Rebuttal: Beryll fluoride Binding Mimics Phosphorylation of Aspartate in Response Regulators

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In my article (3), I have stated that the phosphotransferring state is the most active state of Spo0F and the solution structure of Spo0F:BeF₃⁻ is unlikely to be a good model for the phosphorylated state. In their article, Wemmer and Kern (4) state that they expect the conformational changes induced by BeF₃⁻ on Spo0F to be very similar to those caused by phosphorylation. They also state that helix α 4 is a very likely site for the kinase interactions.

I agree with Wemmer and Kern that the binding of BeF₃⁻ to response regulators mimics phosphorylation. My only disagreement is on the solution structure of Spo0F:BeF₃⁻ (1). All crystallographic studies on response regulators so far show that BeF₃⁻ binding produces conformational changes very similar to those induced by phosphorylation. One of the most significant aspects of the conformational changes is the repositioning of a Thr/Ser residue to a new location where it interacts with a phosphoryl oxygen or a fluorine atom. As I stated in my article, in the Spo0F:BeF₃⁻ solution structure, Thr82 is not suitably positioned for such an interaction. The disagreement between the nuclear magnetic resonance data of Spo0F and the crystallographic data of other response regulators could arise from any of the following reasons. The crystallization process may favor one specific conformational state into the crystal lattice, while multiple conformational states could coexist in the solution. Another possibility is that, in the nuclear magnetic resonance analysis, certain nuclear Overhauser effects are not experimentally observed and therefore the corresponding restraints are absent from the refinement calculations.

Does helix α 4 (residues Leu87 to Leu96) interact with KinA or KinB? The point mutations of residues Leu87, Asp88, and Ile90 on this helix to alanine gave rise to altered phenotypes (2), suggesting the possibility that these residues could have interactions with the kinases, Spo0B, or phosphatases. On the other hand, kinetic analysis showed that the mutation of resi-

due 87 affected the phosphoryl transfer between Spo0F and KinA, while the mutations of 88 and 90 did not have significant effects. Therefore, it appears that KinA may not have extensive interactions with helix α 4, although it plays an important role in the signal transduction of many other pathways. For example, CheY-P uses the α 4- β 5- α 5 surface to bind to FlhM, and a number of other response regulators use this surface to form homodimers en route to transcriptional activation. Spo0F, in contrast, does not use the α 4- β 5- α 5 surface for phosphotransfer to Spo0B (5). Spo0F binding to KinA will also be very similar.

Finally, I wish to make a general comment about the use of the word “activated,” as it is sometimes used as a synonym for “phosphorylated.” In my view, it is incorrect to talk about the activated state without taking the activity of the molecule into consideration. It is true that certain molecules which remain inactive are turned on by phosphorylation. For example, the phosphorylation of the transcription factor Spo0A significantly enhances its binding affinity to DNA. Spo0F, on the other hand, is a messenger, and its role is to carry the message from the kinases to Spo0B by receiving a phosphoryl group from any of the kinases and giving it to Spo0B. In order to carry out this function, Spo0F goes through three distinct states—the unphosphorylated state, the phosphotransferring state, and the phosphorylated state. Of these three states, the phosphotransferring state should be considered the most active state.

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