HPr) \]

in which \( e_1 \), \( e_{I1} \), and \( p \) represent, respectively, the total concentrations of ET, ETI, and HPr, added at zero time. This assumption is in line with the observations (not shown) that, under the conditions measured in Figure 8, the concentrations of PEP (5 mM) and aMglc (2.5 mM) appeared to be saturating, the apparent \( K_M \) for both substrates being lower than 1 mM.

**Steady-State Approximation.** If a steady state may be assumed in all ET, ETI, and HPr complexes mentioned in eq 1–3, the relation between \( v \), \( p \), \( e_1 \), and \( e_{I1} \) will be

\[ p = \frac{1}{v} \left[ \frac{K_{M1}^I}{(e_1 - v/k_2)} \right] + \frac{1}{k_4} \left[ \frac{0.5}{(e_{I1} - v/k_4)} \right] \]  
\[ v = \frac{K_{M1}^I}{(k_2 e_1/v - 1)} + \frac{K_{M11}^I}{(k_2 e_{I1}/v - 1)} + \left( \frac{1}{k_2} + \frac{1}{k_4} \right) \]

in which \( K_{M1}^I = (k_1 + k_3)/k_1 \) and \( K_{M11}^I = (k_3 + k_4)/k_3 \). It should be noted that, in this formula, no assumptions are made whether \( p \), \( e_1 \), or \( e_{I1} \) is in excess with respect to the other two components. As a consequence, the usual assumptions made in Michaelis–Menten kinetics, namely, that the concentration of substrate added equals the free substrate concentration, do not hold. Therefore, the terms \((e_1 - v/k_2)\) and \((e_{I1} - v/k_4)\), which are equal to the steady-state concentrations of ET, ETI, and HPr, respectively, appear in the right-hand side of eq 4. Furthermore, the question whether HPr should be regarded as a substrate or an enzyme is irrelevant for the derivation of eq 4. In fact, eq 4 is identical with the reciprocal rate equation of a ping-pong mechanism if HPr is considered to be an enzyme and ET, ETI, and HPr are the substrates. In that case, HPr would catalyze the phosphoryl group transfer from ET to ETI via the PEP intermediate. Thus, within the assumptions made for the model in Scheme II, eq 4 is the most general equation that describes the relationship between \( v \) and the three components HPr, ET, and ETI, irrespective of their quality and quantity.

**Theoretical Saturation Curve.** In order to explain the curves in Figure 8, it is more convenient to consider \( p \) as a function of \( v \), \( e_1 \), and \( e_{I1} \), as is rewritten in eq 5. Figure 9 shows how

\[ p = \frac{k_2 e_1}{(k_2 e_1/v - 1)} + \frac{k_2 e_{I1}}{(k_2 e_{I1}/v - 1)} + \left( \frac{1}{k_2} + \frac{1}{k_4} \right) \]

a theoretical saturation curve in eq 5 is built up of three separate terms, \( p_1 \), \( p_2 \), and \( p_3 \), which according to eq 3 equal the concentrations of HPr, P:ETI, and the sum of the HPr-enzyme complexes, respectively. It should be noted that, for any given value of \( v \), the contributions \( p_1 \), \( p_2 \), and \( p_3 \) have to be added in order to construct the \( v \) vs. \( p \) plot. The meaning of the separate terms, however, is more easily understood by writing \( v \) explicitly for each of them so that

\[ v = \frac{k_2 e_1}{(1 + K_{M1}^I/p_1)} = \frac{k_2 e_{I1}}{(1 + K_{M11}^I/p_2)} = \frac{k_2 k_4}{(k_3 + k_4)p_3} \]
Table 1: Purification of HPr

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein Content (g)</th>
<th>Purification Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>crude cell extract</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>protamine sulfate</td>
<td>3.6</td>
<td>1</td>
</tr>
<tr>
<td>DEAE pool</td>
<td>0.05</td>
<td>60</td>
</tr>
<tr>
<td>concd DEAE pool</td>
<td>0.05</td>
<td>60</td>
</tr>
<tr>
<td>Sephadex G-75 pool</td>
<td>0.007</td>
<td>286</td>
</tr>
</tbody>
</table>

HPr was purified from 100 g (wet weight) of E. coli K235. Details of the purification procedure are described in the text. The recovery was determined from the data in Figure 8 as described under Discussion. Obtained from the preparation in Figure 8, recombined with saturating amounts of αL-6 glucan.

The two peaks from Figure 1B, recombined.

Origin and Role of αL-6 Glucan. The data presented in this report are the first demonstration that αL-6 glucan polysaccharide can be found in soluble E. coli extracts. The origin of this polysaccharide is not known. Except for the French press step used to rupture the cells, all other handling of the membrane and cell wall components are very gentle. It seems unlikely, therefore, that the material was originally an integral membrane structural component. It is possible that it is an exopolysaccharide coming from the capsule or slime layer which is partially degraded upon rupturing the cells. αL-6 glucan, however, has not been observed previously among E. coli exopolysaccharides (Sutherland, 1977). One trivial explanation is that the polymer is a degradation product of Sephadex, a cross-linked polymer of αL-6 glucans. However, proton NMR showed that the same polysaccharide was present in the HPr pool from the DEAE column (see Figure 1B) which had never been in contact with Sephadex.

The isolation procedure presented above indirectly provides some insight into the origin of the polysaccharide. It is retarded on DEAE-cellulose, while most of the sugar in the crude cell extract is not retarded. This could be explained assuming the polysaccharide carried a negatively charged glucose derivative (e.g., glucose-P) which binds to DEAE. Another explanation is that the retardation on the first DEAE column reflects a noncovalent or labile covalent binding of the polysaccharide to protein which, in turn, binds to DEAE. Obviously, HPr is the most likely protein candidate. On DEAE (Figure 1B), HPr elutes in two peaks, only one of which contains sugar. This elution behavior is not due to heterogeneity in the amino acid composition of HPr as found by Anderson et al. (1971) since (1) on rechromatography over DEAE-cellulose, the pure, sugar-free HPr eluted as a single peak at about 60 mM KCl, the position of the sugar-free peak in Figure 1B; (2) the purified HPr gave a single band on all polyacrylamide gel electrophoresis (see Figure 3).

The elution profile of the Sephadex G-75 column also indicates a binding of αL-6 glucan to HPr. In Figure 4, a portion of the HPr is retarded, eluting as a shoulder after the main HPr peak and overlapping the αL-6 glucan peak. Glucan-free HPr, however, elutes as a single symmetrical peak (see Figure 2B). Altogether, these results suggest a weak binding of αL-6 glucan to HPr.

At the present time, we do not have a clear understanding of the role of the polysaccharide in the PTS. There are, nevertheless, a number of possibilities. (1) The sugar could be originally covalently bound to HPr. The possibility of HPr as a glycoprotein is not unrealistic in light of the osmotic shock experiments of Kundig et al. (1966) which indicate that HPr may be located in the periplasmic space. Viewed from this perspective, the results of our reconstitution studies are quite reasonable. As we reported, the addition of pure αL-6 glucan to pure HPr only partially restored the hyperbola normally found in HPr concentration dependent activity measurements by using impure HPr from crude cell extract. If HPr is a glycoprotein, the polysaccharide will be covalently bound to the HPr in the crude cell extract and, using a reconstituted system where the components are not covalently bound, we would expect a less efficient system and lower activity. (2)
The polysaccharide could also originate in the membrane where it may have a specific role in the binding of P-HPr to membrane-bound E₃. This would correlate with its apparent activation effect which we find in the phosphotransferase activity measurements. (3) If one assumes that the α₁-6 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 α₁-6 helix, has a special function. α₁-6 helices should be much longer than the usual α₁-4 amylase 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 α₁-6 glucan homopolymer.

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References


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

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ABSTRACT: 1H and 31P 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.

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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).

\[
\begin{align*}
\text{PEP}^+ + \text{E}_1 & \rightleftharpoons \text{P-E}_1 + \text{pyruvate} \\
\text{P-E}_1 + \text{HPr} & \rightleftharpoons \text{P-HPr} + \text{E}_1 \\
\text{P-HPr} + \text{F}_{III} & \rightleftharpoons \text{P-F}_{III} + \text{HPr}
\end{align*}
\]

Abbreviations used: PEP, phosphoenolpyruvate; PTS, phosphoenolpyruvate dependent phosphotransferase system; DSS, 2,2-dimethyl-1,3-disilapentane-5-sulfonate; DTT, dithiothreitol; P (as in P-histidine), phospho.