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 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

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

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The aminocacyl-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].

Keywords: ribosomes, RNA structure, antibacterial 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 P\(_3_1\) (a=18.5 Å, c=72.7 Å) and left-handed DNA helices were stacked along a long c-axis in MgCl\(_2\) crystal. That’s entirely different from a usual crystal (P\(_2\)\(_1\)2\(_1\)2\(_1\), a=17.9 Å, b=30.8 Å, c=43.7 Å) obtained from 20mM, 50mM and 250mM Na\(^{+}\) solution. Initial phases of MgCl\(_2\) crystals 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\(_2\)O molecules and 8 Mg\(^{2+}\) binding sites were determined with R=23.3 % and R\(_{free}\)=26.9%. Phosphates of Z-DNA were connected with the other phosphates by hydrogen bonding. The aminoacyl-tRNA decoding site (A site) is a molecular switch in translation accuracy. 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].

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-X-GCG)]\(_2\) (GX9) and [d(CGCGAAT-X-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 immo 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.

References


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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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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