Biochemical Society studentship report 2009

NMR assignment of a troponin C – troponin I chimera and the role of dynamics in muscle regulation

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Background:

The troponin complex is formed from the three troponin molecules C, I and T. Part of the troponin complex contains a regulatory region, which includes a calcium binding site, forming a switch in muscle fibres. The calcium concentration determines whether or not the binding sites will be filled and therefore whether the conformation of the switch is in the on or off state. The C-terminal regulatory region in troponin I is very important but it cannot be visualized by NMR or in crystal structures already known due to the troponin complex being too large, and this region flexible. The regulatory regions can be isolated in a chimera of troponin C – troponin I (Tiroli et al., FEBS J. 272) and have been shown to behave in the same way as in the full troponin complex. This is the chimera which was therefore studied using 2D and 3D nuclear and magnetic resonance (NMR) techniques.

Aims:

1. Produce and purify double labelled troponin C - troponin I chimera to form an NMR sample.
2. Collect 2D and 3D NMR spectrums of the troponin C - troponin I chimera including the region that has not been previously visualized by NMR or in crystal structures already determined.
3. Process and analyse these spectrums using NMRPipe and NMRView before assigning the peaks to residues in the chimera

Description of work:

A sample of the labelled chimera was produced using the bacteria *E.coli* containing a plasmid with the genetic sequence for the chimera. The growth media for the *E.coli* was prepared, once there was enough bacteria present in the culture they were transferred to labelled media and the chimera expression was initiated. This caused the labelled chimera to be produced before being purified using the Q column. The purified chimera was then made into a suitable NMR sample using a calcium containing buffer from which HSQC, HNCACB, CBCACONH and HNCO spectra were collected. These spectra were processed using NMRPipe to correct the phasing and baseline and to extract the area required for analysis and Fourier transform. The spectra were then analysed side by side using NMRView to assign the peaks to the amino acid residues in the chimera.

Results:

The chimera was successfully produced, figure 1 shows the SDS-PAGE of the initial purification of the chimera. A pure sample was acquired by passing through a Q column twice.

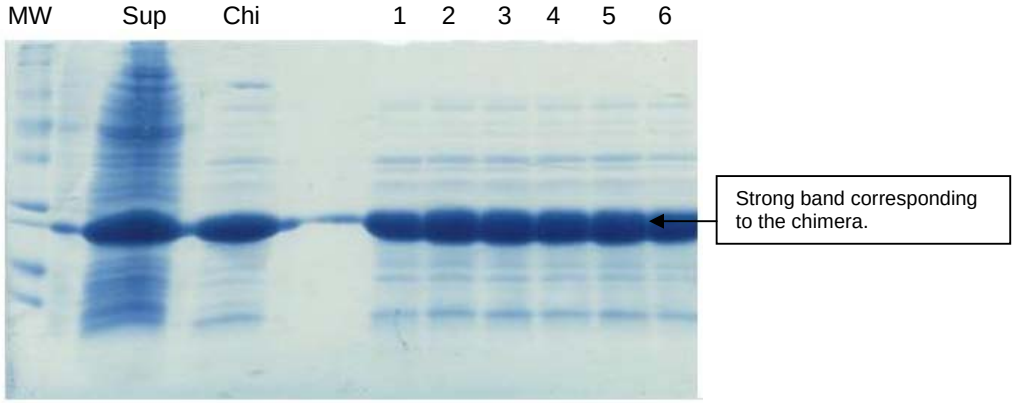

Figure 1:Gel showing the expression of the chimera and the first 6 fractions of the first Q column, the molecular weight standard can also be seen.
MW – molecular weight standard, Sup – Supernatant before dialysis, Chi – sample after dialysis

The pure sample was dialysed into an NMR buffer containing calcium ions, required to maintain the conformation of the chimera in the calcium bound state. The different NMR spectrums were then collected. The initial HNCACB experiment had a lot of noise showing and therefore had to be

repeated to allow the weaker signals to show clearly on the spectrum. The peaks from the HSQC spectrum were then lined up with the matching peaks on the other spectrums and assignments made. Figure 2 shows part of the HNCACB spectrum.

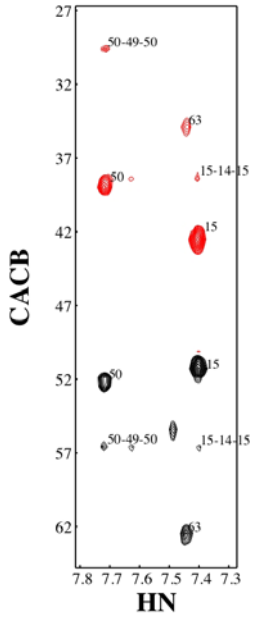

Figure 2: The region of the HNCACB spectra which shows the peaks for residues 15, 50 and 63.

The majority of the early and final parts of the chimera were assigned along with a few of the amino acid residues from the regulatory region which has not been previously studied using NMR. Figure 3 shows the amino acid sequence and the assignments that were made.

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        10         20         30         40         50         60
MASMTDQQAE ARAFLSEEMI AEFKAAFDMF DADGGGDIST KELGTVMRML GQNPTKEELD

        70         80         90        100        110        120
AIIEEVDEDG SGTIDFEEFL VMMVRQMKED AGGAGGKLFD LRGKFKRPPL RRVRMSADAM

       130        140        150        160        170        180
LRALLGSKHK VNMDLRANLK QVKKEDTEKE KDLRDVGDWR KNIEEKSGME GRKKMFEAGE S
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Figure 3: The assigned amino acids are coloured in red.

Future Directions:
It is possible now for the remaining peaks to be assigned in the C-terminal regulatory region of troponin I. This can then be used to visualize troponin I and so the troponin complex will be better understood by being able to fully interpret the NMR relaxation data that has already been collected.

Departures from original proposal:
It was not possible to assign the entire chimera due to time restraints and therefore part of the terminal C regulatory region which has not yet been assigned.

Value of studentship to student:
It has been an invaluable experience for me to undertake this studentship over the summer. Through these experiments I have come to learn more about how to express and purify a protein. It has also been useful to work in a research lab environment so that I now feel more comfortable and would know what to expect should I decide to continue with my current plans of doing a PhD. I found that the most interesting part of the placement for me was learning how to acquire process and then analyse the NMR data. Learning about the different UNIX codes that were needed I feel will be very helpful to me in using other software as well as for the processing of NMR data.

Value of the studentship to the lab:
Through the studentship essential work which relates to the research being conducted was able to be achieved. The collection and analysis of the NMR spectra of the chimera is a very important step towards being able to visualise the regulatory region of the troponin complex. After assignment of the remaining peaks analysis of other spectra of the regulatory region of the troponin chimera will be possible. This work was included in an abstract submitted to the Biophysical Society meeting.