Muscling in on Paramagnetic NMR

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The calcium-mediated regulation of striated muscle contraction is of critical importance in vertebrates, and at the core of this complex regulatory system is Troponin (Tn). In the absence of calcium, this large, flexible protein inhibits actin-myosin interaction. However, upon calcium binding, Tn undergoes a large conformational change, enabling actin-myosin interaction and thus muscle contraction to occur.

The mobility of Tn complicates structural and mechanistic studies of the protein. Therefore, the emerging method of structure analysis, Paramagnetic Relaxation Enhancement NMR (PRE), has been investigated for its application to Tn. The PRE technique requires a paramagnetic compound, which contains an unpaired electron, to be incorporated into the protein for NMR studies. Site directed spin labelling (SDSL) is used to attach a spin label, such as a nitroxide group, to the cysteine residue of the protein via a disulfide bond.

In the 15N-HSQC NMR experiments, the unpaired electron of the paramagnetic nitroxide spin label interacts with nearby spin nuclei, causing a distance dependent increase in relaxation rate of nuclei within a ~25 Å radius. This PRE effect is observed in the HSQC spectra as peak broadening. The distance between the affected nucleus and the spin label can be determined from the magnitude of this broadening, as described by the Solomon–Bloembergen equation (Solomon, 1955). PRE can thus be used to obtain long-range distance constraints, which enables inter-domain movements to be detected. This method is therefore useful in examining the large conformational change that occurs in Tn upon calcium binding.

To examine the suitability of SDSL-PRE for troponin studies, we have produced four single cysteine (Cys) mutants of the small calcium-binding subunit of cardiac Tn, Troponin C (TnC – Cys35, Cys84, Cys94 and Cys136) by site directed mutagenesis. The TnC N15 mutants are labelled with the nitroxide spin label (MTSSL) and HSQC spectra acquired in the paramagnetic and reduced states of the spin label. Potential NMR experiments are to collect PRE data for each mutant in the presence and absence of calcium to compare conformational changes in TnC between the two states. The four TnC mutant cysteine locations provide an overview of the conformational changes that occur in TnC upon calcium binding.

Solomon I. (1955) *Physical Review*, **99:** 559–65.