The activity of a large class of myosin molecules is controlled by a regulatory mechanism built into the molecule itself. As in all muscle myosins, the globular head portion (S1) of the molecule consists of both a motor domain that binds actin and has ATPase activity and an elongated regulatory domain (RD) where two different light chains (LCs) are bound. In molluscous muscles, direct binding of Ca²⁺ ions triggers activity; in vertebrate smooth muscle myosin and some non-muscle myosins, a Ca²⁺-dependent phosphorylation of the molecule acts as the trigger. The unusual state in the regulated myosin is the "off" state of enzymatic activity which appears to require interactions of both heads together with a stable portion of the coiled-coil rod region. In this state, the loss of Ca²⁺ (or dephosphorylation) produces conformational changes in the RD of each head which are transmitted to the motor domains some 100 Å distant.

Here we report the structure of the Ca²⁺-bound form of the RD of scallop myosin refined to 2.0 Å resolution. This proteolytic fragment consists of two calmodulin (CaM)-like LCs that stabilize a long helical heavy chain (HC) fragment. The quality of the electron density is improved sufficiently over that in the previous 2.6 Å structure to establish the precise coordination of the Mg²⁺-ion in the open loop of the regulatory light chain (RLC) allowing us to account for the preferential binding of Mg²⁺ rather than Ca²⁺ in this site. The unusual coordination of the triggering Ca²⁺ site in the closed loop of the essential light chain (ELC) is also accounted for by distinctive structural features of this loop. Because of the network of critical linkages between the Ca²⁺-binding site of the ELC, and both the RLC and HC, the structure implies that in the Ca²⁺-bound "on" state, this domain is likely to be a rigid structure. Correspondingly, we describe how the loss of the Ca²⁺ ion might induce flexibility in the structure. Comparison of the structures of Ca²⁺-bound scallop RD with that of the same region of chicken S1 also shows two places where the HC is bent differently. We describe how these differences might affect the relative positioning of the two motor domains in the molecule. The structural results suggest a model for myosin regulation in which the RD acts as a switch so that in the Ca²⁺-free state, the transient flexibility of the RD permits specific intra-molecular linkages to be made in the myosin molecule that were sterically inaccessible in the Ca²⁺-bound structure. In this "off" state, motions within the motor domain required for activity are effectively switched off. According to this view, regulated myosins belong to a class of structures that require dimerization for the full expression of function.

MS04.13.04 AN OPEN-STATE OF β-ACTIN AND THE SOLID-STATE TRANSFORMATIONS OF PROFILIN-β-ACTIN CRYSTALS
John K. Chik (NIH), Uno Lindberg, Stockholm U. and C.E. Schutt, Princeton U.

The 2.65Å structure of an "open-state" of bovine β-actin reveals many differences to our previously solved "tight-state" structure (C.E. Schutt et al., Nature, 365:810-816 (1993)) such as solvent accessible ATP phosphates. It was also possible to estimate the energy difference between these two states using osmotic pressure. The conformation of β-actin complexed with profilin is sensitive to the environment surrounding the crystal. Profilin-β-actin crystals normally grown in potassium phosphate were sensitive enough to solution condition so as to make them unsuitable for isomorphous heavy-atom methods (Schutt et al., J. Mol. Biol., 209:735-746 (1989)). Transferring the 1.8M potassium phosphate grown crystals into 3.2M ammonium sulphate yielded better diffraction crystals that were also amenable to heavy-atom methods. This transition from potassium phosphate, open-state, to ammonium sulphate, tight-state, buffer caused the c-dimension of the unit-cell to shrink from 185.7Å to 171.9Å without deleterious damage to the crystal. These tight-state crystals lead to the 2.55Å structure of the complex (C.E. Schutt et al., Nature, 365:810-816 (1993)). The structure of the open-state complex was solved using molecular replacement (R=20.1% and Rfree=32.8% from 8-0.002,650). The open- to tight-state transition buries the solvent exposed ATP phosphates, closes the cleft between the domains and rotates sub-domain 2 by 14.7°. Also, as seen in tight-state crystals, open-state β-actins form extensive contacts to symmetry related actins along the 21 axis parallel to the b-direction. This "ribbon" feature is maintained in the open- to tight-state transition. Using osmotic pressure, it was possible change the unit-cell dimensions to a similar degree as seen in the open- to tight-state transition and thus, by inference, provides an experimental estimate of the energy difference between the states. The estimated difference is only a fraction of thermal energy and is consistent with the observed sensitivity of the crystals.


The structure of horse plasma gelsolin to 2.5 Å resolution can be coupled with biochemical and biophysical results to explain the calcium-dependent severing of actin filaments by gelsolin. Gelsolin contains six similarly folded domains organized into two nearly independent halves. Connections between the halves are formed firstly by a long polypeptide loop that covalently links the terminus of S3 to the start of S4, and then by the C-terminal helical tail of the protein reaching back to lie parallel to a long helix in S2. We suggest that the binding of calcium to the second half of gelsolin releases the C-terminal tail from its interactions with S2. The two halves of the gelsolin molecule then can act relatively independently, restrained only by the S0 residue linker, to bind to actin units on opposite sides of an actin filament. We suggest that S2 binds first and positions S1 near to its binding site. The binding of S1 introduces sufficient steric conflict between S1/S3 and the next actin proton in the filament to induce severing. These actions would be mirrored at an actin unit across the filament due to the binding of S4-S6, completing the severing and capping activity. Slack in the lengthy chains that link various domains of gelsolin would enable the required relative motions amongst the segments, without requiring significant changes in the secondary or tertiary structure of any individual domain.