TECHNICAL COMMENT

Response to Comment on "The Pentacovalent Phosphorus Intermediate of a Phosphoryl Transfer Reaction"

Blackburn *et al.* (*1*) state that the phosphorane intermediate observed in the β-phosphoglucomutase (β-PGM) structure reported by Lahiri *et al*. (*2*) was mistaken for a transition state analog formed between enzyme, magnesium trifluoride, and glucose-6-phosphate. Here, we provide five lines of evidence that support the original structure assignment in (*2*) and refute the claim made by Blackburn *et al*. (*1*).

First, the β-PGM–phosphorane complex contains two phosphoryl groups per enzyme subunit. A putative β-PGM–magnesium trifluoride complex, on the other hand, would contain one phosphoryl group per subunit. Bradford protein and Malachite Green phosphate assays, carried out in triplicate on solutions generated from washed crystals, defined an enzyme:phosphate stiochiometry of 1:2 within 10% error. This result is consistent with the phosphorane structure and inconsistent with the magnesium trifluoride model.

Second, published Mg–F bond distances are in the range of 1.9 to 2.0 Å (*3*–*6*), whereas published equatorial P–O bond distances for oxyphosphoranes are 1.7 Å (*7*, *8*). The 1.2 Å resolution structure reported in (*2*), as well as a recent 0.9 Å resolution structure of the same phosphorane intermediate, defines the equatorial P–O bond distance of the C(1)–phosphorane as 1.7 ± 0.1 Å.

Third, an anomalous-difference electron density was calculated using the single-wavelength 1.2 Å dataset and protein model phases only (excluding cofactor and ligand). This electron density map, contoured at 3.5σ, shows electron density of identical magnitude for both atoms assigned as phosphorus. This result is not consistent with the β-PGM–magnesium trifluoride structure because the anomalous scattering from the Mg ion is less than half that of P (at the wavelength of data collection). In addition, if a protein electron density map is calculated on an absolute scale (number of electrons) using only observed amplitudes and protein model phases (excluding ligand), the same number of electrons are present at peaks corresponding to the C(1)P and C(6)P position.

Fourth, crystals of the β-PGM complex are formed in crystallization solutions containing as little as 1 mM ammonium fluoride, yet ammonium fluoride at three times this concentration does not inhibit β-phosphoglucomutase catalysis. This result is consistent with the β-PGM–phosphorane complex, in which fluoride is not bound to the active site. It is inconsistent with a β-PGM–magnesium trifluoride complex, in which fluoride forms a transition state analog in conjunction with Mg ion.

Fifth, the phosphorane intermediate observed in the β-PGM–complex structure has precedent in chemical models (*7*, *8*). The magnesium trifluoride species cited by Blackburn *et al*. (*1*), by contrast, has no proven chemical model. The lone example of magnesium trifluoride is that of the G protein–GDP–magnesium fluoride complex published by Graham *et al.* (*9*), cited in the Blackburn *et al*. comment (*1*). The evidence for this complex is not convincing. Although a proton-induced x-ray emission spectroscopy (PIXE) experiment was carried out to demonstrate a 1:1 P:Mg ratio, there was no determination of the Mg:F ratio. The x-ray structure was determined to 1.8 Å; however, the Mg–F bond length was not reported in the publication. In summary, the study in (*9*) offers no proof of the existence of magnesium trifluoride in solution or bound to the G protein, nor any explanation of why Mg(II) would form magnesium trifluoride in neutral solution or in the active site of an enzyme.

Karen N. Allen
Department of Physiology and Biophysics
Boston University School of Medicine
715 Albany Street, Boston, MA 02118-2394
E-mail: allen@med-xtal.bu.edu

Debra Dunaway-Mariano
Department of Chemistry
University of New Mexico
Albuquerque, NM, 87131
E-mail: dd39@unm.edu

References and Notes

1. G. M. Blackburn, N. H. Williams, S. J. Gamblin, S. J. Smerdon, *Science* **301**, 1184 (2003); www.sciencemag.org/cgi/content/full/301/5637/1184c.
2. S. D. Lahiri, G. Zhang, D. Dunaway-Mariano, K. N. Allen, *Science* **299**, 2067 (2003).
3. H. Hao *et al*., *J. Fluorine Chem.* **155**, 143 (2002).
4. See references 5 to 8 in (3).
5. Y. Zhao, *J. Solid State Chem.* **141**, 121 (1998).
6. A. Caramanian, J.-P. Souron, P. Gredin, A. de Kozak, *J. Solid State Chem.* **159**, 234 (2001).
7. D. E. C. C. Corbridge, *The Structural Chemistry of Phosphorus* (Elsevier, Amsterdam, 1974).
8. R. R. Holmes, "Pentacoordinated Phosphorous," *ACS Monograph 175* (1980), vol. 1.
9. D. L. Graham *et al*., *Chem. Biol.* **9**, 375 (2002).

28 May 2003; accepted 12 August 2003