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

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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

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

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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.

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

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The effect of U-U mismatches on the RNA structure

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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

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

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The aminoacyl-tRNA decoding site (A site) is a molecular switch in the ribosome guaranteeing high translation fidelity. The secondary structure of the A site is conserved except at a few nucleotides between bacteria, mitochondria and eukaryotic cytoplasm. X-Ray analyses have revealed that tertiary structures of the three types of A sites are surprisingly different [1,2]. Therefore, on the basis of the many crystal structures obtained, it is suggested that these three main cell types possess different dynamical states and barriers in the molecular switches controlling translational fidelity, underlying the different evolutionary pressure on decoding. Aminoglycosides are highly effective antibacterial drugs that decrease translation accuracy by binding to the "on" state of the bacterial A-site molecular switch. On the other hand, toxicity to human resulting from the clinical use of aminoglycoside has been considered to originate from the binding of these drugs to the mitochondrial and cytoplasmic A site. Our X-ray analyses have revealed that the binding modes of aminoglycosides to the mitochondrial and cytoplasmic A sites, in which aminoglycosides bind to the "off" states of the A sites, are surprisingly different from those found in the bacterial A site [3].

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Keywords: ribosomes, RNA structure, antibiotic binding

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Crystal structure of Z-DNA d(CGCGCG) complexed with Ca^{2+} ion , and Mg^{2+} ion

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In solution, negatively charged DNA was neutralized by positively charged metal ions such as monovalent Na^+, K^+ and divalent Ca^{2+}, Mg^{2+}. These cations are essential for DNA folding and sometimes decide the helical conformation of DNA by hydrogen bonding networks. We determined crystal structures of DNA hexamer d(CGCGCG) with very high concentration of Mg^{2+} and Ca^{2+} ions by new temperature control technique we developed. Two kinds of DNA crystal were obtained from crystallization solution containing 500mM $MgCl_2$ and 500mM $CaCl_2$ by annealing from 338K to 293K, respectively. X-ray experiment was carried out using synchrotron radiation at BL38B1 in SPring-8. X-ray diffraction data were collected at high resolution (1.2 Å for $MgCl_2$ and 1.1 Å for $CaCl_2$). These crystals belong to new crystal form $P3_2$ (a=b=18.5 Å, c=72.7 Å) and left-handed DNA helixes were stacked along a long c-axis in $MgCl_2$ crystal. That's entirely different from a usual crystal ($P2_12_12_1$, a=17.9 Å, b=30.8 Å, c=43.7 Å) obtained from 20mM, 50mM and 250mM Na^+ solution. Initial phases of $MgCl_2$ crystal were determined by molecular replacement with the program of AMoRe, and structural refinement and model building were carried out with the program of Refmac 5.0 and Coot 0.2, respectively. 68 H_2O molecules and 8 Mg^{2+} binding sites were determined with R=23.3 % and R_{free}=26.9%. Phosphate groups of Z-DNA were connected with the other phosphate group of neighboring Z-DNA via two Mg^{2+} ions. This structural motif would be utilized in storing DNA in compact room with high salt condition.

Keywords: metal ions in biology, DNA crystallography, DNA structure

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X-Ray analyses of DNA duplexes stabilized by bicyclic-C residues

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Base-modified nucleic acids are being evaluated for applications in biotechnology and as therapeutic agents. We found that a nucleotide carrying 7,8-dihydropyrido[2,3-*d*]pyrimidin-2(3H)-one, which is a cytosine derivative with a propene attached at the N4 and C5 atoms (hereafter bicyclic-C or X), increases the stability of DNA duplexes [1]. To establish the conformational effects of X on DNA and to obtain insight into the correlation between the structure and stability of X-containing DNA duplexes, the crystal structures of $[d(CGCGAATT\text{-}X\text{-}GCG)]_2$ (GX9) and $[d(CGCGAAT\text{-}X\text{-}CGCG)]_2$ (AX8) have been determined at 2.9 Å resolutions, respectively. The global and local conformations of the X-containing duplexes and the unmodified duplex are very similar. The X and the counter G bases form a pair in the canonical Watson-Crick geometry, similar to the previously reported GX9* [2]. On the other hand, the final omit map of AX8 suggests that X forms two types of pairs with the counter A residue, one is a wobble type and the other is a Watson-Crick like as the first example. In the former pairing, the adenine base must be protonated, while in the latter, X or A might adopt an imino form through tautomerization. Another interesting finding is that the base stacking interactions at the base pair steps containing the X residues are significantly changed. These changes in stacking areas, however, explain the increased stability of X-containing DNA duplexes.

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Keywords: DNA structure, base modification, DNA stability

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X-ray structure of A and B-DNA under high hydrostatic pressure (up to 2 GPa)

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