Beryll fluoride Binding Mimics Phosphorylation of Aspartate in Response Regulators

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The idea that reversible binding of a small inorganic ion can mimic covalent modification by phosphate is, at first, surprising. However, there is a fairly long history of using such compounds (aluminum fluoride and vanadate as well as beryll fluoride) to form complexes with nucleoside diphosphates at the active sites of ATPases and GTPases (1, 2, 6, 13). Indeed, it was in an attempt to form such a complex with beryll fluoride and ADP at the ATP binding site of the σ^{54} activator NtrC that Dalai Yan et al. found activation of the protein rather than the anticipated inhibition (15). Subsequent detailed investigation showed that addition of BeF_3^- yielded an activity level as high as that from phosphorylation in a direct assay of transcriptional activation by NtrC. The interaction with BeF_3^- is very specific to the active site in the response regulator receiver domain, which is normally activated by phosphotransfer from a histidine kinase as part of a two-component signaling system (14). The aspartate that either is phosphorylated or interacts with the beryll fluoride is part of an active site that greatly enhances the rate of transfer of phosphate from activated phosphodonors, including the phosphohistidine of the kinase. It is the interaction with residues that constitute this active site, including a bound magnesium ion, that facilitates phosphotransfer and beryll fluoride binding.

The instability of the aspartyl phosphoanhydride formed in response regulators makes study of their active forms challenging. By use of a regeneration system initially applied to the chemotaxis-signaling protein CheY (11), a structure of the phosphorylated receiver domain of NtrC was solved by nuclear magnetic resonance (NMR) (8). The structure was subsequently compared with the BeF_3^- complex (7) (for which more-complete data could be collected), with the conclusion that the two structures were the same within the resolution of the measurements and that each was distinctly different from the inactive structure, particularly in the region of helix-4 on the output face of the protein. The structural similarity of the phospho- and BeF_3^- proteins, together with the biochemical assays showing equal activity, indicates that the beryll fluoride complex with NtrC is fully equivalent to the phosphorylated form. The structure of the tetrahedral BeF_3^- in the active site reinforces the previously recognized similarity of the geometries of BeF_3^- and phosphate, including bond lengths and angles. The combined negative charges of the aspartate and BeF_3^- also give a similar charge distribution.

Is this equivalence general? After the initial discovery of

activation of NtrC, several other response regulators were investigated. CheY is a receiver domain involved in signal transfer in bacterial chemotaxis. Phosphorylation by the histidine kinase CheA increases CheY's binding affinity for its target in the flagellar motor, FliM. CheY binding to FliM causes a reversal in the direction of flagellar rotation. Binding of CheY to a peptide from FliM was shown to occur with the full affinity of intact FliM. Measuring tryptophan fluorescence in CheY, we found that BeF_3^- binding led to the same fluorescence change as phosphorylation and we also found the same increase in affinity for binding to the FliM peptide. Furthermore, BeF_3^- binding induced the same NMR chemical shift changes in backbone ^1H and ^{15}N resonances that phosphorylation did (11), again arguing that the induced conformational changes are equivalent. Crystal structures of both BeF_3^- -activated CheY (9) (Fig. 1) and its complex with the FliM peptide (10) were subsequently solved (Fig. 1). Comparison of these with structures of phosphorylated receiver domains from FixJ and Spo0A indicated that the active-site interactions that are specific to the activated forms are equivalent (9). Biochemical assays were also done with the response regulators OmpR and NarL (following changes in DNA binding affinity induced by phosphorylation and BeF_3^- binding [15]), again indicating full equivalence of activation.

In the response regulator DctD, like NtrC an activator of σ^{54} -polymerase, it has been shown that activation leads to a reorganization of a dimeric state of the receiver domain. Crystal structures of both the inactive and BeF_3^- -activated forms were solved, showing that a reorganization of the $\alpha 4$ - $\beta 5$ face leads to different dimer interfaces in the two states and thus to a large change in the relative positions of the monomers. Recently, the structure of the inactive form of a thermophilic σ^{54} activator, NtrC1, was solved. The construct included both the receiver domain and the regulated ATPase domain, and the receiver domain dimer found was identical to that in DctD. Subsequently, the structure of the phosphorylated (active) form of just the receiver domain of NtrC1 was solved, and the dimer found was equivalent to the DctD- BeF_3^- -activated form (3). The details of this reorganization are quite different from those for the other examples, arguing that there is considerable generality in the equivalence of activation by phosphorylation and BeF_3^- complex formation.

The protein Spo0F is part of the pathway regulating spore formation in bacteria such as *Bacillus subtilis*. It is a receiver domain with the same fold as those discussed above (clear by sequence homology and also from NMR [4] and crystal structures [12] that have been previously determined), but it plays a somewhat different role, acting as a phospho-shuttle protein between two kinases rather than controlling an output re-

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FIG. 1. Active-site region of the CheY-BeF₃⁻ crystal structure (9) showing contacts to the berylliofluoride group (green) that mimic those of phosphate as seen in Spo0A and FixJ structures.

sponse directly. A crystal structure for Spo0F in complex with the downstream kinase from *B. subtilis*, Spo0B (16), was solved. A substantial interaction surface between Spo0F and Spo0B positions the histidine in Spo0B, to which phosphotransfer occurs, immediately above the active site of Spo0F, a good position for the phosphotransfer reaction. The conformation of Spo0F in this complex is very similar to that of the free, unphosphorylated protein. In such a phosphorelay system one might expect the initial phosphorylation of Spo0F by the upstream kinases KinA or KinB to decrease the affinity for them and increase the affinity for the next transfer partner, Spo0B, to ensure an efficient phosphotransfer in the biologically desired direction. This modulation in affinity could be accomplished by a conformational change in Spo0F, as it is in other receiver domain-containing proteins.

By use of NMR spectroscopy, it was shown that BeF₃⁻ does form a complex with Spo0F (15) and that its binding induces spectral changes, reflecting a conformational change. The structure of the BeF₃⁻-complexed form was subsequently solved by use of NMR (5). The areas with the largest changes are in the region of helix-4 (the face of the protein used for downstream interactions in previously studied activated receiver domains), in the loops connecting beta strands and helices around the active site, and in helix-1. These are reasonable in the sense that helix-4 may be involved with interactions with KinA/B, while helix-1 and the loops make significant contacts to Spo0B and hence could modulate the affinity of this interaction. However, it is always dangerous to try deduce

energetics from structure, so direct measurements of changes in affinity need to be done to verify that the changes seen with BeF₃⁻ do indeed mimic those of phosphorylation, as expected. Based on the analogy to other receiver domains, we expect that BeF₃⁻ binding will induce a conformational change very similar to that caused by phosphorylation.

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