Structural investigation of a fibronectin Type III -immunoglubulin domain tandem from A-band titin

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Introduction

Single molecules of the giant protein titin extend across half of the muscle sarcomere, from the Z-line to the M-line, and have roles in muscle assembly and elasticity In the A-band titin is integral with thick filaments and in this region the sequence of titin contains fibronectin type III and immunoglobulin-like domains. These are arranged in regular patterns of eleven domain repeats called the large super-repeats. The large super-repeat occurs eleven times and this entire region thus forms nearly half of the titin molecule. We are studying the atomic structure, properties and the inter-domain arrangement of overlapping double and triple domain fragments of the large super-repeat (titin A59-A69 unit) by NMR spectroscopy.

Results

While A67-A68 has a well resolved ^{1}H-^{15}N HSQC spectrum side-chain experiments had poor spectral quality, even with a perdeuterated sample, preventing assignment. As the use of the standard 3D NOESY spectra was impractical, the A67-A68 domain tandem was first investigated by a mixture of chemical shift based structure determination and previously determined NMR structures. The fold of A68 was determined using CS23D based on ^{1}H, ^{15}N and ^{13}C chemical shifts. For A67 we used the NMR structure of A78 [C-terminal domain of the 3LPW], as this is the domain present at the same position as A67 in the next super-repeat. In order to investigate the validity of the model structures the measured and the back-calculated HN Residual Dipolar Couplings values (RDCs) [using a C12E6 - hexanol liquid crystal system] from models were compared for the N- as well as the C-terminal domains. Good correlation ($R > 0.9$) between the back calculated and measured RDC values was observed for resonances with good signal to noise drawn from secondary structure elements and this clearly shows that these structural models are valid as does the good agreement with amide ^{1}H nOe data as well.

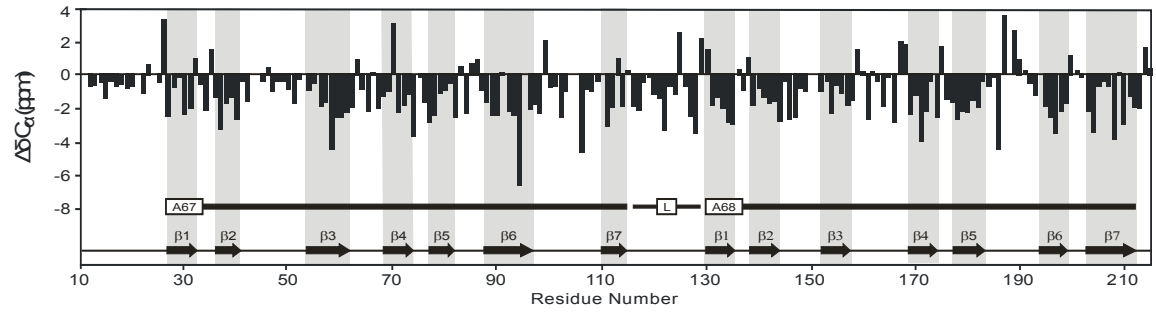

Figure 1: Cα chemical shifts and secondary structure assignments for A67-A68

RDC data only describe domain orientations up to an inversion Cartesian axes which does not change the handedness of alignment tensor frame; giving four possible structural solutions. Structures were calculated using rigid body minimization in using an RDC constraint term. This approach resulted in two physically possible structures. In order to find an unique structure measurements are in progress using a second liquid crystal system to give a second independent tensor frame.

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