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Report:

Introduction. Troponin (Tn), the complex of three subunits (TnC, TnI, and TnT), plays a key role in Ca^{2+} dependent regulation of muscle contraction. To elucidate the interactions between the Tn subunits and the conformation of TnC in the Tn complex, we have determined the crystal structure of TnC in complex with the N-terminal fragment of TnI (TnI_{1-47}).

Data collection. The crystals belong to the space group $P3_221$, with the unit cell dimensions $a=b=46.9$Å, $c=152.3$Å. The data for the native TnC/TnI_{1-47} complex and its PCMBS (p-chloro-mercuribenzenesulfonate) derivative at three distinct wave lengths (including one at the absorption edge of the Hg atom) have been collected from the two single frozen crystals (100°K) at beam line BM14 (ESRF, Grenoble).

Structure determination. The structure was solved by the SIR/MAD technique. The major heavy atom site of the PCMBS derivative was located in both isomorphous and anomalous difference Patterson maps. The overall figure of merit was 0.85 at 2.8Å resolution. The phases were improved by solvent flattening and histogram matching. The rough orientation of the TnC lobes (PDB entry 1TOP) in the final electron density map was determined manually. The TnC model was then rebuilt to achieve the best fit to the electron density, and the interlobe linker region, and the TnI_{1-47} a-helix were modeled.

Refinement. The model was refined with the program X-PLOR. The refinement converged to a crystallographic R-factor of 22.2% (R-free=32.6%).

Results and discussion. In the TnC/TnI_{1-47} complex, the central, connecting a-helix observed in the structure of uncomplexed TnC (TnC_{free}) is unwound at the center and bent by 90°. As a result, the TnC in the complex has a compact globular shape with direct interactions between the N- and C-terminal lobes, in contrast to the elongated dumb-bell shaped molecule of uncomplexed TnC. The 31-residue long TnI_{1-47} α-helix stretches on the surface of TnC and stabilizes its compact conformation by multiple contacts with both TnC lobes. The amphiphilic C-terminal end of the TnI_{1-47} a-helix is tightly bound in the hydrophobic pocket of the TnC C-lobe through 38 van der Waals interactions. The results indicate the major difference between integrated (TnC) and isolated (calmodulin) Ca^{2+} receptors. The TnC/TnI_{1-47} structure suggests the model for a novel regulatory TnI segment bound to TnC and implies the mechanism of how Tn regulates the muscle contraction.

- References.

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2. Vassylyev, D.G., Takeda, S., Wakatsuki, S., Maeda, K. & Maeda, Y. (1998) submitted to *Proc. Natl. Acad. Sci. USA.*