P04.04.211

Acta Cryst. (2008). A**64**, C297

Small angle scattering: The regulatory domains of cardiac C-protein and their complex with F-actin

Cy M. Jeffries[1], Andrew E Whitten[1,2], Samantha P Harris[3], Jill Trewhella[1]

[1]The University of Sydney, School of Molecular and Microbial Biosciences, Building G08. Cnr. Butlin Ave and Maze Cr. Camperdown, Sydney, New South Wales, 2006, Australia, [2]Australian Nuclear Science Technology Organisation, Lucas Heights, NSW, Australia., [3]University of California Davis, California, U.S.A., E-mail：cjef6222@mail.usyd.edu.au

Cardiac myosin binding protein C (cMyBP-C) is a multidomain accessory protein of muscle sarcomeres that plays a significant role in maintaining regular heart function. Four domains at the N-terminus of cMyBP-C, C0-C1-m-C2, modulate the rate/extent of actomyosin cross-bridge formation in response to a number of inotropic stimuli (as in fight-or-flight). Point mutations in C0-C1-m-C2 cause significant disruptions to the cardiac cycle and the development of familial hypotrophic cardiomyopathy (FHC). We have used small-angle X-ray scattering (SAXS) to determine the average shape of C0-C1-m-C2. The domains are arranged in an extended conformation that is sufficient to span actomyosin cross-bridge distances. Atomic models have been constructed that fit the SAXS data and show that the sites of FHC causing mutations occur along one side of the molecule. Consequently, we suggest that a myosin S2 interaction interface exists along the length of the C1, m- and C2- domains. The SAXS based model is in keeping with the current view that the N-terminal domains of cMyBP-C effect cross-bridge kinetics via an 'on-off' interaction with myosin S2 which ultimately fine tunes the position of myosin heads during contraction. However, recent studies indicate that C0-C1-m-C2 also binds directly to actin. Small-angle neutron scattering (SANS) experiments provide direct evidence for this interaction (Whitten et al., poster presentation, this conference). The combined SAXS/SANS data support a model in which the m- and C2- domains adopt a conformation that extends away from the fiber long axis and into the interfilament space. Thus the first four N-terminal domains of cMyBP-C are uniquely poised to effect heart muscle contraction via a combined interaction with both myosin S2 and actin.

Keywords: SAXS/SANS, myosin binding protein C, F-actin complex

P04.04.212

Acta Cryst. (2008). A**64**, C297

A small-angle neutron contrast variation study of the complex of actin and myosin binding protein C

Andrew E Whitten[1,2], Cy M Jeffries[2], Samantha P Harris[3], Jill Trewhella[2]

[1]Australian Nuclear Science and Technology Organisation, Bragg Institute, PMB 1, Menai, NSW, 2233, Australia, [2]School of Molecular and Microbial Biosciences, University of Sydney, NSW, 2006, Australia, [3]Section of Neurobiology, Physiology and Behaviour, University of California, Davis, CA, 95616, USA, E-mail：andrew.whitten@ansto.gov.au

Small-angle neutron scattering using contrast variation has been used to investigate the nature of the interaction between cardiac myosin binding protein C (cMyBP-C) and actin. Scattering data were collected from samples prepared by adding a construct composed of the four N-terminal domains of cMyBP-C (C0-C1-m-C2; designated C0C2), to a solution of G-actin (monomeric actin). The scattering data show that actin and C0C2 interact, inducing the formation of F-actin (filamentous actin) under conditions in which G-actin should be prevalent. As the length of the filament is much larger than those probed by the scattering experiment, only cross-sectional information can be extracted from the scattering data. In an approach analogous to that developed by Svergun for 3D modelling [1], we have modelled the cross-section of the filamentous complex against the contrast variation data. This modelling shows that the C0C2 binds symmetrically to the actin filament, projecting outwards on either side. Using a structure of C0C2 determined from small-angle X-ray scattering ([2] and poster by Jeffries at this conference), and a model structure for the actin filament, we were able to construct a 3D model of the complex that is consistent with all of the experimental data. The C0C2-actin interaction has been inferred from physiological studies in, among others, the Harris lab, but direct evidence has been lacking. The scattering data provide direct evidence of this interaction that has important implications for the role of cMyBP-c in the regulation of acto-myosin interactions and muscle contraction.

[1] D. I. Svergun, *Biophys. J.* (1999), 76, 2879-2886.
[2] C. M. Jeffries, *et al., J. Mol. Biol*. (2008), 377, 1186-1199.

Keywords: small-angle neutron scattering, actin, myosin binding protein C

P04.05.213

Acta Cryst. (2008). A**64**, C297

The effect of U-U mismatches on the RNA structure

Agnieszka Kiliszek, Ryszard Kierzek, Wlodzimierz Krzyzosiak, Wojciech Rypniewski

Institute of Bioorganic Chemistry, Polish Academy of Sciences, ibch@ibch.poznan.pl, Poznan, Wielkopolska, 61 704, Poland, E-mail：kiliszek@ibch.poznan.pl

mRNA of the human gene Dmpk (dystrophin myotonin protein kinase) contains tracks of CUG repeats in 3'UTR. Their role in the non-coding region is unclear. However,biochemical studies have revealed that tracks can form double-stranded regions which can be recognised by proteins. This suggests that they play a regulatory role. It is also postulated that they are involved in the pathogenesis of myotonic dystrophy. The number of repeats are variable. Usually it is about 50 but it may increase to as much as 250 or more. That long double-stranded CUG tracks become pathogenic. It is possible that they interact with larger number of proteins molecules than usual. Thus depleting the number of free proteins in the cell. In effect some cellular processes may be deregulated. We have determined the crystal structure of short duplexes containing CUG repeats in C2 space group at 1.23 Å resolution. Duplex contains typical C-G and G-C pairs which are interrupted by U-U mismatches. Although the structure contains non-canonical base pairs, the RNA helix adopts typical A-form. All detailed structural aspects of the CUG tracks will be discussed as well as possible mechanism of their recognition by proteins.

Keywords: RNA, RNA structure, disease

P04.05.214

Acta Cryst. (2008). A**64**, C297–298

Crystal structures of the bacterial, mitochondrial and cytoplasmic A-site molecular switches

Jiro Kondo, Eric Westhof

Universite Louis Pasteur, IBMC-CNRS, 15 rue Rene Descartes,