Crystal Structure of Enzyme I of the Phosphoenolpyruvate Sugar Phosphotransferase System in the Dephosphorylated State*

Received for publication, August 19, 2009, and in revised form, September 25, 2009 Published, JBC Papers in Press, September 28, 2009, DOI 10.1074/jbc.M109.057612

Anselm E. Oberholzer, Philipp Schneider, Christian Siebold¹, Ulrich Baumann², and Bernhard Erni³

From the Departement für Chemie und Biochemie, Universität Bern, Freiestrasse 3, CH-3012 Bern, Switzerland

The bacterial phosphoenolpyruvate (PEP) sugar phosphotransferase system mediates sugar uptake and controls the carbon metabolism in response to carbohydrate availability. Enzyme I (EI), the first component of the phosphotransferase system, consists of an N-terminal protein binding domain (EIN) and a C-terminal PEP binding domain (EIC). EI transfers phosphate from PEP by double displacement via a histidine residue on EIN to the general phosphoryl carrier protein HPr. Here we report the 2.4 Å crystal structure of the homodimeric EI from *Staphylococcus aureus*. EIN consists of the helical hairpin HPr binding subdomain and the phosphorylatable $\beta\alpha$ phospho-histidine (P-His) domain. EIC folds into an $(\beta\alpha)_8$ barrel. The dimer interface of EIC buries 1833 Å² of accessible surface per monomer and contains two Ca²⁺ binding sites per dimer. The structures of the *S. aureus* and *Escherichia coli* EI domains (Teplakov, A., Lim, K., Zhu, P. P., Kapadia, G., Chen, C. C., Schwartz, J., Howard, A., Reddy, P. T., Peterkofsky, A., and Herzberg, O. (2006) *Proc. Natl. Acad. Sci. U.S.A.* 103, 16218–16223) are very similar. The orientation of the domains relative to each other, however, is different. In the present structure the P-His domain is docked to the HPr binding domain in an orientation appropriate for in-line transfer of the phosphate to the active site histidine of the acceptor HPr. In the *E. coli* structure the phospho-His of the P-His domain projects into the PEP binding site of EIC. In the *S. aureus* structure the crystallographic temperature factors are lower for the HPr binding domain in contact with the P-His domain and higher for EIC. In the *E. coli* structure it is the reverse.

The bacterial phosphoenolpyruvate sugar phosphotransferase system (PTS)⁴ mediates sugar uptake and controls the

carbon metabolism in response to carbohydrate availability. It consists of four functional phosphoprotein units that sequentially transfer phosphoryl groups from phosphoenolpyruvate (PEP) to the transported sugars (for reviews, see Refs. 1 and 2). The transport of PTS sugars is driven by the tight coupling of solute translocation with solute phosphorylation (group translocation). The control functions rely on the ratio of the phosphorylated to the dephosphorylated state of certain PTS proteins. This ratio reflects the difference between supply of phosphate by PEP and availability of solutes that consume phosphate during their uptake. The ratio increases in the absence of sugar, and it decreases when PTS substrates are taken up and/or the PEP supply is reduced.

The four PTS units which occur as either subunits or domains are phosphorylated at histidine or cysteine residues. Enzyme I (EI) is the first protein at the top of the phosphotransferase cascade. It transfers phosphate from PEP to HPr, the general high energy phosphate carrier protein of the PTS. HPr then phosphorylates the different PTS sugar transporters (EII, enzymes II) at the bottom of the cascade (Scheme 1).

SCHEME 1

EIIs can be grouped into four structurally diverse (super)families (3, 4), which have in common that they consist of three functional units, namely the phosphate transferring EIIA and EIIB and the solute-translocating, membrane-spanning EIIC subunit/domain (5, 6). PTS of variable size with respect to the number EIIs (between none and more than 20) occur in 70% of all eubacteria (3) and in some archaeobacteria (7) but not in eukaryotes.

EI of the PTS is one of the best conserved bacterial proteins with only minimal similarity to animal proteins (for review, see Ref. 8). It is a homodimer of 64-kDa subunits that consists of an N- and a C-terminal domain (EIN and EIC). The x-ray structures of full-length EI of *Escherichia coli* and *Staphylococcus carnosus* (9, 10), of the EIN domain from *E. coli* (11), and of EIC from *Thermoanaerobacter tengcongensis* (12) have been solved. Also known is the solution structure of the complex between EIN and HPr (13–15). EIN consists of the α -helical HPr binding subdomain that provides the docking site for HPr and the α/β P-His subdomain with its phosphoryl-acceptor histidine (His-191). The P-His domain carries the phosphate from PEP bound

* This work was supported by the Swiss National Science Foundation Grant 3100A0-105247.

The atomic coordinates and structure factors (code 2wqd) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (<http://www.rcsb.org/>).

The nucleotide sequence reported in this paper has been submitted to the Swiss Protein Database under Swiss-Prot accession no. P51183.

¹ Present address: Division of Structural Biology, University of Oxford, Henry Wellcome Building of Genomic Medicine, Roosevelt Drive, Oxford OX3 7BN, United Kingdom.

² To whom correspondence may should be addressed. Tel.: 41-31-6314320; Fax: 41-31-6314887; E-mail: ulrich.baumann@ibc.unibe.ch.

³ To whom correspondence may should be addressed. Tel.: 41-31-6314346; Fax: 41-31-6314887; E-mail: erni@ibc.unibe.ch.

⁴ The abbreviations used are: PTS, phosphotransferase system; PEP, phosphoenolpyruvate; HPr, histidine containing phosphocarrier protein of the PTS; EI, enzyme I of the PTS; EIN, N-terminal domain of EI (comprises HPr binding and P-His (sub)domains); EIC, C-terminal PEP binding domain of EI; P-His domain, phosphohistidine domain of EI; *Ec*, *E. coli*; *Sa*, *S. aureus*; EII,

sugar-specific transporters of the PTS; PPK, pyruvate phosphate dikinase; Ack, acetate kinase; MR, molecular replacement.

to EIC to the active site His of HPr. The P-His domain has also been designated as a swiveling domain (10, 16). EIC is a compact eight-stranded (β/α)₈ barrel. It contains the PEP binding site (17, 18) and provides the dimerization interface of EI. The PEP binding domain EIC and the P-His subdomain of EIN exhibit 30% sequence identity with the respective domains of the pyruvate phosphate dikinase (PPDK, Swiss-Prot accession no. P22983 and PDB codes 1kbl and 2r82 (16, 19–22), whereas the four-helix-bundle HPr binding domain of EIN is unrelated to the nucleotide binding domain of PPDK.

In vitro experiments indicated that a phosphoryl group can be transferred to EI not only from PEP but also from ATP and acetyl phosphate through the phosphorylated intermediate of acetate kinase (AcK) and that phosphoryl transfer between AcK and Enzyme I is reversible (23, 24).

Equilibrium and rate constants of the EI monomer dimer transition vary strongly with protein and substrate concentrations, pH, temperature, and other *in vitro* parameters. The K_a decreases with decreasing temperature (cold sensitivity) and increasing ionic strength (ionic interactions). K_a increases in the presence of Mg^{2+} and PEP (25, 26). The phosphoryl group is transferred from PEP to the P-His domain of the same protein subunit, and intersubunit transfer does not occur (10, 27). Nevertheless, only dimeric EI can be autophosphorylated, whereas phosphate is exchanged between HPr and the monomeric EIN domain (28). It has been suggested that EI activity controls overall PTS sugar transport (29) and that EI activity itself is controlled by the monomer-dimer equilibrium. It is not clear yet which physiological signal, for instance which allosteric effector, could shift the monomer-dimer equilibrium *in vivo* and, thus, regulate EI activity (26) and how the apolar dimerization interface of EIC is protected against denaturation/aggregation, for instance by heterodimerization with an as yet unknown protein. It is noteworthy that the compact C-terminal dimerization domain is thermally and proteolytically unstable in full-length EI of *E. coli* as well as when expressed separately, whereas the monomeric, highly flexible EIN domain is resistant (30).

Here we present the x-ray structure of EI from *Staphylococcus aureus* (SaEI, Swiss-Prot accession no. P51183) at 2.4 Å resolution. The structures of the SaEI and the *E. coli* EI (EcEI) (sub)domains are very similar. Their orientations relative to each other, however, are different. In the *E. coli* structure the P-His domain is oriented toward EIC thus that the phosphorus of the phosphohistidine and the oxygens of the PEP analog oxalate come to within 3–4 Å distance. In the present structure the P-His domain is oriented in the alternative conformation, namely docked to the HPr binding domain with its active site His perfectly oriented for an in-line transfer of the phosphate to the acceptor HPr.

EXPERIMENTAL PROCEDURES

Expression, Purification, and Crystallization—Plasmids encoding SaEI(H191A/C365S) were constructed as described previously (17). SaEI(H191A/C365S) was expressed in *E. coli* TH074($\Delta ptsI$), which carries a deletion of codons 131–258 of *ptsI* (31). Cells were grown in Luria-Bertani broth at 37 °C to an A_{550} of 0.8 and induced by 0.1 mM isopropyl- β -thiogalactoside. After 2.5 h of induction cells were harvested, resuspended in buffer A (20 mM Tris-HCl, pH 8.5, 1 mM EDTA, 2 mM dithio-

TABLE 1

Data collection and refinement statistics for enzyme I of *S. aureus*

Space group	P3 ₂ 2 ₁
Unit cell parameters	
<i>a</i> = <i>b</i> , <i>c</i> (Å)	98.34, 105.11
Molecules/asymmetric unit	1
Data collection (XDS)	
Wavelength (Å)	1.5418
Resolution range ^a	35.91–2.4 (2.54–2.4)
No. observations (unique)	448,666 (23,409)
Completeness %	100 (100)
R_{sym} %	8.7 (46.0)
$I/\sigma(I)$	29.6 (6.9)
Refinement (Phenix)	
Resolution range (Å)	35.91–2.40
No. reflections working set	21,659
No. reflections test set	1,747
No. non-hydrogen atoms	4,486
Ions	1
Solvent water molecules	163
R/R_{free} (%)	23.4/29.5
Root mean square deviation bond length (Å)	0.003
Root mean square deviation bond angles (degree)	0.674
Ramachandran plot	
Most favored (%)	94.9
Allowed (%)	3.2
Disallowed (%)	1.9

^a The values in parentheses of resolution range, completeness, R_{sym} , and $I/\sigma(I)$ correspond to the outermost resolution shell.

^b $R_{sym} = \sum_{hkl} \sum_j |I(hkl)_j| / \langle I(hkl) \rangle - \langle I(hkl) \rangle / (\sum_{hkl} \sum_j I(hkl)_j)$, where $I(hkl)_j$ is the *j*th measurement of the intensity of the unique reflection (*hkl*), and $\langle I(hkl) \rangle$ is the mean overall symmetry-related measurement.

threitol), and disrupted with a French pressure cell. Insoluble material and membranes were removed by centrifugation (60 min, 150,000 × *g*). The supernatant was applied on a High Q column (Bio-Rad) and eluted with a linear gradient of 0–600 mM NaCl. The EI-containing fractions were pooled, dialyzed against buffer A, applied on a Resource Q (Amersham Biosciences) column, and eluted with a NaCl gradient. EI was further purified by gel filtration using a Superdex 200 (Amersham Biosciences) column equilibrated in buffer B (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 2 mM dithiothreitol, 20 mM CaCl₂). The peak fractions were pooled, dialyzed against buffer C (5 mM Tris-HCl pH 7.5, 2 mM dithiothreitol, 20 mM CaCl₂), and concentrated in a Centrprep-50 concentrator to 27 mg of protein/ml.

22.3 μ l of SaEI was mixed with 7.6 μ l of buffer C to a final protein concentration of 20 mg/ml, added to 10 μ l of crystallization buffer containing 0.2 M KCl, 20% (w/v) polyethylene glycol 3350, and preincubated at 32.5 °C for 5 min. Crystals were grown at 20 °C by vapor diffusion in hanging drops using 4 μ l of the preincubated solution. Crystals ($\sim 200 \times 200 \times 200$ - μ m³ size) were cryo-protected by the addition of 15% (v/v) 2-methyl-2,4-pentanediol and flash-cooled in a nitrogen stream at 110 K. Diffraction data to 2.4 Å resolution were collected on a RaxisIV image plate area detector mounted on a Rigaku RU300 rotating-anode generator operating at 100 mA and 50 kV. Data were integrated and scaled with XDS (32, 33). Data collection statistics are reported in Table 1.

Structure Determination—SaEI crystallized in space group P3₂2₁ with one molecule in the asymmetric unit. The structure was solved by molecular replacement (MR) using the program PHASER (34) employing the EcEI P-His domain residues (2–21, 151–232) (PDB code 2hwg) (10), the EI HPr binding

domain residues (36–144) from *S. carnosus* (PDB code 2hro) (9), and the EIC from *T. tengcongensis* (PDB code 2bg5) (12) as search models. An initial model could be built manually into the resulting electron density. The model was completed by iterative cycles of restrained refinement and model building using COOT (35) and PHENIX (36). The final model includes residues 2–571 of 572. 94.9% of the residues are in the most favored, 3.2% are in the allowed, and 1.9% are in the disallowed region of the Ramachandran plot. Refinement statistics are given in Table 1. Figs. were prepared using PYMOL.

RESULTS AND DISCUSSION

Expression and Crystallization—Because the EIs of *E. coli* and *T. tengcongensis* did not afford diffraction quality crystals, EIs from seven other species were expressed in *E. coli* and purified for crystal screening. EI from *Bacillus subtilis*, *Bacillus stearothermophilus*, *Borrelia burgdorferi*, *Enterococcus faecalis*, *Haemophilus influenzae*, and *S. aureus* could be expressed in soluble form. They complemented glucose phosphotransferase activity of *E. coli* TH074 (*ptsI*[−]) and displayed from 4 to 7% of *E. coli* EI phosphotransferase activity in the heterologous *in vitro* phosphotransferase assay with *E. coli* PTS protein subunits (37). The recombinant EI domain of the *E. coli* multidomain protein EI^{Ntr} (*ptsP*, codons 171–748) formed inclusion bodies, and the EI domain of the *Pseudomonas aeruginosa* multidomain protein FruB (residues 301–956) was not expressed. After optimization, EI from *S. aureus* (*SaEI*) afforded the best crystals diffracting to 4.0 Å resolution (37). The same *SaEI* with an N-terminal histidine tag did not afford x-ray quality crystals.

To further improve the protein homogeneity of *SaEI* (judged by symmetry and sharpness of the gel filtration peak), two non-conserved cysteines predicted to be at the protein surface, Cys-365 and Cys-557 of the C-terminal domain, were mutated to Ser and Ala alone and in combination (Fig. 1). Of eight mutants, the C365S mutation improved crystal quality to a diffraction of 3.5 Å. In addition to Cys-365, the phosphorylatable His-191 was mutated to alanine to avoid sample heterogeneity due to partial EI autophosphorylation. The double mutant EI(H191A/C365S) eventually afforded crystals that diffracted to 2.4 Å resolution.

Structure Determination—Crystals of *SaEI*(H191A/C365S) belonged to the trigonal space group P3₂21 ($a = b = 98.34$, $c = 105.11$) with one monomer in the asymmetric unit. Only a few crystals diffracting up to 2.4 Å could be obtained, whereas the majority of crystals grown in the same conditions diffracted poorly. The structure was determined by MR. A solution for the EIC domain could be easily determined by PHASER with EIC of *T. tengcongensis* (PDB code 2bg5) as the search model. The structure of the N-terminal domain, however, could be found neither with the model of the recombinant EIN (PDB code 1zym (11)) nor with EIN of the full-length EI (PDB code 2hwg (10)), probably because of the intrinsic mobility of the P-His subdomain relative to the HPr binding subdomain. An initial MR solution of poor Z-scores (<5) was obtained when the two subdomains (HPr binding domain of *S. carnosus*, PDB code 2hro; P-His domain of *E. coli*, PDB code 2hwg) were used as independent search models. Many runs of MR using differently trimmed search probes followed by evaluation of the resulting models by several rounds of refinement and inspection of annealed omit maps were

necessary to identify correct solutions for the P-His and HPr binding subdomains. The correctness of the model was confirmed by calculation of an anomalous difference Fourier map, which showed peaks higher than 4 σ above the mean for 13 (10 in EIN, 3 in EIC) of 21 sulfur atoms.

Residues 2–571 could be built into the electron density (Fig. 1). Residues Lys-208, Phe-301, Lys-416, and three short stretches corresponding to residues 151–155 (hinge I of EIN), 304–308, and 341–356 (in EIC β/α loops 2 and 3) showed poor density in the 2F_o – F_c map. Only the main chain could be traced, whereas the side chains remained invisible. These residues were modeled as alanines for the structure refinement. Contrary to expectation, Cys-365, which was mutated to a serine to improve crystallization, is buried and inaccessible from the surface. Cys-365 is not conserved and is located in a loop far from the EIC-EIN interdomain interface and at a distance of 19 Å from the PEP binding site. In view of this it is unlikely that the C365S mutation has an effect on the domain conformation.

Overall Structure—The *SaEI* monomer model shows the three (sub)domains (Fig. 2); that is, the N-terminal HPr binding subdomain (green, residues 27–148), the P-His subdomain (yellow, residues 2–23, 150–255), which transfers the phosphoryl groups from PEP to HPr (11), and the C-terminal PEP binding domain EIC (cyan/blue, residues 260–571). The HPr binding domain consists of four helices arranged in two hairpins; the P-His domain exhibits an α/β fold, and the PEP binding domain exhibits a ($\beta\alpha$)₈ barrel fold. HPr binding and P-His subdomains are linked by two crossovers of the polypeptide backbone (hinge I, residues 24–37 and 145–156) and together form the EIN domain (Figs. 1 and 2). The P-His domain is linked with the PEP binding domain through a short aperiodic segment (hinge II, residues 260–271).

The N-terminal Domain—In the N-terminal domain of *SaEI*, the P-His and HPr binding subdomains assume the same relative orientation as in the crystal structure of the stand-alone *EcEIN* fragment (11) and in the NMR structure of the complex between *EcEIN* and *EcHPr* (14). NMR dipolar coupling measurements (38) indicated that this conformation remains unchanged irrespective of whether EIN, HPr, or both are phosphorylated or not. It appears that this is a minimal energy conformation of the ternary complex between HPr, HPr binding, and P-His domains. When HPr is docked *in silico* to the model of *SaEI* in the orientation given by the NMR solution structure (14, 39) (Fig. 3C), the distance between N ϵ 2 of His-191 and N δ 1 of His-15 of HPr can be reduced to 3.7 Å. This is less than the 4.1 Å O–P–O distance of the pentacoordinate phosphate in phosphoglucumutase near the transition state (40). To determine this distance, Ala-191 (mutation to prevent autophosphorylation) was replaced *in silico* with a histidine, and the rotamer conformation was adjusted such that a hydrogen bond between N δ 1 of His-191 and O ϵ 1 of the invariant Glu-127 was formed (in analogy to similar motifs observed in other PTS proteins (41–43)). However, the excellent local fit notwithstanding, HPr could not bind to EI in exactly the conformation shown in the model (Fig. 3C) because of a clash with the flexible C-terminal extensions the EIC ($\beta\alpha$)₈ barrel. This clash can be relieved by a small displacement of EIN and EIC around the hinge II between EIN and EIC (residues 260–271).

FIGURE 1. Sequence alignment of EI from *S. aureus* (STAAU) and *E. coli*. The EI subdomains are color coded as follows: yellow, P-His domain; green, HPr binding domain; cyan, PEP binding domain. The hinges I and II (–) are red. Note that the P-His and HPr binding domains are connected by two crossovers of the polypeptide chain (hinge I). Also indicated are the linker helix (~~), the mutated residues H191A and C365S, the active site Cys-502 of the PEP binding site, the three Ca^{2+} binding residues of the PEP binding domain, and those residues (*) that are disordered in the x-ray structure.

As mentioned above, it was found that *EcEI* was *in vitro* phosphorylated not only by PEP but also by AcK with either ATP or acetyl phosphate as the phosphoryl donors (23, 24). It was assumed that a phospho-AcK intermediate acts as phosphoryl donor toward *EcEI*. Such a mechanism appears unlikely today, first because AcK catalyzes the direct in-line transfer between ATP and acetate and not a double displacement with a covalent AcK-phosphate intermediate (44). Second, the P-His domain of EI and the substrate binding site of AcK lack any structural complementarity. One possibility to explain the observation is phosphorylation by Ac-P of the surface-accessible active site His-191 of the P-His domain.

Such nonenzymatic phosphorylation of active site histidines by Ac-P was observed with two-component response regulator proteins (45, 46).

The C-terminal Domain—The PEP binding domain *SaEI*C features a $(\beta\alpha)_8$ barrel with three extensions on the C-terminal side of the barrel. The atomic coordinates of the monomer superimpose well with those of the crystal structure of *T. tengcongensis* EIC (53% amino acid sequence identity) (12) and *E. coli* EIC (48% sequence identity, Fig. 1), resulting in a root mean square deviation of 0.95 Å for the paired C^α atoms. Two monomers from adjacent asymmetric units form the physiological dimer (Fig. 2, A and B). The contact interface between the

FIGURE 2. **Structure of EI.** A and B show ribbon diagrams of the EI dimer. The subunits are shaded in color and gray; the HPr binding domain is green, the P-His domain plus EIN-EIC linker helix is yellow, and the PEP binding domain is cyan/blue. The residues Ala-191 (H191A mutation) and Cys-504 are shown as stick models. Two Ca^{2+} are shown as magenta spheres. C, enlargement of the Ca^{2+} binding site is seen in the same orientation as in B. Atomic colors are as follows: red, oxygen; blue, nitrogen; gray, carbon. The imaginary Fourier map is depicted in chicken wire representation and contoured at 3 (blue) and 5 (yellow) S.D. above the mean. Thr-397 and Asn-399 from monomer 1 and Gln-477 from monomer 2 are shown as stick models.

FIGURE 3. **Comparison of the SaEI and EcEI.** A and B, shown are crystallographic temperature factors. A, the P-His domain is bound to the HPr-binding domain, and as a consequence, the HPr-binding domain has small temperature factors (blue), and the PEP binding domain has large temperature factors (red). B, the P-His domain is bound to the PEP binding domain, and the temperature factors are small for the latter and large for the HPr binding domain. C and D, semitransparent surface representation of EI to which HPr (magenta) is docked to the HPr binding domain (green) in the orientation, given by the NMR solution structure (PDB code 3eza) (14, 39). C, His-191 (yellow) of the P-His domain projects toward His-15 (red) of HPr. D, His-191 projects toward the inhibitory oxalate (yellow) of the PEP binding domain (10). No correction was made for the steric clash between the EIC domain (cyan) and the *in silico*-docked HPr (magenta).

two C-terminal domains buries $1833 \text{ \AA}^2$ of solvent-accessible surface per monomer. Although the SaEIC and *T. tengcongensis* EIC protomers superimpose well, the protomer orientation in the dimer differs by a 7° rotation around the normal to the crystallographic dimer axis.

An anomalous difference Fourier map showed two peaks above 5σ over mean within the dimer interface of SaEIC (Fig. 2C). We assigned the difference Fourier peaks to Ca^{2+} ions because an anomalous signal at the $\text{CuK}\alpha$ x-ray emission wavelength is expected of Ca^{2+} only but not of any other positively charged ion of the crystallization buffer. 20 mM Ca^{2+} was present because this concentration was beneficial for the homogeneity of EI during purification.

Ca^{2+} is located in an electron-rich cation hole at the edge of the dimer interface where it is coordinated by the side chain oxygens of Thr-397 and Asn-399 from one and Gln-477 of the second subunit (Fig. 2C). The three residues are conserved (S/T, N/Q/E, Q) but are not invariant in homologous EIs. It was shown that Mg^{2+} alone and Mg^{2+} plus PEP together shift the monomer-dimer equilibrium toward the dimeric form and stabilize EcEIC by 7 and 14°C , respectively, against thermal unfolding. The stoichiometry of Mg^{2+} binding was estimated to be two Mg^{2+} per monomer with K_d values of 0.39 and 5.8 mM (25). The high affinity Mg^{2+} occupies the PEP binding site of EcEIC, where it coordinates two oxygen atoms of the inhibitor oxalate and one oxygen of the His-189 phosphoryl group on the P-His domain (10). The low affinity Mg^{2+} might complex to the interfacial site, which has been identified by the Ca^{2+} . Ca^{2+} improved purification and crystallization. Mg^{2+} may affect other stability parameters by the same mechanism.

FIGURE 4. Comparison of domain motions in EI and PPDK. EI, HPr (magenta) is docked to the HPr binding domain (green) in the conformation given by the NMR solution structure of the EIN-HPr complex (PDB code 3eza) (14, 39). Conformation II, shown is EI with the P-His domain (yellow) projecting into the PEP binding site of EIC (cyan). X, hypothetical intermediate; the P-His and EIC domains have dissociated by a rotation around hinge II (red) that is located at the C-terminal end of the linker helix (schematic). In this intermediate HPr is not yet associated with the P-His domain. Conformation I, the HPr binding domain has snapped back to the P-His domain by a rotation and a bending motion of hinge I (red) located between the P-His domain and the HPr binding domain. PPDK, Conformation II, the P-His domain (yellow) projects into the PEP binding site (cyan). Conformation I, the P-His domain has swung to the nucleotide binding domain (NBD, green) by a rotation (swiveling motion) around the hinge (red) located at the N-terminal end of the linker helix (schematic). Only one protomer of EI and PPDK is shown.

Conformation of the EI Dimer—The P-His domain is in contact with the HPr binding domain of EIN (Fig. 2, A and B). If HPr is *in silico*-docked to the HPr binding domain (Fig. 3C) in the orientation given by the NMR solution structure of the EIN-HPr complex (14, 39), then the ternary complex between the P-His domain, the HPr binding domain, and HPr buries ~ 860 Å² of accessible surface area. The free energy of this interaction corresponds to an experimental complex dissociation constant of $5 \mu\text{M}$ (38). For comparison, about 920 Å² of solvent-accessible surface are buried between the P-His and the EIC domains when the phosphorylated His-191 projects into the PEP binding site of EIC (Fig. 3, B and D) (10).

A comparison of the crystallographic temperature factors between *SaEI* and *EcEI* shows that the factors are low for the domains in contact with the P-His domain and increased in the uncomplexed domains (Fig. 3, A and B). For example, loops $\beta\alpha 2$ and $\beta\alpha 3$ of EIC are well ordered when in contact with the phosphorylated P-His domain as in *EcEI* (Fig. 3B) but display poor electron density without this contact as in *SaEI* (Fig. 3A). These two loops also participate in the EIC dimer interface. The free energy of the ternary contact (EIC·EIC·phospho-P-His domain) could account for the stronger protomer association of phospho-EI (26) in comparison with the binary contact (EIC·EIC) of dephosphorylated EI. The $\beta\alpha 2$ and $\beta\alpha 3$ loops of *T. tengcongensis* EIC are well defined even in the absence of a P-His domain. They

may “freeze” at room temperature, which is 50°C below the physiological value of 75°C (47).

Conformation Changes—The three x-ray structures that have been solved so far show EI in three clearly different conformations. In *SaEI* the P-His domain and the HPr binding domain are orientated such that the phosphate can be transferred from His-191 to the active site His-15 of HPr (Fig. 4A, conformation I). In *EcEI* the P-His domain projects into the PEP binding site of EIC (Fig. 4B, conformation II) and is aligned to accept the phosphate from PEP (10). In *S. carnosus* EI (9) the three domains are by and large dissociated.

The *E. coli* and *S. aureus* models represent two alternative and functionally relevant conformations (I and II). Assuming that the shortest path between conformations I and II also is the energetically most favorable one, the transition can be decomposed into two small rigid body motions (Fig. 4, A and B). First, the P-His domain disengages from the PEP binding site. This step involves an anticlockwise rotation of 45° around hinge II (residues

260–271) between the P-His and the EIC domain (Fig. 4B). In the second step the HPr binding domain, which was displaced away from EIC in conformation II, snaps back toward the P-His domain to afford conformation I (Fig. 4A). Snapping involves a 60° twist and a gentle bending of the hinge I (residues 24–37 and 145–156) between the P-His and the HPr binding domains (Figs. 1 and 4, A and B). It is noteworthy that the $\alpha\beta$ core of the P-His domain and the conspicuous C-terminal “linker helix” (residues 235–259, Figs. 1 and 4) move as a single rigid body. The short turn (residues 232–234) between the $\alpha\beta$ core and the linker helix does not function as a flexible hinge.

EI shares two homologous domains with the PPDK, namely the PEP binding (EIC) and the P-His domains (16). The HPr binding domain of EIN and the nucleotide binding domain of PPDK, however, have completely different folds and different locations relative to the PEP binding domain. PPDK is shown in Fig. 4, C and D, in an orientation such that the $(\beta\alpha)_8$ cores of the PEP binding domains (cyan) of PPDK and EI are aligned. The HPr binding domain (green) with docked HPr (magenta) of EI is located at five o’clock above the picture plane (Fig. 4A), and the nucleotide binding domain of PPDK (green) is located at two o’clock behind the picture plane (Fig. 4C).

In EI the PEP binding domain and the HPr binding domain move relative to each other such that the distance between the phosphoryl donor PEP and the acceptor His-15 of HPr

varies between 17 Å for *SaEI* (conformation I, P-His domain projects toward His15 of HPr) and 35 Å for *EcEI* (conformation II, P-His domain projects into the PEP binding site) (Fig. 4, A and C). The P-His domain (yellow) first rotates around hinge II, and then the acceptor (complex of HPr binding domain and HPr) moves around hinge I toward the P-His domain (compare Fig. 4, B with A). The linker helix remains tightly associated with the β/α core of the P-His domain (yellow) of EI.

In PPDK, the PEP binding and nucleotide binding domains are coupled, and the 45 Å distance between the phosphoryl donor PEP and the acceptor AMP-PP does not change (Fig. 4, C and D). Only the P-His domain moves from the donor to the acceptor. The conformational changes of PPDK are described in detail by Lim *et al.* (22). The P-His domain of PPDK first disengages from the PEP binding site and then rotates anti-clockwise around the hinge (red) (Fig. 4, from D to C). The PPDK hinge is formed by two antiparallel peptide segments. The first connects the nucleotide binding domain with the P-His domain. The second connects the P-His domain with the N terminus of the linker helix (Fig. 4, C and D). The linker helix of PPDK remains tightly associated with the $(\beta/\alpha)_8$ core of the PEP binding domain (cyan) and not with the P-His domain as in EI. Thus, only domain folds are conserved between EI and PPDK but not the structural elements that govern their dynamic interaction.

In summary, modest rigid body motions of the P-His domain relative to the EIC PEP binding domain and of the HPr binding relative to the P-His domain suffice to transfer phosphate from PEP to HPr. Compared with the compact structures of *EcEI* and *SaEI*, the three (sub)domains of *S. carnosus* EI (83% sequence identity with *S. aureus*) are stretched over a length of 110 Å (9). The P-His domain shows weak electron density and does not contact the PEP and HPr binding domains. The open structure may represent one of many short-lived conformers as postulated in Patel *et al.* (26), because there is no evidence that the shortest reaction pathway (outlined in Fig. 4) also is the energetically most favorable one. Alternatively this elongated conformation may be caused by crystal lattice contacts.

Acknowledgment—The help of Priska Bähler is gratefully acknowledged.

REFERENCES

- Görke, B., and Stülke, J. (2008) *Nat. Rev. Microbiol.* **6**, 613–624
- Deutscher, J., Francke, C., and Postma, P. W. (2006) *Microbiol. Mol. Biol. Rev.* **70**, 939–1031
- Barabote, R. D., and Saier, M. H., Jr. (2005) *Microbiol. Mol. Biol. Rev.* **69**, 608–634
- Saier, M. H., Hvorup, R. N., and Barabote, R. D. (2005) *Biochem. Soc. Trans.* **33**, 220–224
- Robillard, G. T., and Broos, J. (1999) *Biochim. Biophys. Acta* **1422**, 73–104
- Siebold, C., Flükiger, K., Beutler, R., and Erni, B. (2001) *FEBS Lett.* **504**, 104–111
- Bolhuis, H., Palm, P., Wende, A., Falb, M., Rampp, M., Rodriguez-Valera, F., Pfeiffer, F., and Oesterhelt, D. (2006) *BMC Genomics* **7**, 169
- Ginsburg, A., and Peterkofsky, A. (2002) *Arch. Biochem. Biophys.* **397**, 273–278
- Márquez, J., Reinelt, S., Koch, B., Engelmann, R., Hengstenberg, W., and Scheffzek, K. (2006) *J. Biol. Chem.* **281**, 32508–32515
- Tepljakov, A., Lim, K., Zhu, P. P., Kapadia, G., Chen, C. C., Schwartz, J., Howard, A., Reddy, P. T., Peterkofsky, A., and Herzberg, O. (2006) *Proc. Natl. Acad. Sci. U.S.A.* **103**, 16218–16223
- Liao, D. I., Silverton, E., Seok, Y. J., Lee, B. R., Peterkofsky, A., and Davies, D. R. (1996) *Structure* **4**, 861–872
- Oberholzer, A. E., Bumann, M., Schneider, P., Bächler, C., Siebold, C., Baumann, U., and Erni, B. (2005) *J. Mol. Biol.* **346**, 521–532
- Garrett, D. S., Seok, Y. J., Peterkofsky, A., Clore, G. M., and Gronenborn, A. M. (1997) *Biochemistry* **36**, 4393–4398
- Garrett, D. S., Seok, Y. J., Peterkofsky, A., Gronenborn, A. M., and Clore, G. M. (1999) *Nat. Struct. Biol.* **6**, 166–173
- Peterkofsky, A., Wang, G., Garrett, D. S., Lee, B. R., Seok, Y. J., and Clore, G. M. (2001) *J. Mol. Microbiol. Biotechnol.* **3**, 347–354
- Herzberg, O., Chen, C. C., Kapadia, G., McGuire, M., Carroll, L. J., Noh, S. J., and Dunaway-Mariano, D. (1996) *Proc. Natl. Acad. Sci. U.S.A.* **93**, 2652–2657
- García-Alles, L. F., Flükiger, K., Hewel, J., Gutknecht, R., Siebold, C., Schürch, S., and Erni, B. (2002) *J. Biol. Chem.* **277**, 6934–6942
- Seok, Y. J., Lee, B. R., Gazdar, C., Svenson, I., Yadla, N., and Peterkofsky, A. (1996) *Biochemistry* **35**, 236–242
- Herzberg, O., Chen, C. C., Liu, S., Tempczyk, A., Howard, A., Wei, M., Ye, D., and Dunaway-Mariano, D. (2002) *Biochemistry* **41**, 780–787
- Wei, M., Li, Z., Ye, D., Herzberg, O., and Dunaway-Mariano, D. (2000) *J. Biol. Chem.* **275**, 41156–41165
- Xu, Y., Yankie, L., Shen, L., Jung, Y. S., Mariano, P. S., Dunaway-Mariano, D., and Martin, B. M. (1995) *Biochemistry* **34**, 2181–2187
- Lim, K., Read, R. J., Chen, C. C., Tempczyk, A., Wei, M., Ye, D., Wu, C., Dunaway-Mariano, D., and Herzberg, O. (2007) *Biochemistry* **46**, 14845–14853
- Fox, D. K., and Roseman, S. (1986) *J. Biol. Chem.* **261**, 13487–13497
- Fox, D. K., Meadow, N. D., and Roseman, S. (1986) *J. Biol. Chem.* **261**, 13498–13503
- Patel, H. V., Vyas, K. A., Mattoo, R. L., Southworth, M., Perler, F. B., Comb, D., and Roseman, S. (2006) *J. Biol. Chem.* **281**, 17579–17587
- Patel, H. V., Vyas, K. A., Savtchenko, R., and Roseman, S. (2006) *J. Biol. Chem.* **281**, 17570–17578
- García-Alles, L. F., Alfonso, I., and Erni, B. (2003) *Biochemistry* **42**, 4744–4750
- Seok, Y. J., Lee, B. R., Zhu, P. P., and Peterkofsky, A. (1996) *Proc. Natl. Acad. Sci. U.S.A.* **93**, 347–351
- Weigel, N., Kukuruzinska, M. A., Nakazawa, A., Waygood, E. B., and Roseman, S. (1982) *J. Biol. Chem.* **257**, 14477–14491
- LiCalsi, C., Crocenzi, T. S., Freire, E., and Roseman, S. (1991) *J. Biol. Chem.* **266**, 19519–19527
- Hesterkamp, T., and Erni, B. (1999) *J. Mol. Microbiol. Biotechnol.* **1**, 309–317
- Kabsch, W. (1993) *J. Appl. Crystallogr.* **26**, 795–800
- Kabsch, W. (1988) *J. Appl. Crystallogr.* **22**, 916–924
- McCoy, A. J., Grosse-Kunstleve, R. W., Storoni, L. C., and Read, R. J. (2005) *Acta Crystallogr. D Biol. Crystallogr.* **61**, 458–464
- Emsley, P., and Cowtan, K. (2004) *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2126–2132
- Adams, P. D., Grosse-Kunstleve, R. W., Hung, L. W., Ioerger, T. R., McCoy, A. J., Moriarty, N. W., Read, R. J., Sacchettini, J. C., Sauter, N. K., and Terwilliger, T. C. (2002) *Acta Crystallogr. D Biol. Crystallogr.* **58**, 1948–1954
- Siebold, C. (2002) *Structural Studies on Proteins of the Bacterial Phosphotransferase System*. Ph.D thesis, University of Bern
- Suh, J. Y., Cai, M., Williams, D. C., Jr., and Clore, G. M. (2006) *J. Biol. Chem.* **281**, 8939–8949
- Suh, J. Y., Cai, M., and Clore, G. M. (2008) *J. Biol. Chem.* **283**, 18980–18989
- Lahiri, S. D., Zhang, G., Dunaway-Mariano, D., and Allen, K. N. (2003) *Science* **299**, 2067–2071
- Christen, S., Srinivas, A., Bähler, P., Zeller, A., Pridmore, D., Bieniossek, C., Baumann, U., and Erni, B. (2006) *J. Biol. Chem.* **281**, 23129–23137
- Nunn, R. S., Marković-Housley, Z., Génovésio-Taverne, J. C., Flükiger, K.,

Structure of *S. aureus* Enzyme I

- Rizkallah, P. J., Jansonius, J. N., Schirmer, T., and Erni, B. (1996) *J. Mol. Biol.* **259**, 502–511
43. Zurbriggen, A., Jeckelmann, J. M., Christen, S., Bieniossek, C., Baumann, U., and Erni, B. (2008) *J. Biol. Chem.* **283**, 35789–35796
44. Gorrell, A., Lawrence, S. H., and Ferry, J. G. (2005) *J. Biol. Chem.* **280**, 10731–10742
45. Lukat, G. S., McCleary, W. R., Stock, A. M., and Stock, J. B. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 718–722
46. Klein, A. H., Shulla, A., Reimann, S. A., Keating, D. H., and Wolfe, A. J. (2007) *J. Bacteriol.* **189**, 5574–5581
47. Xue, Y., Xu, Y., Liu, Y., Ma, Y., and Zhou, P. (2001) *Int. J. Syst. Evol. Microbiol.* **51**, 1335–1341