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G. Michael Blackburn, *et al.*

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

The claim by Lahiri *et al.* (1) to have identified the pentacovalent phosphorus intermediate of a phosphoryl transfer reaction addresses an important, long-disputed problem: whether the enzyme-catalyzed transfer of phosphate is a dissociative or associative process. The apparent observation of a pentaoxy-phosphorane suggests that the transfer occurs through an associative, addition-elimination process and contrasts with the solution mechanism, which is dissociative in character and has little bond order to the nucleophile and leaving group in the transition state (2).

However, the apical P–O bond lengths for this trigonal bipyramidal intermediate (2.0 and 2.1 Å) are significantly longer than those in small-molecule phosphorane structures [e.g., 1.65 Å and 1.67 Å for pentacyclohexyloxy-phosphorane (3)]. These distances are not unreasonable for a transition state structure with partial apical bonds (4), but by definition this is not a stable species. Even at 93 K, the half-life for decomposition of an activated complex is $<10^{-11}$ s, so this cannot be a kinetically trapped transition state. To be thermodynamically viable, such a TS complex would need to be comparable in stability with the starting and product complexes, which implies that the kinetic barrier to reaction has disappeared (and so the transfer reaction should become ultrafast at low temperature) and that the thermodynamic properties of the transition state are very unusual (because the change in temperature from 291 to 93 K would have to lead to a decrease in $\Delta G^\ddagger$ of some 12 kcal mol $^{-1}$ for the reaction within the ES complex). From a structural point of view, we were also concerned that

some of the attributes of various Fourier maps described by Lahiri *et al.* (1) did not seem consistent with a PO_3^{n-} moiety.

Is there an alternative, better interpretation of these data? The crystallization conditions used by Lahiri *et al.* (1) contained 10 mM Mg^{2+} and 100 mM NH_4F . Our published results (5) have shown that MgF_3^- is a good mimic of the transition state of phosphoryl transfer reactions and can be formed under conditions such as those employed by Lahiri *et al.* (1). Our crystal structure of a small GTPase complexed with its GTPase-activating protein (GAP) shows that MgF_3^- sits in the active site and adopts a trigonal bipyramidal arrangement with similar apical bond lengths (2.0 and 2.3 Å) to the nucleophile and leaving group as those assigned to the "phosphorane" above. It therefore seems plausible that the PGM structure contains an MgF_3^- rather than a PO_3^{n-} species. We further note that fluoride catalyses the dephosphorylation of a phosphoenzyme without affecting activity (6), furnishing a kinetic pathway for loss of phosphate from the PGM complex.

We have previously confirmed that our G protein complex really contained the MgF_3^- moiety by proton-induced x-ray emission spectroscopy (PIXE) (5). This technique is capable of establishing directly the ratio of P/Mg in the crystals of the PGM complex in question. If its structure is indeed a phosphorane intermediate, the P/Mg ratio will be 2:1; if it is an MgF_3^- complex, the ratio will be 1:2.

The high-resolution crystallographic data of Lahiri *et al.* (1) are fully compatible (7) with our analysis that the PGM structure is

likely to contain an MgF_3^- transition state analog, rather than to represent the high-energy phosphorane intermediate suggested in (1). This change in interpretation is crucial, because the structural analog can be interpreted with equal validity as a mimic of the transition state for a concerted reaction, as a phosphorane intermediate, or as a metaphosphate species in a dissociative process. The difference between these systems is simply a few tenths of an angstrom in the apical bond distances and cannot be resolved solely on the basis of a model structure. Therefore, we believe that the catalytic mechanism of PGM remains unidentified.

Seeing may be believing (8)—but only when you truly apprehend the object itself!

G. Michael Blackburn
Nicholas H. Williams

Krebs Institute
University of Sheffield
Sheffield, S3 7HF, UK

E-mail: g.m.blackburn@shef.ac.uk;
n.h.williams@shef.ac.uk

Steven J. Gamblin
Stephen J. Smerdon

National Institute for Medical Research
Mill Hill
London, NW7 1AA, UK

E-mail: sgambli@nimr.mrc.ac.uk;
ssmerdo@nimr.mrc.ac.uk

References and Notes

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