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Chemical shift assignments of domain 4 from the phosphohexomutase from *Pseudomonas aeruginosa* suggest that freeing perturbs its coevolved domain interface

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Abstract

A domain needed for the catalytic efficiency of an enzyme model of simple processivity and domain–domain interactions has been characterized by NMR. This domain 4 from phosphomannomutase/phosphoglucomutase (PMM/PGM) closes upon glucose phosphate and mannose phosphate ligands in the active site, and can modestly reconstitute activity of enzyme truncated to domains 1–3. This enzyme supports biosynthesis of the saccharide-derived virulence factors (rhamnolipids, lipo-polysaccharides, and alginate) of the opportunistic bacterial pathogen *Pseudomonas aeruginosa*. ^1H , ^{13}C , and ^{15}N NMR chemical shift assignments of domain 4 of PMM/PGM suggest preservation and independence of its structure when separated from domains 1–3. The face of domain 4 that packs with domain 3 is perturbed in NMR spectra without disrupting this fold. The perturbed residues overlap both the most highly coevolved positions in the interface and residues lining a cavity at the domain interface.

Keywords

Domain interactions; NMR spectroscopy; Multi-domain enzyme; Phosphomannomutase; Phosphoglucomutase

Biological context

The interplay of domains is important for the activity of enzymes. One comparatively simple, processive enzyme utilizing multiple domains is phosphomannomutase/phosphoglucomutase (PMM/PGM), which processes sugars in carbohydrate biosynthesis (Regni et al. 2006). Its catalysis (Naught and Tipton 2001, 2005), structure (Regni et al. 2002), ligand-binding (Regni et al. 2004), evolution (Shackelford et al. 2004; Lee et al.

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2012), and domain movement (Regni et al. 2006; Sarma et al. 2012; Luebbering et al. 2012) have been characterized in a bacterial representative of the α -D-phosphohexomutase superfamily. The enzyme catalyzes reversible phosphoryl transfer between glucose 6-phosphate and glucose 1-phosphate (G1P), or between mannose 6-phosphate and mannose 1-phosphate (M1P) (Ray and Roscelli 1964; Naught and Tipton 2001, 2005). *Pseudomonas aeruginosa* employs PMM/PGM in biosynthesis of the three kinds of saccharide-derived molecules associated with its virulence, namely rhamnolipids, lipo-polysaccharides, and alginate (Olvera et al. 1999), which are important for infiltration of airway barriers (Zulianello et al. 2006), stimulating a host response, and impeding oxygen diffusion in the lung (Hassett 1996), respectively.

Crystal structures revealed four domains of bacterial PMM/PGM (Regni et al. 2002). C-terminal domain 4 (D4) contributes the phosphate binding site that provides most of the hydrogen bonding to the phosphoryl group of singly phosphorylated hexose ligands (Regni et al. 2004, 2006). D4 comprises a sheet of six anti-parallel β -strands that faces the cleft and two α -helices, which are 3 or 4.5 turns in length and distal from the active site (Regni et al. 2002). Full or partial closure of the catalytic cleft upon phosphohexose substrate/product binding involves rotation of D4 about a hinge at the linker joining it to domain 3 (D3) (Regni et al. 2006). A half-open state suggested that a similar rotation of D4 with partial opening of the cleft could accommodate the 180° reorientation of the hexose-1,6-bisphosphate intermediate on the enzyme implicated by kinetics and mechanism of the enzyme (Naught and Tipton 2005). Wild-type PMM/PGM limits the flexibility of the hinge between D3 and D4 with a proline in order to optimize activity (Schramm et al. 2008). Part of the surfaces of D3 and D4 in contact were recently demonstrated to have coevolved in the PMM/PGM family (Lee et al. 2012).

D4, expressed separately from the enzyme, reconstituted limited activity of bacterial PMM/PGM truncated to domains 1–3 (D1–3) (Schramm et al. 2010). Chemical shift assignments are nearly complete from backbone through side chains of D4. They indicate the preservation of the structure and folding of D4. The spectra, peak lists, and protein are attractive for training and methods development exercises. The side chain assignments may aid assignment of methyl resonances in the intact enzyme. Interestingly, the chemical shift perturbations of D4 from separating it from the enzyme map to the face of D4 that packs with D3, and include the key coevolved residues and cavity-lining residues of the interface.

Methods and experiments

Protein expression and purification

D4 from PMM/PGM was expressed and purified with a yield of 160 mg/l, as described (Schramm et al. 2010), but with the use of ^{13}C glucose (Sigma-Isotec) supplemented by 5 % (v/v) ^{13}C , ^{15}N -labeled Celtone CN (Cambridge Isotope Laboratories). Its N-terminal hexahistidine tag added 20 residues to the 95 residues of D4. The D4 sample was dialyzed against 50 mM MOPS (pH 7.4), 1 mM MgCl_2 , and 0.1 % NaN_3 , and concentrated up to 2.1 mM by Bradford assay. D_2O was added to 5 % (v/v).

NMR spectroscopy

NMR spectra were acquired at 37 °C with a Bruker Avance III 800 MHz spectrometer with TCI cryoprobe. NMR spectra were processed with Bruker Topspin 2.1. Assignment of backbone amide, C α , and C β resonances relied on a suite of triple resonance spectra expedited by the BEST approach: HNCA, HN(CO)CA, HNCO, HN(CA)CO, HNCACB, and HN(CO)CACB (Lescop et al. 2007). Backbone resonances were interpreted with CARA (Keller 2004), SAGA (Crippen et al. 2010), and Sparky (Goddard and Kneller 2000). Ambiguities remaining in sequential connections were resolved using (H)N(CAN)NH (Weisemann et al. 1993). Assignment of aliphatic ^1H and ^{13}C resonances used (H)CC(CO)NH, H(CCO)NH (Montelione et al. 1992; Grzesiek et al. 1993), HCCH-TOCSY (Kay et al. 1993), and constant-time ^{13}C HSQC (Vuister and Bax 1992; Hallenga and Lippens 1995). Aromatic side chain assignments were determined using (HB)CB(CG)CDHD and (HB)CB(CGCDCE)HE spectra (Yamazaki et al. 1993) joined by ct-TROSY-(H)CCH-COSY of the aromatic region (Pervushin et al. 1998). CCPNmr Analysis (Vranken et al. 2005) was used to assign the side chain resonances. Referencing of ^1H , ^{13}C , and ^{15}N assignments was indirectly to DSS (Edison et al. 1994). DEVise software was used to analyze all chemical shifts.

Structural assessments

To locate secondary structural elements in the sequence, the $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ chemical shifts were interpreted using the Perl script called SSP with default parameters and ^{13}C referencing adjustment turned on (Marsh et al. 2006). The NMR peak assignments were submitted to the CS23D server, which uses NMR chemical shifts to refine homology models based on sequence-matched structural templates (Wishart et al. 2008).

Assignments and data deposition

Figure 1 presents the ^{15}N HSQC spectrum of PMM/PGM D4 at pH 7.4 that contains 95 native residues and N-terminal hexahistidine tag. Two groups of amide peaks overlap: (i) S369 with S423 and (ii) A391 with L441 and the leucine from the N-terminal tag. Their assignments were sorted out using the (H)N(CAN)NH and HNCACB spectra. Assignments of backbone and side chain ^1H , ^{13}C , and ^{15}N resonances are nearly complete, but for D424, T425, T426 with amide peaks not identified. The resonances of over 83 % of assignable side chain and backbone atoms were assigned (Fig. S1 of Supplementary Material). Assignments of side chain amide and amine groups were neglected. Validation was performed with BMRB validation software. The chemical shifts have been deposited in the BioMagResBank (<http://www.bmr.b.wisc.edu>) under accession code 19044.

The spectra and peak pick lists proved useful in training of graduate students and are available upon request. As samples of D4 are appealing for methods implementation and development, its expression construct is also available upon request.

Intact structure with perturbed domain interface

We compared the secondary structure propensity of isolated D4 with that in the intact enzyme) using their respective $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ shifts and a recent algorithm (Marsh et al.

2006). The trends in the isolated form of D4 support the retention of both α -helices and five of the six β -strands (Fig. S2 of Supplementary Material). The chemical shift assignments of the isolated form of D4 were submitted to the CS23D server in order to refine a homology model with the chemical shifts (Wishart et al. 2008). The resulting model of D4 separated from PMM/PGM is very similar to the crystallographic coordinates of D4 in the apo state of the full length enzyme (PDB code 1K35) (Fig. 2b). Small secondary structure propensities at the N-terminus (Fig. S2 of Supplementary Material) and the deviation there in the NMR-enhanced structural model (Fig. 2b) suggest loss of the N-terminal β -strand in isolated D4. The N-terminus is presumably perturbed by replacement of its linkage to D3 with a His tag. The agreement of the crystal structure of the enzyme, the NMR-enhanced model of isolated D4 (Fig. 2b), and their secondary structures (Fig. S2 of Supplementary Material) gives the dominant impression that the isolated form of D4 has retained its structure overall and that it folds autonomously.

We further mapped where D4 may adjust when separated from PMM/PGM by locating the accompanying perturbations in NMR spectra of backbone amide and carbonyl groups ($\omega_{\text{HNC}'}$). The larger of these spectral perturbations with $\omega_{\text{HNC}'} > 0.3$ lie at the N-terminus, four β -strands, and a loop (Fig. 2). These perturbations from separation from the enzyme map to the face that packs with D3 in the enzyme (blue and cyan dots and sticks in Fig. 3). This suggests that the packing of these two domains may have small effects on the interior face of D4. The similarity of the β -sheet where it would contact D3 in the enzyme suggests the structural perturbations in this region by separating D4 from the enzyme (Fig. 2b) to be minimal. Among the perturbed positions, L419, V430, and R432 show the highest level of coevolution (Lee et al. 2012), implying their functional importance in a network across the interface between D3 and D4 (Lee et al. 2012). Also among the perturbed residues of the interface are W417, R432, and F433 which surround the cavity named H2 between D3 and D4 (Chuang et al. 2010) (Fig. 3). When the enzyme closes upon a phosphosugar ligand, the H2 cavity collapses as D3 and D4 move into closer contact (Chuang et al. 2010; Regni et al. 2006). Without ligand in the active site, D4 reorients rotationally 6–10 % faster than D1–D3 (Sarma et al. 2012). The effects of isolating D4 could parallel its increased separation from D3 in intact enzyme without ligand. This is suggested by comparing the locations of chemical shift perturbations from isolation of D4 with those induced in the enzyme by a hexose-phosphate inhibitor in a preliminary titration. In both cases, the most large peak perturbations map to the central three strands of the β -sheet facing D3 (Sarma and Van Doren, unpublished). Thus, the chemical shift perturbations from releasing D4 from the enzyme appear to coincide with its functionally critical and adjustable interface with D3 in the enzyme.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Chuang GY, Mehra-Chaudhary R, Ngan CH, Zerbe BS, Kozakov D, Vajda S, Beamer LJ. Domain motion and interdomain hot spots in a multidomain enzyme. *Protein Sci.* 2010; 19(9):1662–1672.10.1002/pro.446 [PubMed: 20589904]
- Crippen G, Rousaki A, Revington M, Zhang Y, Zuiderweg E. SAGA: rapid automatic mainchain NMR assignment for large proteins. *J Biomol NMR.* 2010; 46(4):281–298.10.1007/s10858-010-9403-2 [PubMed: 20232231]
- Edison AS, Abildgaard F, Westler WM, Mooberry ES, Markley JL. Practical introduction to theory and implementation of multinuclear, multidimensional nuclear magnetic resonance experiments. *Methods Enzymol.* 1994; 239:3–79. [PubMed: 7830587]
- Goddard, TD.; Kneller, DG. SPARKY. University of California; San Francisco: 2000.
- Grzesiek S, Anglister J, Bax A. Correlation of backbone amide and aliphatic side-chain resonances in $^{13}\text{C}/^{15}\text{N}$ -enriched proteins by isotropic mixing of ^{13}C magnetization. *J Magn Reson, Ser B.* 1993; 101:114–119.
- Hallenga K, Lippens G. A constant-time ^{13}C - ^1H HSQC with uniform excitation over the complete ^{13}C chemical shift range. *J Biomol NMR.* 1995; 5(1):59–66.10.1007/bf00227470 [PubMed: 22911436]
- Hassett DJ. Anaerobic production of alginate by *Pseudomonas aeruginosa*: alginate restricts diffusion of oxygen. *J Bacteriol.* 1996; 178(24):7322–7325. [PubMed: 8955420]
- Kay LE, Xu G-Y, Singer AU, Muhandiram DR, Forman-Kay JD. A gradient-enhanced HCCH-TOCSY experiment for recording side-chain ^1H and ^{13}C correlations in H_2O samples of proteins. *J Magn Reson.* 1993; 101:333–337. Series B.
- Keller, R. PhD Swiss. Federal Institute of Technology; Zurich: 2004. Optimizing the process of nuclear magnetic resonance spectrum analysis and computer aided resonance assignment.
- Lee Y, Mick J, Furdai C, Beamer LJ. A coevolutionary residue network at the site of a functionally important conformational change in a phosphohexomutase enzyme family. *PLoS ONE.* 2012; 7(6):e38114.10.1371/journal.pone.0038114 [PubMed: 22685552]
- Lescop E, Schanda P, Brutscher B. A set of BEST triple-resonance experiments for time-optimized protein resonance assignment. *J Magn Reson.* 2007; 187(1):163–169. 10.1016/j.jmr.2007.04.002. [PubMed: 17468025]
- Luebbering EK, Mick J, Singh RK, Tanner JJ, Mehra-Chaudhary R, Beamer LJ. Conservation of functionally important global motions in an enzyme superfamily across varying quaternary structures. *J Mol Biol.* 2012; 423(5):831–846. 10.1016/j.jmb.2012.08.013. [PubMed: 22935436]
- Marsh JA, Singh VK, Jia Z, Forman-Kay JD. Sensitivity of secondary structure propensities to sequence differences between alpha- and gamma-synuclein: implications for fibrillation. *Protein Sci.* 2006; 15(12):2795–2804.10.1110/ps.062465306 [PubMed: 17088319]
- Montelione TG, Lyons AB, Emerson SD, Tashiro M. An efficient triple resonance experiment using carbon-13 isotropic mixing for determining sequence-specific resonance assignments of isotopically-enriched proteins. *J Am Chem Soc.* 1992; 114:10974–10975.
- Naught LE, Tipton PA. Kinetic mechanism and pH dependence of the kinetic parameters of *Pseudomonas aeruginosa* Phosphomannomutase/phosphoglucosyltransferase. *Arch Biochem Biophys.* 2001; 396(1):111–118. [PubMed: 11716469]
- Naught LE, Tipton PA. Formation and reorientation of glucose 1,6-bisphosphate in the PMM/PGM reaction: transient-state kinetic studies. *Biochemistry.* 2005; 44(18):6831–6836. [PubMed: 15865428]
- Olvera C, Goldberg JB, Sanchez R, Soberon-Chavez G. The *Pseudomonas aeruginosa* algC gene product participates in rhamnolipid biosynthesis. *FEMS Microbiol Lett.* 1999; 179(1):85–90. [PubMed: 10481091]

- Pervushin K, Riek R, Wider G, Wüthrich K. Transverse relaxation-optimized spectroscopy (TROSY) for NMR studies of aromatic spin systems in ^{13}C -labeled proteins. *J Am Chem Soc.* 1998; 120(25):6394–6400.10.1021/ja980742g
- Ray WJ Jr, Roscelli GA. A kinetic study of the phosphoglucomutase pathway. *J Biol Chem.* 1964; 239:1228–1236. [PubMed: 14165931]
- Regni C, Tipton PA, Beamer LJ. Crystal structure of PMM/ PGM: an enzyme in the biosynthetic pathway of *P. aeruginosa* virulence factors. *Structure.* 2002; 10(2):269–279. [PubMed: 11839312]
- Regni C, Naught LE, Tipton PA, Beamer LJ. Structural basis of diverse substrate recognition by the enzyme PMM/PGM from *P. aeruginosa*. *Structure.* 2004; 12:55–63. [PubMed: 14725765]
- Regni C, Schramm AM, Beamer LJ. The reaction of phosphohexomutase from *Pseudomonas aeruginosa*: structural insights into a simple processive enzyme. *J Biol Chem.* 2006; 281(22): 15564–15571. [PubMed: 16595672]
- Sarma AVS, Anbanandam A, Kelm A, Mehra-Chaudhary R, Wei Y, Qin P, Lee Y, Berjanskii MV, Mick JA, Beamer LJ, Van Doren SR. Solution NMR of a 463-residue phosphohexomutase: domain 4 mobility, substrates, and phosphoryl transfer defect. *Biochemistry.* 2012; 51(3):807–819.10.1021/bi201609n [PubMed: 22242625]
- Schramm AM, Mehra-Chaudhary R, Furdul CM, Beamer LJ. Backbone flexibility, conformational change, and catalysis in a phosphohexomutase from *Pseudomonas aeruginosa*. *Biochemistry.* 2008; 47(35):9154–9162. [PubMed: 18690721]
- Schramm AM, Karr D, Mehra-Chaudhary R, Van Doren SR, Furdul CM, Beamer LJ. Breaking the covalent connection: chain connectivity and the catalytic reaction of PMM/PGM. *Protein Sci.* 2010; 19(6):1235–1242.10.1002/pro.402 [PubMed: 20512975]
- Shackelford GS, Regni CA, Beamer LJ. Evolutionary trace analysis of the alpha-D-phosphohexomutase superfamily. *Protein Sci.* 2004; 13(8):2130–2138. [PubMed: 15238632]
- Vranken WF, Boucher W, Stevens TJ, Fogh RH, Pajon A, Llinas M, Ulrich EL, Markley JL, Ionides J, Laue ED. The CCPN data model for NMR spectroscopy: development of a software pipeline. *Proteins Struct Funct Bioinf.* 2005; 59(4):687–696. 10. 1002/prot.20449.
- Vuister GW, Bax A. Resolution enhancement and spectral editing of uniformly ^{13}C -enriched proteins by homonuclear broadband ^{13}C decoupling. *J Magn Reson.* 1992; 98:428–435.
- Weisemann R, Ruterjans H, Bermel W. 3D Triple-resonance NMR techniques for the sequential assignment of NH and ^{15}N resonances in ^{15}N - and ^{13}C -labelled proteins. *J Biomol NMR.* 1993; 3:113–120. [PubMed: 8448431]
- Wishart DS, Arndt D, Berjanskii M, Tang P, Zhou J, Lin G. CS23D: a web server for rapid protein structure generation using NMR chemical shifts and sequence data. *Nucleic Acids Res.* 2008; 36(Web Server issue):W496–W502.10.1093/nar/gkn305 [PubMed: 18515350]
- Yamazaki T, Foreman-Kay JD, Kay LE. Two-dimensional NMR experiments for correlating $^{13}\text{C}\beta$ and ^1H chemical shifts of aromatic residues in ^{13}C -labeled proteins via scalar couplings. *J Am Chem Soc.* 1993; 115:11054–11055.
- Zulianello L, Canard C, Köhler T, Caille D, Lacroix J-S, Meda P. Rhamnolipids are virulence factors that promote early infiltration of primary human airway epithelia by *Pseudomonas aeruginosa*. *Infect Immun.* 2006; 74(6):3134–3147. 10.1128/iai. 01772-05. [PubMed: 16714541]

Fig. 1.
 ^{15}N HSQC spectrum of 2 mM D4 from PMM/PGM (pH 7.4, 310 K, and 800 MHz ^1H frequency) labeled with the backbone assignments

Fig. 2.

Preservation of fold of freed D4 and perturbation of its face that packs with D3 in PMM/PGM. **a** The amide and carbonyl peaks of D4, isolated or in full-length enzyme (BMRB entry 17602) (Sarma et al. 2012), are compared as $\omega_{\text{HNC}}' = [(\omega_{\text{HN}})^2 + (\omega_{\text{N}}/6)^2 + (\omega_{\text{C}}'/2.4)^2]^{1/2}$. **b** Plotted are the homology model refined by CS23D using the chemical shifts of the isolated state of D4 in solution (*green*) superimposed with D4 from the apo form of intact PMM/PGM (*orange*, PDB code 1K35) (Regni et al. 2002). The phosphate binding site is marked with C α -C β -C γ atoms and *purple*. The residues with $\omega_{\text{HNC}}' \geq 0.3$ from **a** are colored *red*

Fig. 3.

Perturbation of the face that packs with D3 in PMM/PGM. The interface between D4 (*blue*) and D3 (*red*) from the apo-enzyme (PDB code 1K35) coincides with: (1) residues with NMR spectral perturbations from separation of D4 from the enzyme (from Fig. 2), (2) a coevolved network, and (3) a cavity. Side chains and dots are drawn for D4 residues with NMR peaks perturbed ($\omega_{\text{HNC}'} \geq 0.3$ in Fig. 2a) by separating it from PMM/PGM. Cyan additionally indicates either high degree of coevolution (*pink labeling*) (Lee et al. 2012) or contact with the solvent mapped cavity named H2 (*italics*) (Chuang et al. 2010)