Using NMR to study the formation of complex between enzyme 1^{NTR} and NPr

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Enzyme I^{Ntr} (EIN^{Ntr}) and NPr are the first two enzymes in a newly discovered nitrogen-based phosphorylation transfer pathway in *E. coli*.[1] There is a paralogous EIN^{sugar} and HPR involved in the phosphorylation pathway that transfers sugars across bacterial membranes. The crystal structure of the EIN^{Ntr} and the NMR structure of NPr show that the structures of the protein components in these two distinct pathways look alike. Therefore we propose that the substrate specificity in these two pathways depends on the detail of their respective molecular interactions. Using NMR we probed the forces that drive the EIN^{Ntr}:NPr complex formation, changes in protein dynamic upon formation of the complex, and transient encounter complex population in the EIN^{Ntr}:NPr system.

Using the crystal structure of free EIN^{Ntr} and the NMR structure of free NPr,[2] we determined the complex structure using residual dipolar couplings (RDCs) and chemical shift perturbations (CSPs) as ambiguous distance restraints between the two interfaces. RDCs were also used to validate the individual starting structures of EIN^{Ntr} and NPr. For the paralogous EIN^{sugar}:HPR complex, there is little change in structure upon binding.[3] If this was also the case for EIN^{Ntr} and NPr, RDCs for the complex should also fit the structures in the free form. This was indeed true for NPr, whereas the crystal structure of EIN^{Ntr} required a rotation of the α and α-β domains with respect to each other in order to fit the RDCs.The EIN^{Ntr}:NPr complex provides clear evidence for specific molecular interactions.

It is known that EIN^{sugar} and HPR sample transient encounter complexes[4] as well as a more stable minor complex[5] prior to productive binding. EIN^{Ntr} has a higher binding affinity to NPr than the EIN^{sugar}:HPR complex[1] and was therefore not expected to form these encounter complexes. PRE experiments are very sensitive to transient nonspecific complexes whereas RDCs represent an average so describe only the major, productive complex. We measured paramagnetic relaxation enhancement (PRE) data for our EIN^{Ntr}:NPr complex. PRE experiments were collected by mutation of three sites on NPr (T6C, E45C and E74C) and disulfide addition of a nitroxide spin-label MTSL. Our RDC and PRE data did not agree with each other, confirming the presence of encounter complexes. We therefore use the PRE data to describe the initial stages of complex formation by calculating an ensemble of EIN^{Ntr}:NPr encounter complexes.

The structure of the EIN^{Ntr}:NPr complex, their dynamic behavior, and the ensemble distribution of their transient encounter complexes can be compared to the homologous EIN^{sugar}:HPR complex to reveal the mechanism that prevents cross-over between the two phosphorylation pathways.

References

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