The Crystal Structure of Uncomplexed G-Actin in the ADP State

L. R. Otterbein, P. Graceffa, R. Dominguez

Boston Biomedical Research Institute, Watertown, MA U.S.A.

Background and Significance

Actin represents the major component of the thin filament of muscle cells and the cytoskeletal system of nonmuscle cells. Monomeric actin (G-actin) assembles under physiological salt concentrations to form polymers (F-actin), a phenomenon that has so far prevented its crystallization in an uncomplexed form. Its three-dimensional structure, however, has been revealed from complexes with three different actin-binding proteins (ABP) that block its polymerization: DNase I,[1] profilin,[2] and gelsolin.[3]

An F-actin filament model was also constructed by fitting the structure of the monomer into low-resolution x-ray fiber diffraction from oriented gels of F-actin.

We have determined the first x-ray crystal structure of actin alone at 1.54 Å resolution. The structure corresponds to the ADP state and displays a dramatic conformational change in subdomain 2 resulting from Pi release. Such a conformational change has been predicted to play a critical role in controlling the dynamics of the actin filaments in the cells.

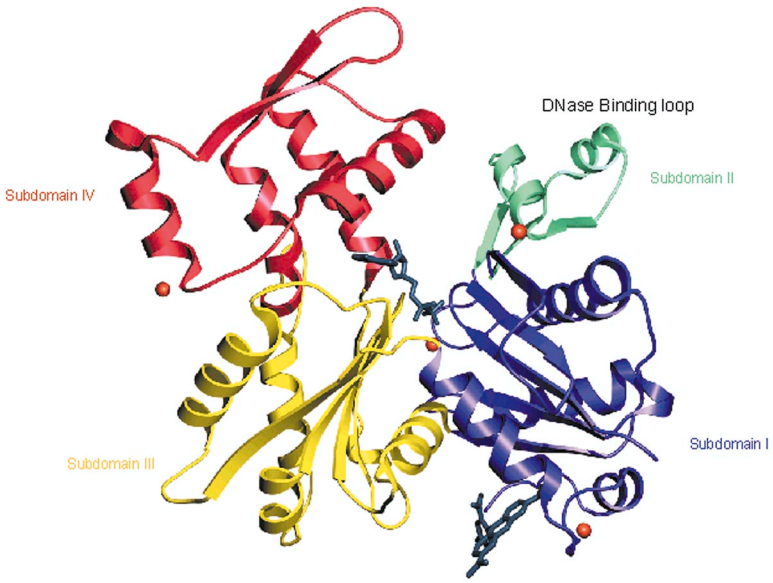

FIG 1. Ribbon representation of the uncomplexed actin structure in the ADP state. Actin subdomains are represented in different colors: subdomains 1 (purple), 2 (green), 3 (yellow), and 4 (red). A molecule of ADP is bound in the active site in association with a Ca^{2+} ion. The molecule used to prevent actin polymerization (TMR) during crystallization is covalently bound near the C-terminus.

Methods and Materials

Rabbit skeletal α-actin was chemically modified with TMR at Cys374, which blocks polymerization. The crystals were grown using the hanging-drop vapor diffusion method. They belong to space group C2 with unit cell dimensions a = 112.8 Å, b = 37.5 Å, c = 85.3 Å, β = 108.3°, and typically measured ~800 x 50 x 10 µm.

Crystals of G-actin were collected at the BioCARS beamline 14-BM-C and IMCA-CAT beamline 17-ID. The data sets were processed using the programs DENZO and SCALEPACK.

The structure was solved by molecular replacement and refined to 1.54 Å resolution using the programs wARP and REFMAC. The final model displays good stereochemistry and R_{free} = 2 2.3 and $R_{facto\ r}$ = 17.9.

Results

Overall, the structure of uncomplexed actin (Fig. 1) is similar to previous actin structures obtained from complexes with DNase I,[1] profilin,[2] and gelsolin.[3]

Major differences occur in the orientation of subdomain 2 and, to a lesser extent, that of subdomain 4. A major difference is observed in the so-called DNase I binding loop within subdomain 2 (residues His40-Gly48), which in our ADP structure is folded as a α-helix but in previous actin structures is either disordered or folded as a β-turn. In the DNase I complex, in particular, this loop is attached as an additional strand to a β-sheet in DNase I. It is unusual to observe such secondary structure transition within the same protein. The ability of some polypeptides to undergo α/β transitions has recently attracted significant attention because of their postulated role in a number of misfolding diseases associated with the accumulation of insoluble fibrils known as amyloids. Notice that this secondary structure transition occurs in addition to the rigid body rotation of the entire subdomain 2. Taken together, these changes place some of the amino acids at the top of subdomain 2, notably those around Val45, some 14 Å apart from their location in the actin ATP structures. Close inspection of the ADP-actin structure reveals that minor reorientations of some of the side chains around the nucleotide γ-phosphate site (more significantly Ser14) become gradually amplified into larges displacements of the DNase I binding loop at the top of subdomain 2. Our results suggest that a conformational change in actin subdomain 2, resulting from the release of the nucleotide γ-phosphate, may be responsible for controlling the ATP-dependent dynamics of the actin filaments in the cells.

The structure also opens the door to future structure determinations of actin complexes with actin binding proteins such as myosin.

A manuscript describing this work has been submitted for publication to Science.

Acknowledgments

We thank the staff members at BioCARS beamline 14-BM and IMCA-CAT beamline 17-ID for their helpful assistance during data collection. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-ENG-38. Use of the BioCARS Sector 14 was supported by the National Institutes of Health (NIH), National Center for Research Resources, under grant number RR07707.

Beamline 17-ID in the facilities of the Industrial Macromolecular Crystallography Association Collaborative Access Team

(IMCA-CAT) is supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with the Illinois Institute of Technology (IIT), executed through IIT's Center for Synchrotron Radiation Research and Instrumentation. Support for one of the authors provided by NIH grants R01 AR46524 (RD) and March of Dimes grant 5-FY99-774 (RD).

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