Rebuttal: Conformational Changes of Spo0F along the Phosphotransfer Pathway

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In our article (3), we argue that formation of BeF_3^- complexes with receiver domains leads to conformational changes that mimic phosphorylation. In the opposing article, Varughese (2) argues that this might not be the case for Spo0F. As with any structure, the model produced should generate ideas for further experiments to test specific aspects of the interpretation. The functional equivalence of BeF_3^- complex formation and phosphorylation does remain to be directly tested for Spo0F; solving a crystal structure of the BeF_3^- complex of Spo0F with Spo0B might be a good test.

In our original paper, we did use the term “activated state” for BeF_3^- -Spo0F to imply that its conformation is functionally equivalent to the phosphorylated form. This conclusion was based on significant structural changes seen when BeF_3^- -Spo0F is compared to Spo0F, some of which are similar to the changes seen upon activation in other response regulators. In contrast, the structure of Spo0F in the Spo0F:Spo0B cocrystal is almost identical to that of uncomplexed Spo0F.

There are aspects of the BeF_3^- -Spo0F structure that, as Varughese points out, differ from structures of phosphorylated receiver domains, particularly the detailed structure around the active site. It is well known that certain types of interaction, e.g., the interaction between the Lys104 amino group and the BeF_3^- (or phosphate) or the contact between the O^γ of Thr82 and O^δ of Asp, cannot be identified by nuclear magnetic resonance (NMR), and that crystallography provides more accurate information. We examined this issue in some detail (1), finding that NMR data for NtrC’s receiver domain are consistent with the active site geometry defined by crystallography

but are not sufficient to define this geometry. Fortunately, the conformational changes away from the active site have been consistently defined by both NMR and crystallography, and the active-site structure determined by diffraction for both phosphorylated and BeF_3^- -modified receiver domains are quite consistent and can probably be safely modeled in NMR structures.

However, crystal contacts can trap one conformation from the ensemble existing in solution. Varughese’s comments on variability in the position of Tyr106 in CheY show this.

Varughese makes a statement regarding constitutively active CheY mutants. In the absence of kinetic studies of conformational changes in these variants, no statement can be made about the energy barrier between the ground state and activated states. The statement that can be made is that the difference in free energy between the ground and activated states is smaller in the variants than in the wild type.

Published diffraction and NMR data for unphosphorylated Spo0F do not agree on the structure of helix-4. We felt the solution structure was less likely to be perturbed from the real structure in vivo and hence made our comparison to it. This region has been shown to be flexible in many receiver domains; it may be that no single conformation properly represents it. Our comparison was between NMR in a solution of Spo0F and NMR in a solution of Spo0F- BeF_3^- ; the NMR data suggest that there is a difference in the solution structures at the beginning of helix-4 with and without BeF_3^- .

REFERENCES

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