Recombinant expression of the cardiac troponin I fragment, cTnI[135-209], that controls cardiac contraction

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Introduction

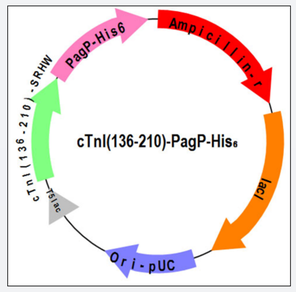
Intrinsically disordered regions (IDRs) are protein segments that lack a fixed 3D structure. They are susceptible to post-translational modifications like proteolysis and phosphorylation, making them highly important in cellular signal transduction and the regulation of numerous biological processes.

Cardiac troponin I (cTnI) is a key protein that turns contraction on and off with every heartbeat. cTnI[135-209] binds to actin to block actin-myosin interaction and shut off muscle contraction.

In the absence of actin, cTnI[135-209] is intrinsically disordered and susceptible to proteolytic degradation. We express human cTnI [135-209] in *E. coli*, using a novel fusion partner, PagP, to direct it to dense insoluble aggregates known as inclusion bodies.

Materials and Methods

Plasmid map for bacterial expression vector



Expression of fusion protein in insoluble inclusion bodies in *E. coli*

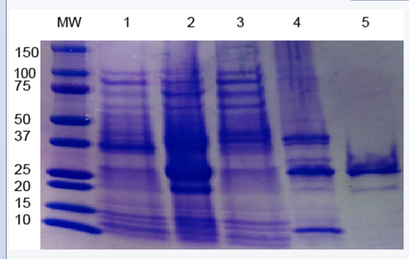
Solubilization in 6 M Gdn-HCl and purification using Ni-affinity chromatography

Ni^{2+}-catalyzed cleavage to release the fusion partner PagP

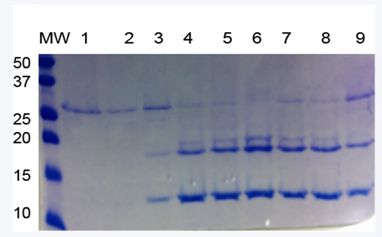
Removal of insoluble PagP-His_6 from the soluble target protein

Ni^{2+}-catalyzed cleavage

Results



Production and purification of cTnI (135-209)-PagP-His_6.
1: Whole cells before induction.
2: Whole cells after IPTG induction.
3: Soluble fraction after cell lysis.
4: Insoluble fraction dissolved in 6 M Gdn-HCl.
5: Fusion protein purified from nickel affinity column (double band correponds to folded and unfolded forms of PagP).



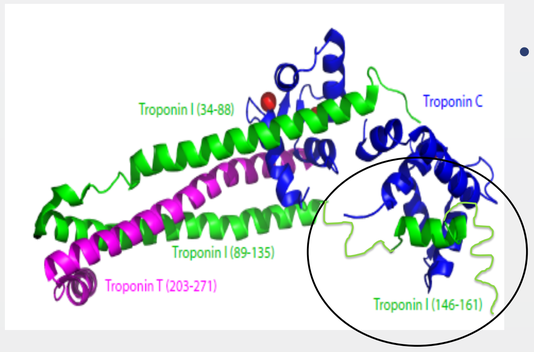
Different conditions for Ni-catalyzed cleavage of cTnI (135-209)-PagP-His_6.
All taken at 4 hours.
1: Purified protein before cleavage
2: pH 7.0, at 45°C
3: pH 7.5, at 45°C
4: pH 8.0, at 45°C
5: pH 8.5, at 45°C
6: pH 9.0, at 45°C
7: pH 9.0, at 37°C
8: pH 9.0, at 30°C
9: pH 9.0, at 25°C

cTnI (135-209) is robustly produced, cleaved, and purified from its fusion partner, PagP-His_6. The final yield was 26 mg protein from 14 g wet cell pellet using 2 L M9 minimal media (10 g glucose per L).

Complete cleavage of cTnI(135-209)-PagP-His_6 was obtained at pH 9.0 after 4 hours of incubation at 45°C.

Discussion

Here we present a novel method for mass producing any intrinsically disordered protein (IDR), using cTnI[135-209] as an example.



- Unstructured cTnI [135-209] within the cardiac troponin complex

Expression in *E. coli* inclusion bodies is an old technique for producing large quantities of peptides or proteins. In 1982, Eli Lilly patented a such a technique to produce recombinant insulin peptide for clinical use. The newer method presented here offers several advantages:

Insulin Humulin R	cTnI[135-209]	Advantages
Fusion partner: TrpΔLE, 17-amino acid sequence (MKAIFVLKGSLDR DPEF)	Fusion partner: PagP, 161-amino acid membrane protein	*Larger fusion partner directs larger IDRs to inclusion bodies (>120AA)
CNBr cleavage ↓ - Met -	Ni cleavage ↓ - SRHW-	*More specific cleavage sequence *Allows internal methionine residues in target protein
Conventional chromatography	Ni affinity chromatography	*Easier and more effective purification

Future directions

We have used our protein expression system to produce large amounts of ^{15}N, ^{13}C-labeled cTnI [135-209] for further study using multinuclear multidimensional nuclear magnetic resonance spectroscopy. We are working to determine its 3D structure bound to actin. This will explain how it inhibits muscle contraction and how mutations in this region give rise to heritable cardiomyopathies.

References

- Krezel, A. *et al*. Sequence-specific Ni(II)-dependent peptide bond hydrolysis for protein engineering. Combinatorial library determination of optimal sequences. *J. Am. Chem. Soc.* **132,** 3355-3366 (2010)
- Hwang, P. M., Pan, J. S. & Sykes, B. D. A PagP fusion protein system for the expression of intrinsically disordered proteins in Escherichia coli. *Protein Expr. Purif.* **85,** 148-151 (2012).