Three-dimensional structure of the *Escherichia coli* phosphocarrier protein III^{glc}

(crystal structure/protein phosphorylation/signal transduction)

DAVID WORTHYLAKE*, NORMAN D. MEADOW†, SAUL ROSEMAN†, DER-ING LIAO‡, OSNAT HERZBERG‡, AND S. JAMES REMINGTON*§

*Department of Physics and Institute of Molecular Biology, University of Oregon, Eugene, OR 97403; †McCollum–Pratt Institute, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218; and ‡Center for Advanced Research in Biotechnology, Maryland Biotechnology Institute, University of Maryland, 9600 Gudelsky Drive, Rockville, MD 20850

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ABSTRACT The crystal structure of a proteolytically modified form of the *Escherichia coli* phosphocarrier and signal transducing protein III^{glc} has been determined by multiple isomorphous and molecular replacement. The model has been refined to an *R*-factor of 0.166 for data between 6- and 2.1-Å resolution with an rms deviation of 0.020 Å from ideal bond lengths and 3.2° from ideal bond angles. The molecule is a β -sheet sandwich, with six antiparallel strands on either side. Several short distorted helices line the periphery of the active site, which is a shallow extremely hydrophobic depression ≈ 18 Å in diameter near the center of one face. The side chains of the active site histidine residues 75 and 90 face each other at the center of the depression, with the N3 positions exposed to solvent, separated by 3.3 Å in an excellent position to form adducts with phosphate. Chloroplatinate forms a divalent adduct with both histidyl side chains, suggesting that the phosphodonor reaction might proceed through a similar transition state. The hydrophobic patch forms the primary crystal contact, suggesting a mode of association of III^{glc} with other components of the phosphoenolpyruvate-dependent phosphotransferase system.

The phosphoenolpyruvate:glycose phosphotransferase system (PTS) catalyzes the transport and phosphorylation of a number of simple sugars. In *Escherichia coli*, glucose uptake results from a sequential phosphate transfer reaction involving histidine residues of four PTS proteins: phosphoenolpyruvate $\rightarrow$ enzyme I $\rightarrow$ histidine-containing protein (HPr) $\rightarrow$ III^{glc} $\rightarrow$ II^{glc} $\rightarrow$ glucose. The PTS also regulates chemotaxis to PTS sugars, adenylate cyclase, certain non-PTS permeases, and the transcription of some operons (for reviews, see refs. 1–6). Genetic (7) and biochemical (2) studies show that these regulatory phenomena are controlled by the *crr* gene product III^{glc} (*M_r*, 18,099). The current model suggests that III^{glc} and/or *P*-III^{glc} interact with several proteins and that it is the ratio *P*-III^{glc}/III^{glc} that may be the determining factor in PTS-mediated regulation.

III^{glc} contains two histidine residues, His-75 and His-90. Only His-90 is required for the acceptance of *P* from *P*-HPr (8), but both are required for *P*-III^{glc} to act as a donor to a glucose analogue. Cell extracts contain two electrophoretically distinguishable forms of III^{glc}, III^{glc}_{fast} and III^{glc}_{slow}. The former is the product of an endopeptidase that cleaves the N-terminal heptapeptide from III^{glc}_{slow} (9). III^{glc}_{fast} accepts the phosphoryl group from HPr but has only 3% of the phosphodonor activity of the intact protein, suggesting that the N terminus is important in the interaction with II^{glc}. An NMR study (10) indicated that there are no detectable structural

differences between the two forms of III^{glc} and that the N-terminal 18 amino acids are disordered in solution.

To investigate the structural basis for the diversity of functions of this unusual protein, we have crystallized III^{glc}_{fast}. In this paper, we present a refined atomic model of III^{glc}_{fast}.

METHODS

III^{glc}_{fast} was isolated from an overproducing strain of *E. coli* by a modification of the procedure as described (11). *p*-Aminobenzamidine was omitted from all solutions, resulting in the production of III^{glc}_{fast} by endogenous protease. III^{glc}_{fast} and III^{glc}_{slow} were partially resolved by the DEAE-Sephacrose 6B-CL column and, in a third step, were chromatographed on a 21.5 $\times$ 150 mm Bio-Gel DEAE-5-PW HPLC column (Bio-Rad) equilibrated with 10 mM Tris SO₄, pH 7.5/0.5 mM EDTA/5% (vol/vol) glycerol (buffer A) and run at 4 ml/min. The sample (10 ml, containing no more than 50 mg of III^{glc}) was dialyzed against buffer A and transferred to the column, which was washed with 80 ml of buffer A and then with 140 ml of 0.1 M KCl in buffer A. III^{glc} was eluted with a 480-ml gradient from 0.1 M KCl in buffer A to 0.3 M KCl in buffer A, which resolved III^{glc}_{fast} and III^{glc}_{slow}. III^{glc} purified by this method was >99% pure as determined by quantitative scanning of heavily loaded SDS/PAGE gels.

Crystallization was achieved by hanging-drop vapor diffusion at room temperature. Drops of protein solution (5 μ l, 10–15 mg/ml in 25 mM Tris-HCl, pH 7.0) were mixed with an equal volume of precipitant [20% (wt/wt) polyethylene glycol (PEG; *M_r*, 8000)/400 mM sodium acetate/100 mM cacodylic acid, pH 6.6] and placed over wells in tissue culture plates (Linbro) containing 1 ml of precipitant. Rhombohedral crystals of maximum dimensions of 0.4 $\times$ 0.4 $\times$ 0.4 mm were obtained in a few days and were stored in 20% PEG 8000/100 mM Pipes/400 mM sodium acetate, pH 6.6. Heavy atom derivatives were prepared by soaking crystals in a modified storage solution. Data were collected on film using the oscillation method (12) and on a Xuong–Hamlin multiwire detector using graphite monochromated copper K- α radiation. Heavy atom positions were refined using the phase refinement module of the PROTEIN package by W. Steigemann (Max-Planck Institute for Biochemistry, Munich).

A multiple isomorphous replacement (MIR) electron density map calculated using four derivatives revealed the approximate location of the molecules in the unit cell but was otherwise uninterpretable. At this point, the crystal structure

Abbreviations: MIR, multiple isomorphous replacement; HPr, histidine-containing protein; PTS, phosphoenolpyruvate:glycose phosphotransferase system.

§To whom reprint requests should be addressed.

†The atomic coordinates and structure factors have been deposited in the Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973 (reference 1F3G).

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Table 1. Data collection statistics and heavy atom parameters

Compound	Reflections collected, no.	Unique reflections, no.	R_m^*	Phasing power [†]	Heavy atom parameter			O [‡]
					X	Y	Z	
Native crystal	15,038	8279	0.021	—				
PtCl ₄ [§]	11,749	4437	0.070	2.44	0.918	0.034	0.200	6.2
UOAC	37,014	9911	0.054	1.71	0.000	0.000	0.263	5.1
UOAC site 2					0.761	0.141	0.477	1.1

* $R_m = \sum_h \sum_i |I_{i,h} - \langle I_{i,h} \rangle| / \sum_h \sum_i I_{i,h}$, where $\langle I_{i,h} \rangle$ is the mean intensity of the i th observation.

[†]rms heavy atom scattering divided by rms lack of closure.

[‡]Fractional coordinates, occupancy on an arbitrary scale.

[§]Crystal soaking conditions: for PtCl₄, 2 mM K₂PtCl₄ in 20% PEG 8000/0.4 M sodium acetate/0.1 M Pipes, pH 6.5 (3 days); for UOAC (uranyl acetate), saturated uranyl acetate in standard storage solution (2 days). The PtCl₄ derivative data were collected on film and have lower resolution and redundancy than the area detector data sets.

of *Bacillus subtilis* III^{glc} at 2.2-Å resolution became available (ref. 13; also referred to as enzyme IIa, as it is the C-terminal domain of the glucose permease; ref. 14). It has ≈42% sequence identity with *E. coli* III^{glc}. We were unable to locate the *B. subtilis* model in the unit cell using standard molecular replacement procedures (15), so the original four-derivative electron density map was used to position the model. The position and orientation of the model were accurately determined using a six-dimensional real space search program written for the purpose, in which the α -carbon backbone was rotated in steps of 5° and translated in steps of 2 Å in a box 20 Å on a side centered on a region of high electron density. The search function was the sum of the electron density values nearest each α -carbon position.

Difference electron density maps calculated using model phases revealed that three of the four derivatives differed only in minor sites, with the major binding site on the threefold axis and had been incorrectly modeled. Two of these data sets were discarded. The two remaining derivatives led to a readily interpretable electron density map. Model building proceeded with the FRODO program (16) in both MIR and combined phase (17) electron density maps, the latter being of higher quality. In later stages $2F_o - F_c$ and $F_o - F_c$ maps (where F_o and F_c are the observed and

calculated structure amplitudes) calculated with model phases were inspected. Automated crystallographic refinement was performed with the TNT package (18). Structural overlays were performed with the OVLAP program by W. S. Bennett (Max-Planck-Gesellschaft IASMB, Hamburg, F.R.G.) using the method of Rossmann and Argos (19). Accessible surface areas were calculated as described (20) using a probe radius of 1.4 Å.

RESULTS

Data Collection and Model Building. The space group is *R*3 with $a = 80.3$ and $c = 86.6$ Å (hexagonal indexing). A single molecule in the asymmetric unit gives a crystal packing parameter $V_m = 3.0$ Å³/Da. Data were collected to the limit of the diffraction pattern (Table 1). The native data set is 68% complete to 2.1-Å resolution; however, only 10% were observed in the 2.2- to 2.1-Å shell. A 3-Å resolution MIR map calculated with the two derivatives in Table 1 had a overall figure of merit of 0.64 (Fig. 1).

The real space search locating the *B. subtilis* III^{glc} model in the unit cell showed a single prominent peak 13 standard deviations above the mean. The model was refined for 3 cycles of rigid-body refinement and 17 cycles of positional

FIG. 1. Stereoview of a segment of the double isomorphous replacement electron density map calculated using the derivatives listed in Table 1 at 3-Å resolution. The contour cage is drawn at one standard deviation of the map. Residues 88–92 from the refined model are superimposed.

FIG. 2. Stereo α -carbon diagram of *E. coli* III^{glc}, with selected side chains. The view is into the active site (His-75 and -90, enhanced). Three phenylalanine residues and a number of valines and isoleucines form a pronounced hydrophobic surface ≈ 18 Å in diameter surrounding the histidines with Asp-94 and Asp-38 at one edge.

refinement to a residual (*R*) factor of 0.37 for the resolution range of 8.0–3.0 Å.

Inspection of both MIR and combined phase maps revealed that the C-terminal section from residue 140 on was substantially displaced and the N-terminal 17 residues were disordered, so these were deleted from the *B. subtilis* model, as were side chains that were different. Seven rounds of manual model building and TNT refinement led to a model with a conventional crystallographic *R* factor of 0.166 for 7737 reflections observed between 6- and 2.1-Å resolution. Individual isotropic temperature factors were refined. The rms deviation from ideal bond lengths is 0.020 Å and from bond angles is 3.2°. All peptide (Φ , Ψ) conformational angles are in or very close to the “allowed” regions of a Ramachandran diagram.

No electron density is apparent for the residues preceding residue 18 and is weak for Gly-18 as well, so residues 19–168 have been included in the model. Fifteen water molecules have been assigned at locations where density greater than 3

standard deviations appeared in an $F_o - F_c$ map, providing at least one hydrogen bond could be made to the protein.

Molecular Architecture. Fig. 2 is an α -carbon backbone diagram with selected side chains of *E. coli* III^{glc} looking into the active site. The polypeptide backbone forms a thin antiparallel β sandwich and has a fold that appears to be unique. It may be roughly described as a parallelepiped about 30 Å on a side, but substantially thinner from face to face. The active site side of the molecule consists of six antiparallel strands, with the usual curvature, and the opposite side of the molecule shows a surprising pair of Ω -shaped loops, arranged about a local twofold axis. Two of these loops (residues 107–119 and 152–163) are Ω -loops (21), except both contain 3 residues of β -sheet. A third Ω -loop forms part of the active site (residues 71–76). The topology is shown in Fig. 3, highlighting the approximate twofold axis with regard to secondary structure. If residue 54 were connected instead to residue 134 (dashed lines in Fig. 3), two protomers of identical topology with about 80 residues each result. A 179.7° rotation superimposes 46 pairs of α -carbons with an rms deviation of 2.03 Å ($E1 = E2 = 3.0$ Å, see ref. 19 for definition of $E1$ and $E2$). Of these 46 pairs, 11 are identical in sequence, primarily in 2 pairs of peptides that interact with each other across the molecular twofold axis (bars mark identities):

59-MVAPVDGTI	112-AVGVDTVIEF
22-IIAPLSGEI	155-SVTVGTPVIRI

Perhaps III^{glc} evolved from a dimer of identical subunits and gene duplication and rearrangement resulted in the present structure.

Two segments of the paired loops forming the “back” of the molecule make several hydrogen bonds with the active site sheet and may be considered to be additional strands in that sheet; however, unlike β -barrel structures, there is no other main chain interaction between the active site sheet and the “back” side of the molecule. Several short, kinked, or distorted α - and 3_{10} -helices and β -turns are located peripheral to the active site sheet and form a ridge surrounding the two histidines. Most prominent of these are residues 32–45 [an amazing structure consisting of a 3_{10} -helix (residues 32–36), a γ -turn (residues 36–38), and an α -helix (residues 38–45) antiparallel to the 3_{10} -helix]; residues 94–103 (a 3_{10} -helix followed by two β -turns), and an α -helix (residues 123–131).

FIG. 3. Schematic diagram of the β -sheet topology of III^{glc} highlighting the approximate twofold symmetry of the fold. β -Sheet strands are indicated with arrows, and the irregular helices or important turns are indicated with cylinders of arbitrary size. The six β -sheet strands in the center form the active site face of the molecule and the two pairs of loops (right and left) form the back. The dashed lines indicate a rearrangement that results in formation of a symmetrical dimer, suggesting that gene duplication and rearrangement might have resulted in the present structure. The letter H marks the approximate positions of the active-site histidine residues.

FIG. 4. Stereo diagram of the active site of III^{glc} . Nitrogen and oxygen atoms are indicated with solid circles, and carbon is indicated with open circles. The thin bonds represent hydrogen bonds to the N1 position of the histidine rings. The contour density cage shows the $(\text{PtCl}_4\text{-native})$ difference electron density map at 3-Å resolution contoured at +4 standard deviations, showing reaction of PtCl_4^{2-} at the N3 positions of both histidines.

A comparison of the structures of *E. coli* and *B. subtilis* III^{glc} will be presented elsewhere.

Active Site. The active site is quite remarkable (Figs. 2 and 4). Surrounding the two histidines is a ring of hydrophobic residues ≈ 18 Å in diameter, including three phenylalanine side chains (Phe-41, -71, and -88) that are roughly coplanar. Several valine (Val-39, -40, -46, -73, and -96) and isoleucine (Ile-45 and -93) residues complete the ring. His-75 and -90 are adjacent, with the N3 nitrogens 3.3 Å apart. Only N3 of His-75 is accessible to solvent (accessible surface area, 2.2 Å²). His-90 is more exposed, with C1, C2, and N3 having 1.3, 2.2, and 13.9 Å², respectively. The histidine ring orientations could be determined as a result of hydrogen bonding. His-90 N1 is bonded to the carbonyl oxygen of Gly-92 (2.7 Å) and His-75 N1 is bonded to O γ 1 of Thr-73 (3.1 Å). The position and orientation of the two histidine side chains is fixed by these hydrogen bonds and steric interactions with neighboring side chains and the main and side chains of residues 95–96. This orientation appears to be perfect to allow formation of either monovalent or divalent adducts. In Fig. 4, the active site is presented along with a $(\text{PtCl}_4\text{-native})$ difference electron density map. Chloroplatinate reacts simultaneously at both N3 positions, forming a divalent adduct. Unrefined Pt–N bond lengths estimated from the native structure and the heavy atom position are 2.3 Å, with an N–Pt–N bond angle of 91° (although slight shifts of the imidazole rings would make this larger). Inspection of a $2F_o - F_c$ PtCl_4 density map revealed no evidence for large shifts of protein atoms upon reaction. The amide nitrogens of residues 94 and 95 project toward the pocket where the

phosphate group would be and may form an “oxyanion hole.”

Asp-94 and -38, which are conserved in the *B. subtilis* III^{glc} , are at the edge of the hydrophobic patch. Neither directly interacts with the histidines (Fig. 2). These residues may be important for the reaction but could also serve to confer specificity in macromolecular interactions, for example, by formation of salt bridges.

Intermolecular Contacts. The unit cell contains a close-packed trimer of III^{glc} forming a limited number of polar contacts with neighboring trimers (Table 2). The trimer is formed by van der Waals interactions of the active site hydrophobic patch of one monomer with that of another (Fig. 5). Residues 36–39 on one molecule contact Phe-71, Phe-88, and His-90 of a threefold-related molecule, with Val-39 of one molecule close to His-90 of the next. Trimer formation may not be physiologically relevant, but the type of interaction is an example of that which might occur with partner molecules during the phosphotransfer reaction or in regulatory interactions.

DISCUSSION

III^{glc} has multiple roles in *E. coli*. It acts as part of a phosphate shuttle between HPr, II^{glc} , and ultimately glucose. However, the phosphorylation state of III^{glc} apparently regulates several other metabolic pathways and is part of a signal transduction pathway reflecting the concentration of extracellular PTS sugar (6). III^{glc} therefore interacts with several different macromolecules. There are examples of two mechanisms for how such intermolecular interactions might be controlled by phosphorylation (22). Glycogen phosphorylase (23) has been shown to undergo large structural changes in response to phosphorylation that clearly effect the control of enzymatic activity. On the other hand, *E. coli* isocitrate dehydrogenase does not undergo structural perturbations upon phosphorylation (24), and it was demonstrated by mutagenesis that a negative charge at the location of the phosphate group is sufficient to inactivate the enzyme (25). Our results suggest that regulation by III^{glc} will fall into the latter class of mechanisms.

Reaction of the active-site histidines with chloroplatinate anion does not cause large structural perturbations. Thus, formation of a monovalent bond with phosphate or a divalent transition state intermediate with both histidines should not cause large structural changes. We propose the hydrophobic patch to be a site with which III^{glc} can interact with other molecules by relatively nonspecific van der Waals interactions. The affinity of a particular partner may simply depend on whether a negative charge at the center of the interaction

Table 2. Crystal contacts

Reference molecule	Neighbor	Distance, Å	Symmetry operator
Asp-35 OD1	Lys-69 NZ	3.00	1
Pro-37 CG	Glu-72 OE1	3.34	1
Pro-37 C	Phe-71 CE1	3.48	1
Ile-45 CD1	Ile-45 CD1	2.70	1
Lys-147 NZ	Glu-160 OE1	2.52	2
Lys-147 NZ	Glu-160 OE2	2.46	2
Glu-148 OE2	Asn-57 N	3.36	2
Glu-148 OE2	Val-158 CG2	3.13	2
Glu-148 OE1	Val-158 CG2	3.26	2
Glu-148 CD	Val-158 CG2	3.08	2
Leu-149 O	Asn-57 OD1	3.01	2

Only contacts shorter than 3.5 Å are listed. Symmetry operator 1 is $1 - Y, X - Y, Z$; symmetry operator 2 is $Y - X + 2/3, 4/3 - X, Z + 1/3$.

FIG. 5. Stereoview of one of the threefold-related intermolecular contacts stabilizing the trimer that forms the unit cell of the rhombohedral lattice. Residues 36–39 of one molecule (open bonds) form a van der Waals interaction with the hydrophobic patch on a threefold-related molecule (solid bonds).

site can be accommodated by the partner and, consequently, upon the phosphorylation state of III^{glc}. The hydrophobic ring may also enhance electrostatic interactions, which may be important in the phosphotransfer reaction (for example, by stabilizing neutral histidines) or conferring interaction specificity.

Two interacting surfaces the size of the hydrophobic patch on III^{glc} would bury roughly 500 Å² upon complex formation. The hydrophobic effect, estimated to be 20–25 cal·mol⁻¹·Å⁻² of buried surface (e.g., ref. 26), leads to an estimated dissociation constant of 1 μM at standard state. The *K_i* of III^{glc} for glycerol kinase is 4–10 μM, depending on pH (27), in reasonable agreement with this crude estimate. The pH dependence of the binding is consistent with the suggestion that electrostatic interactions may be important for specificity of binding.

The crystal structure of III^{glc} is in good agreement with the secondary structure assignments arrived at by solution NMR studies (10, 11), suggesting that crystallization does not cause major structural perturbations. We arrived at similar conclusions regarding the positioning of the active site histidine residues, the distorted nature of the helices, the hydrogen bonding pattern of the antiparallel sheet, and the disorder of the N-terminal residues. The phosphate group on *P*-III^{glc} is labile, making it likely that NMR techniques will be required to characterize this structure.

The fold of HPr has been determined by NMR by Klevit and Waygood (28) who commented that the active site histidine is located in a neutral to hydrophobic area and suggested that the hydrophobicity of this area might be important for the interaction of HPr and III^{glc}, in agreement with our proposal.

We have obtained several crystal forms of the regulatory complex of III^{glc} (both slow and fast forms) with glycerol kinase. The structure will be of interest in light of the hypotheses presented here.

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1. Meadow, N. D., Fox, D. K. & Roseman, S. (1990) *Annu. Rev. Biochem.* **59**, 497–542.

2. Meadow, N. D., Kukuruzinska, M. A. & Roseman, S. (1984) in *The Enzymes of Biological Membranes*, ed. Martonosi, A. (Plenum, New York), Vol. 3, 2nd Ed., pp. 523–559.
3. Stock, J. B., Ninfa, A. J. & Stock, A. M. (1989) *Microbiol. Rev.* **53**, 450–490.
4. Saier, M. H., Jr. (1989) *Microbiol. Rev.* **53**, 109–120.
5. Postma, P. W. & Lengler, J. (1985) *Microbiol. Rev.* **49**, 232–269.
6. Roseman, S. & Meadow, N. D. (1990) *J. Biol. Chem.* **265**, 2993–2996.
7. Saffen, D., Presper, K. A., Doering, T. L. & Roseman, S. (1987) *J. Biol. Chem.* **262**, 16241–16253.
8. Presper, K. A., Wong, C.-Y., Liu, L., Meadow, N. D. & Roseman, S. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4052–4055.
9. Meadow, N. D., Coyle, P., Komoryia, A., Anfinsen, C. B. & Roseman, S. (1986) *J. Biol. Chem.* **261**, 13504–13509.
10. Pelton, J. G., Torchia, D. A., Meadow, N. D., Wong, C.-Y. & Roseman, S. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3479–3483.
11. Pelton, J. G., Torchia, D. A., Meadow, N. D., Wong, C.-Y. & Roseman, S., *Biochemistry*, in press.
12. Schmid, M. F., Weaver, L. H., Holmes, M. A., Gruetter, M. G., Ohlendorf, D. H., Reynold, R. A., Remington, S. J. & Matthews, B. W. (1981) *Acta Crystallogr. A* **47**, 701–710.
13. Liao, D.-I., Kapadia, G., Reddy, P., Saier, M. H., Jr., Reizer, J. & Herzberg, O., *Biochemistry*, in press.
14. Dean, D. A., Reizer, J., Nikaido, H. & Saier, M. H. (1990) *J. Biol. Chem.* **265**, 21005–21010.
15. Crowther, R. A. & Blow, D. M. (1967) *Acta Crystallogr.* **23**, 544–548.
16. Jones, T. A. & Thirup, S. (1986) *EMBO J.* **5**, 819–822.
17. Hendrickson, W. A. & Lattmann, E. E. (1970) *Acta Crystallogr. B* **26**, 136–143.
18. Tronrud, D. E., Ten Eyck, L. F. & Matthews, B. W. (1987) *Acta Crystallogr. A* **43**, 489–501.
19. Rossmann, M. G. & Argos, P. (1977) *J. Mol. Biol.* **109**, 99–129.
20. Lee, B. & Richards, F. M. (1971) *J. Mol. Biol.* **55**, 379–400.
21. Leszczynski, J. & Rose, G. (1986) *Science* **234**, 849–855.
22. Johnson, L. N. (1991) *Curr. Biol.* **1**, 17–19.
23. Sprang, S. R., Acharya, K. R., Goldsmith, E. J., Stuart, D. I., Varvill, K. M., Fletterick, R. J., Madsen, N. B. & Johnson, L. N. (1988) *Nature (London)* **336**, 215–221.
24. Hurley, J. H., Dean, A. M., Thorsness, P. E., Koshland, D. E. & Stroud, R. M. (1990) *J. Biol. Chem.* **265**, 3599–3602.
25. Hurley, J. H., Dean, A. M., Sohl, J. L., Koshland, D. E. & Stroud, R. M. (1990) *Science* **249**, 1012–1016.
26. Janin, J. & Chothia, C. (1990) *J. Biol. Chem.* **265**, 16027–16030.
27. Novotny, M. J., Frederickson, W. L., Waygood, E. B. & Saier, M. H. (1985) *J. Bacteriol.* **162**, 810–816.
28. Klevit, R. E. & Waygood, E. B. (1986) *Biochemistry* **25**, 7774–7781.