PROTEIN CONFORMATIONS AND INTERACTIONS IN BIOCHEMICAL REGULATION

Small-angle neutron scattering, with contrast variation, has provided key insights into the regulation of protein activities. Life functions at the molecular level via a large number of highly regulated molecular networks through which messages are communicated so that cells can maintain vital ongoing functions and also can respond as needed to external stimuli. Proteins are the workhorses of life carrying out all the functions required for survival, growth and reproduction. They are responsible for motility, transport, signal transduction, catalysis, protein synthesis and degradation, energy capture, transport and conversion, damage recognition and repair, replication and transcription, etc. These activities all must be strictly coordinated, and there are a variety of mechanisms for regulating protein function that are key to healthy functioning. One of the most common regulatory mechanisms of protein activity is the addition, or removal, of phosphate groups from hydroxyl groups on proteins. Protein phosphorylation reactions are catalyzed by a family of enzymes called the protein kinases of which several hundred have been identified to date. Protein kinase activities themselves are frequently regulated by "second messengers" that are released into the cellular cytoplasm in response to a "first messenger" signal, such as a hormone binding to a cell surface receptor. Two commonly used second messengers are divalent calcium ions and cyclic nucleotides. These messengers modulate kinase activities generally by binding to intermediary regulatory proteins that then either bind to or modulate their interaction with the kinase such that the kinase activity is switched on or off.

Small-angle scattering from proteins in solution gives information on their overall shapes and is particularly sensitive to domain movements as well as protein-protein associations. In the case of neutron scattering, the differences in the scattering properties of hydrogen and deuterium allow one to use specific deuterium labeling with contrast variation to extract structural data on individual components within complexes. We have used neutron scattering with contrast variation to characterize the conformational transitions and associations in the activation mechanism of two model kinases; the Ca^{2+}/calmodulin-dependent kinase myosin light chain kinase (MLCK), and the cyclic nucleotide (cAMP)-dependent protein kinase.

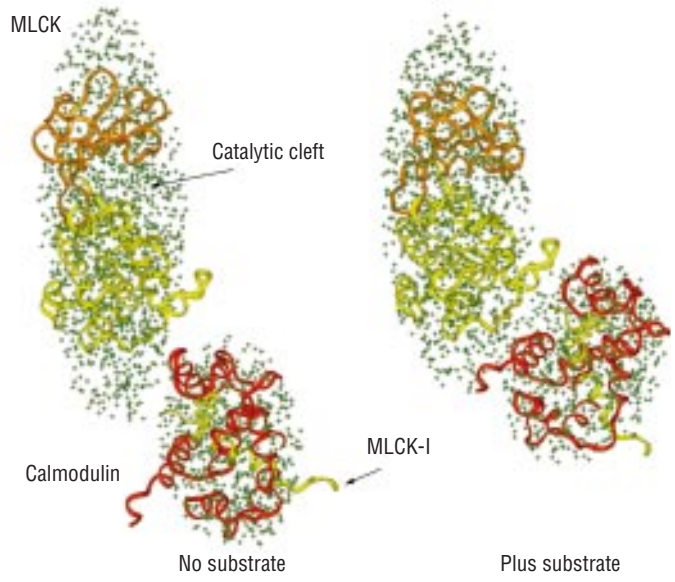

FIGURE 1. Ellipsoid models derived from the neutron scattering data for $4Ca^{2+}$•calmodulin•MLCK complexes with (right) and without (left) substrates. Superimposed within the ellipsoids are the known structures for the upper (orange ribbon) and lower (yellow ribbon) lobes of the MLCK catalytic core. Within the smaller ellipsoid Calmodulin is represented as a red ribbon, with the MLCK calmodulin-binding domain (MLCK-I) in yellow. Upon substrate binding there is movement of the calmodulin closer to the catalytic cleft. At the same time the catalytic cleft of MLCK closes, presumably about its substrate.

In our neutron scattering studies of the Ca^{2+}/calmodulin/MLCK activation mechanism we have determined the conformational transitions undergone by both the kinase and calmodulin upon complex formation [1], and the effects of substrate binding on the complex [2]. These experiments were performed using samples prepared with the MLCK enzyme complexed with deuterated calmodulin. Data were measured for the complex in solvents having a range of D_2O levels. The basic scattering functions for calmodulin and MLCK within the complex, as well as the cross term, were extracted from these neutron data. Uniform two-ellipsoid models were used to aid in the interpretation of the scattering data. Figure 1 shows the conformations of calmodulin and MLCK in the complex with and without substrate as determined by the neutron scattering experiment. By fitting the high-resolution crystal or NMR structures available on the components of these complexes into our ellipsoid models we have been able to gain new insights into the molecular basis for the kinase regulation. Figure 2 summarizes

Jill Trewhella, Joanna K. Krueger, Glenn A. Olah, and Jinkui Zhao
Los Alamos National Laboratory
Los Alamos, NM 87545

James T. Stull
University of Texas
Southwestern Medical Center
Dallas, TX 75235

Donald A. Walsh
University of California at Davis
Davis, CA 95616

the information derived from the solution scattering studies concerning the activation mechanism.

Our more recent solution scattering studies of the cAMP-dependent protein kinase [3] have revealed the quaternary structure of this kinase which has two identical catalytic and two identical regulatory subunits that bind the cAMP second messenger. This binding results in the dissociation and activation of the catalytic subunits. Again by fitting the high resolution structures into the molecular envelops defined by the neutron data, we have been able to compare and contrast the different regulatory mechanisms for the highly conserved catalytic core of the two kinases we have studied thus far.

Our neutron contrast variation studies provide critical information about the dynamic conformational transitions underlying the regulatory mechanisms we are studying. The ability to study the global shapes of the component structures within complexes in which there is inherent flexibility provides a critical framework into which higher resolution structural data on the individual components can be fit. These types of problems cannot be studied by high-resolution crystallography when flexibility inhibits crystallization. Neither can they be studied by NMR when the structures are larger than ~ 40 kDa. Thus neutron small-angle scattering and contrast variation fills an important niche that helps to assemble the molecular jigsaw puzzles that we need to solve in order to understand the way in which molecular networks operate. This understanding is key to medical and biotechnology applications that utilize biomolecules and their unique properties.

REFERENCES

[1] J. K. Krueger, G. A. Olah, S. E. Rokop, G. Zhi, J. T. Stull and J. Trewhella, "The Structure of $4Ca^{2+}$ "Calmodulin and a Functional Myosin Light Chain Kinase in the Activated Complex," Biochemistry **36**, 6017 (1997).

[2] J. Krueger, G. Zhi, J. T. Stull and J. Trewhella, "Neutron Scattering Studies Reveal Further Details of the Ca^{2+}/Calmodulin-Dependent Activation Mechanism of Myosin Light Chain Kinase," in press.

[3] J. Zhao, E. Hoyer, S. Boylen, D. A. Walsh and J Trewhella, "Quaternary Structure of the cAMP-Dependent Protein Kinase by Neutron Contrast Variation," in press.

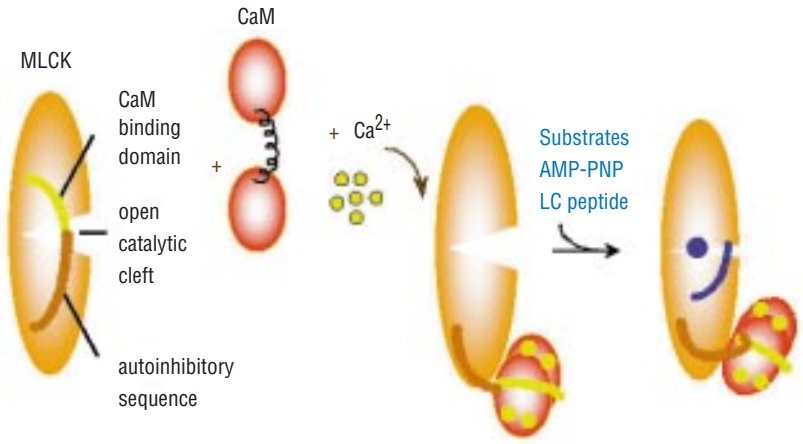

FIGURE 2. Schematic summarizing the sequential conformational transitions for calmodulin activation of MLCK. In its inactive conformation, MLCK maintains an open catalytic cleft. Upon binding $4Ca^{2+}$, calmodulin undergoes a conformational collapse as it interacts with hydrophobic residues at each end of the calmodulin-binding sequence that forms a helix. Substrate binding induces closure of the kinase catalytic cleft. Thus the fully activated complex is formed.