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 \( \langle 10^{-11} \text{ s} \rangle \), 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 \( \Delta G^\circ \) 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\(_4^{3-}\) moiety.

Is there an alternative, better interpretation of these data? The crystalization conditions used by Lahiri et al. (1) contained 10 mM Mg\(^{2+}\) and 100 mM NH\(_4\)F. 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\(_4^{3-}\) species. We further note that fluoride catalyses the dephosphorylation of a phosphoenzym 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!

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References and Notes
5. D. L. Graham et al., Chem. Biol. 9, 375 (2002); coordinates deposited with PDB code 1OW3.
7. The proximate amino acids (V9, D10, A115, K145) make shorter hydrogen bonds to the equatorial fluorines than those described in (1).

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