Solution Scattering Studies of Protein Conformations and Interactions in Biochemical Regulation

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Small-angle solution scattering, using both x-rays and neutrons, has provided key insights into the regulation of kinase activity. Small-angle scattering from proteins in solution is particularly sensitive to domain movements, as well as protein-protein associations. We have characterized the conformational transitions and associations in the activation mechanism of the Ca^{2+}/calmodulin-dependent kinase myosin light chain kinase (MLCK), as well as in two cyclic nucleotide-dependent protein kinases. The kinase family, with > 500 members identified to date, has a highly conserved catalytic core that consists of a large mostly alpha-helical domain and a smaller predominantly beta-structured domain. The cleft between the large and small domains encompasses all the elements of the protein required for phosphorylation of protein substrates. The structure of the catalytic subunit of the cAMP-dependent dependent kinase was the first of this class of enzymes to be solved by x-ray crystallography, and this first structure was of the enzyme with a bound peptide pseudosubstrate. We used small-angle x-ray scattering to show that the effect of pseudosubstrate binding was to close the catalytic cleft, thus bringing all of the elements required for catalysis together in close proximity to the substrate binding sites.[1] We further showed that this cleft closure was achieved via a hinge formed by a pair of glycine residues that are highly conserved in the kinase family tree branches that contain the calmodulin-dependent and cyclic nucleotide-dependent protein kinases. Catalytic activity in these kinases is commonly regulated by inhibitory mechanisms that use a pseudosubstrate sequence either as part of an auto-regulatory domain in the case of the calmodulin-dependent kinases, or from a regulatory partner as is the case for the cyclic nucleotide-dependent protein kinases. Binding of Ca^{2+} to calmodulin (CaM) which then binds to the kinase, or cyclic nucleotide binding to a regulatory partner, releases the inhibition. In our solution scattering studies of the Ca^{2+}/CaM/MLCK activation mechanism, we have evaluated the Ca^{2+}-dependent binding of MLCK to the enzyme showing that binding occurs with substoichiometric Ca^{2+} concentrations,[2] determined the conformational transitions undergone by both the kinase and CAM upon complex formation,[3] and the effects of substrate binding on the complex.[4] Our solution scattering studies of the cAMP-dependent protein kinase have revealed the quaternary structure of the kinase, which has two identical catalytic and two identical regulatory subunits, and also has revealed information on the conformation of the catalytic subunit in its inhibited state.[5] Our studies of the cGMP-dependent protein kinase have elucidated information on the conformational transitions induced by cGMP binding and subsequent release of this kinase inhibition.[6] Small-angle scattering instrumentation developments at synchrotron facilities have significantly accelerated the rate at which these types of experiments can be done, and thus have enabled us to systematically track the conformational transitions and associations within specific activation pathways.

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