Removing the regulatory N-terminal domain of cardiac troponin I diminishes incompatibility during bacterial expression

Zhi-Bin Yu, Jiang-Ping Jin *

Section of Molecular Cardiology, Evanston Northwestern Healthcare and Northwestern University Feinberg School of Medicine, Evanston, IL 60201, USA

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Abstract

Troponin I (TnI) is a muscle-specific protein and plays an allosteric function in the Ca\(^{2+}\) regulation of cardiac and skeletal muscle contraction. Expression of cloned cDNA in Escherichia coli is an essential approach to preparing human TnI and mutants for structural and functional studies. The expression level of cardiac TnI in E. coli is very low. To reduce the potential toxicity of cardiac TnI to the host cell, we constructed a bi-cistronic expression vector to co-express cardiac TnI and cardiac/slow troponin C (TnC), a natural binding partner of TnI and a protein that readily expresses in E. coli at high levels. The co-expression moderately increased the expression of cardiac TnI although a high amount of TnC protein was produced from the bi-cistronic mRNA. The use of an E. coli strain containing additional tRNAs for certain low bacterial usage eukaryotic codons improved the expression of cardiac TnI. Modifications of two 5’-regional codons that have predicted low usages in bacterial cells did not reproduce the improvement, indicating that not the 5’ but the overall codon usage restricts the translational efficiency of cardiac TnI mRNA in E. coli. However, deletion of the cardiac TnI-specific N-terminal 28 amino acids significantly improved the protein expression independent of the host cell tRNA modifications. The results suggest that the regulatory N-terminal domain of cardiac TnI is a dominant factor for the incompatibility in bacterial cells, supporting its role in modulating the overall molecular conformation.

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Muscle contraction is regulated by intracellular Ca\(^{2+}\) via the thin filament-based troponin–tropomyosin system. Troponin is a striated muscle-specific protein complex contains three subunits: the Ca\(^{2+}\)-binding subunit troponin C (TnC), the tropomyosin binding subunit troponin T (TnT) and the inhibitory subunit troponin I (TnI) [1,2]. During muscle contraction, cytoplasmic Ca\(^{2+}\) rises and binds to TnC to induce a series of conformational changes in the troponin complex that release the inhibition of actomyosin ATPase and activate force development [3]. Three homologous TnI genes (cardiac, fast skeletal muscle, and slow skeletal muscle) have evolved in vertebrates to encode muscle-type-specific TnI isoforms [4]. Primary structures of cardiac, fast and slow skeletal muscle TnI isoforms are highly conserved. The main structural difference is a cardiac TnI-specific ~30 amino acids N-terminal extension [5].

Expression of cloned cDNA in bacteria is widely used in the preparation of TnI proteins for structural and functional studies. This approach is especially critical to the preparations of human TnI and site-specific mutations. A challenge here is the low expression level of cardiac TnI in Escherichia coli [6]. Although fusion protein expressions may increase the yield, TnI is an allosteric protein [3,5] in which any fusion peptide may affect its structure and function. To avoid the need of removing the fusion peptide after purification, direct expression of non-fusion TnI protein is...
desirable for accurate structural and functional characterizations. In the present study, we investigated the factors that limit the expression of cardiac TnI in *E. coli*. To reduce the potential toxicity of cardiac TnI to the host cell, we constructed a bi-cistronic expression vector to co-express cardiac TnI and cardiac/slow TnC, a natural binding partner of TnI and a protein that readily expresses in *E. coli* at very high levels [7]. To examine the effect of prokaryotic usage of eukaryotic codons on the translational efficiency of cardiac TnI mRNA, we compared expression in a host *E. coli* strain containing additional tRNAs for certain low bacterial usage eukaryotic codons with that in host bacteria containing only *E. coli* tRNAs. The co-expression with TnC only moderately increased the expression of cardiac TnI although a high amount of TnC protein was produced from the bi-cistronic mRNA. The use of tRNA-enhanced *E. coli* host to increase the usage of eukaryotic codons did improve the level of expression. To test whether the usage of codons near the 5′-end of the mRNA makes a major contribution to the restriction of cardiac TnI expression in bacterial cells [8,9], we modified two 5′ codons that have predicted low usages in *E. coli*. However, the modification of only 5′ codons did not reproduce the beneficial effect of improving the overall codon usage. In contrast, deletion of the N-terminal 28 amino acids of cardiac TnI significantly improved the protein expression in the absence of host tRNA modification. The results suggest that the N-terminal region of cardiac TnI is a dominant factor for the incompatibility during bacterial expression. With the regulatory role of the N-terminal domain of cardiac TnI, the results support its function through modulating the overall molecular conformation.

Materials and methods

Reverse transcription-coupled polymerase chain reaction cloning of cDNAs encoding human cardiac TnI and human cardiac TnC

Two microgram of total RNA from adult human ventricular muscle was used as template for cloning of cDNAs encoding cardiac TnI and cardiac/slow TnC by reverse transcription-coupled polymerase chain reaction (RT-PCR). As described previously [10], total first strand human cardiac cDNA was synthesized using avian myeloblastosis virus reverse transcriptase with an oligo-dT primer. Using the cDNA as templates, PCR was carried out using two pairs of oligo nucleotide primers flanking the coding regions of cardiac TnI and cardiac/slow TnC, respectively. For each pair, the upstream forward primer was synthesized according to the published sequence flanking the translation initiation codon [11,12]. The downstream reverse primer was designed as a complementary sequence to the region flanking the translation termination codon. Double-stranded cDNAs encoding human cardiac TnI and human cardiac/slow TnC were obtained by 35 cycles of PCR using 90% Taq DNA polymerase and 10% Pfu DNA polymerase with proofreading activity (Stratagene) to reduce the rate of random mutations.

At restriction enzyme cloning sites constructed in the PCR primers, the human cardiac TnI cDNA was digested with *Neol* and *Bam*HI, purified by agarose gel electrophoresis, and recovered by the Prep-A-Gene glass beads-binding method (Bio-Rad Laboratories) according to the manufacturer’s protocol. The cDNA insert was ligated to *Neol*- *Bam*HI-cut pET3d plasmid, a T7 RNA polymerase-based prokaryotic expression vector (Novagen). After transformation of JM109 *E. coli* cells, ampicillin-resistant colonies containing the recombinant plasmids were screened by PCR using T7 primer recognizing an upstream sequence in the pET3d vector [13] and the insert-specific reverse primer used in the cDNA amplification. The cloned human cardiac TnI cDNA was confirmed by dyeoxy chain termination DNA sequencing at a service facility and transferred as an *XhoI*- *Bam*HI fragment together with the upstream Shine-Dalgarno ribosome-binding sequence [14] to another T7 RNA polymerase-based expression vector pAE04 [15].

The human cardiac/slow TnC cDNA amplified by PCR was first cloned into the pCR4-TOPO vector using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Transformed bacterial colonies that were ampicillin-resistant and β-galactosidase-negative were screened by PCR using T3 and T7 primers flanking the cDNA insert to identify cDNA clones with the anticipated size. After confirmation by DNA sequencing, the cloned human cardiac/slow TnC cDNA was isolated by restriction enzyme cleavage at the *Neol* site built in the 5′ cloning primer and an *EcoR*I site in the vector sequence, gel purified as above, and sub-cloned into *Neol*- *Bam*HI-cut pET3d expression plasmid for protein expression. This was done by first ligating the compatible *Neol* sites and then filling-in the 5′-non-compatible ends followed by Klenow DNA polymerase reaction followed by blunt-end ligation.

**Construction of bi-cistronic expression vector**

Together with TnI, TnC is one of the three subunits of muscle troponin complex and a protein naturally binds TnI with high affinity [7]. Mean- time, TnC is a eukaryotic protein that can readily express at very high levels in *E. coli*. To investigate whether co-expression of cardiac TnI and TnC would form binary protein complex in the host cell and reduce cardiac TnI’s toxicity, we constructed a bi-cistronic expression vector to test the effect on the level of protein expression. As illustrated in Fig. 1, 50 ng of *Neol*- *Bam*HI-cut pET3d plasmid DNA was mixed with *Neol*- *EcoR*I-cut human cardiac TnI cDNA and *XhoI*-*Bam*HI-cut human cardiac TnI cDNA downstream of a Shan-Dalgarno ribosomal binding sequence from pET3d vector at 1:3:3 molar ratio in a 10 μL reaction. After T4 DNA ligase treatment of the three fragments mixture at 16 °C overnight to join the compatible sticky *Neol* ends and *Bam*HI ends, the reaction was diluted three times with double distilled water and Klenow DNA polymerase and dNTP substrates were added to fill-in the *EcoR*I and *XhoI* ends. The reaction mixture was then boosted with T4 DNA ligase and reaction buffer and incubated at room temperature for 4 h for the second step blunt end ligation. The ligation products were used to transform competent JM109 *E. coli* and the ampicillin-resistant colonies were screened by PCR for the presence of both cardiac TnI and cardiac TnC cDNA inserts in tandem orientation downstream of the T7 promoter site (Fig. 1). The recombinant plasmid was verified by restriction endonuclease mapping.

5′-codon modifications in human cardiac TnI cDNA

To investigate the possibility that low usages of the 5′ codons [8,9] of human cardiac TnI mRNA may hamper the expression in *E. coli*, we tested the effect of 5′-regional codon modifications on the bacterial expression. PCR mutagenesis was carried out to replace two codons in the 5′-region of human cardiac TnI cDNA, which are predicted to have low usage in *E. coli*. As illustrated in Fig. 2, an oligo nucleotide primer was synthesized to introduce base substitutions that convert codons Gly285 Arg286 from GGG and AGG to GGU and CGU, respectively. On wild type human cardiac TnI cDNA template cloned in pET3d plasmid, 25 cycles of PCR was performed with the mutagenesis primer and T7 primer in the vector sequence to amplify a 118-bp DNA fragment. This DNA fragment containing the base modifications was purified by agarose gel and recovered by the Prep-A-Gene method as above. The double stranded DNA fragment was directly used as a mega-primer pairing with the downstream E8R reverse primer in a second PCR using wild type human cardiac TnI cDNA template to generate full-length human cardiac TnI cDNA containing the codon modifications. This biased PCR with low efficiency was performed for 30 cycles and yielded a detectable amount of full-length modified cardiac TnI cDNA. After gel purification to remove the wild type cardiac TnI plasmid DNA, a third PCR of 25
cycles using T7 and E8R primers was carried out to further amplify the modified cardiac TnI cDNA. After restriction enzyme cuts at the 5′/HindIII site and 3′/EcoRI site, the cDNA was inserted into XbaI–EcoRI cut pAED4 vector. After transformation of JM109 E. coli, recombinant plasmid clone was screened by PCR and the codon-modified cDNA coding template was verified by DNA sequencing.

Prokaryotic expression plasmid encoding N-terminal truncated mouse cardiac TnI

To investigate whether the N-terminal region of the cardiac TnI polypeptide chain resulted in incompatibility in bacterial cells, we characterized the expression of an N-terminal truncated mouse cardiac TnI in which the N-terminal 28 amino acids are deleted [16]. The 5′-truncated mouse cardiac TnI cDNA cloned in pET3d plasmid was sequenced and verified by small-scale protein expression in E. coli [17] and Western blot identification as described below.

Expression of human cardiac TnI in E. coli culture

The recombinant expression plasmids encoding wild type or modified cardiac TnI alone or bi-cistronically together with cardiac/slow TnC were used to transform chemically treated competent BL21(DE3)pLysS [13] or tRNA-modified BL21(DE3)CodonPlus-RP (Stratagene) E. coli cells using standard procedures. The transformed bacterial cells were selected on LB agar plates containing 100 μg/ml ampicillin and 25 μg/ml chloramphenicol at 37 °C overnight. The freshly transformed cells were cultured in 2× tryptone-yeast broth containing 100 μg/ml ampicillin and 12.5 μg/ml chloramphenicol at 37 °C with vigorous shaking. Growth of the liquid culture was monitored by measuring O.D. at 600 nm. The cultures were induced with 0.4 mM isopropyl-1-thio-β-D-galactoside (IPTG) at various early- and mid-log phase growth time points. After three additional hours of culture at 37 °C, the bacterial cells were harvested by centrifugation at 4 °C and lyzed for SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting analysis.

SDS–polyacrylamide gel electrophoresis and Western blotting

Transformed bacterial cells were lyzed in SDS–PAGE sample buffer containing 2% SDS, 1% β-mercaptoethanol, 10% glycerol, 0.1% bromo-
Statistical analysis was done by Student’s computer program. Two-dimensional densitometry of SDS-gel and tetrazolium substrate solution as previously described above, and developed in 5-bromo-4-chloro-3-indolylphosphate/nitro blue (Sigma Chemical Co.) at room temperature for 1 h, washed again as abated with alkaline phosphatase-labeled anti-mouse IgG second antibody Tris-buffered saline containing 0.5% Triton X-100 and 0.05% SDS, incubated with alkaline phosphatase-labeled anti-mouse IgG second antibody (Sigma Chemical Co.) at room temperature for 1 h, washed again as above, and developed in 5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium substrate solution as previously described [18].

After blocking in 1% bovine serum albumin, the nitrocellulose membranes were incubated with an anti-cardiac TnI monoclonal antibody (mAb) TnI-L [19] at 4 °C overnight. The membranes were then repetitively washed under high stringency using Tris-buffered saline containing 0.5% Triton X-100 and 0.05% SDS, incubated with alkaline phosphatase-labeled anti-mouse IgG second antibody (Sigma Chemical Co.) at room temperature for 1 h, washed again as above, and developed in 5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium substrate solution as previously described [18].

Data analysis

DNA and protein sequence analysis was done using the DNAStar computer program. Two-dimensional densitometry of SDS-gel and Western blot was carried out using the NIH Image program version 1.61. Statistical analysis was done by Student’s t-test.

Results and discussion

The higher copy number pAED4 vector gives a higher expression of cardiac TnI than that of pET3d

Fig. 3a demonstrates the specific detection of cardiac TnI and cardiac/slow TnC expressed in E. coli culture by SDS-PAGE and TnI-L mAb Western blotting. Under standard culture and induction conditions, only a low level of human cardiac TnI protein was detected in transformed BL21(DE3)pLysS E. coli culture by Western blotting using the TnI-L mAb (Fig. 3b). Between the low level expressions, pAED4 vector produced a higher expression than that of pET3d vector (Fig. 3b and Table 1). We have observed by the yields of plasmid DNA preparation from parallel transformed bacterial cultures as well as agarose gel direct quantification of the plasmid DNA extracted from bacterial cells (as normalized by the level of ribosomal RNAs) that pAED4 plasmid produces a copy number in the transformed E. coli cell approximately twice as that of pET3d (data not shown). Therefore, the higher level of cardiac TnI expression by pAED4 vector suggests an advantage of using high copy number expression vectors, especially for the production of hard-to-express proteins in E. coli culture.

Bi-cistronic co-expression with TnC improves the expression level of cardiac TnI

One hypothesis that we tested was that free cardiac TnI protein might be toxic to the host bacterial cell. Considering that TnC naturally binds TnI with high affinity [6] and is a protein that readily expresses in E. coli at high levels, we examined whether co-expression of cardiac TnI with TnC would allow the formation of TnI–TnC binary protein complex in the bacterial cell and reduce the amount of free cardiac TnI to prevent the potential toxicity. To ensure co-expression in all transformed bacterial cells, we constructed a bi-cistronic expression vector encoding human cardiac TnI and human cardiac/slow TnC in a single mRNA (Fig. 1). When the bi-cistronic expression plasmid was used to transform BL21(DE3)pLysS E. coli, co-expression of cardiac TnI and cardiac/slow TnC was achieved upon IPTG induction (Fig. 3c). The co-expression with TnC had a beneficial effect on the expression level of cardiac TnI (Fig. 3c and Table 1), supporting the hypothesis that the presence of TnC could reduce the incompatibility of cardiac TnI with the host E. coli cell.

Similar to that seen in previous studies [20–22], the improvement of cardiac TnI expression by using the TnC–TnI bi-cistronic vector was moderate (Table 1). The high level expression of TnC indicates the presence of an abundant amount of the bi-cistronic mRNA in the bacterial cell and the co-expression with cardiac TnI did not affect the translational efficiency and accumulation of human cardiac TnC protein in E. coli. Same as that upstream of the cardiac TnC cDNA, the Shan-Dalgaro sequence upstream of the cardiac TnI cDNA was from pET3d vector. Accordingly, the translations of TnC and TnI cistrons were directed by identical ribosomal binding sites which should allow similar levels of translational initiation. Therefore, the significantly lower level of cardiac TnI protein expression comparing to that of TnC suggests that the formation of binary TnI–TnC complex in the bacterial cell, although conveyed the moderate protective effect, is of low efficiency. The chemical environment in bacterial cells is apparently different from that of the muscle cells and may not be suitable for high affinity binding between TnC and TnI. On the other hand, native troponin complex is known to resist to high salt buffers [23]. Therefore, the ineffective binding between TnI and TnC in bacterial cells might not be simply due to the solution environment but result from ineffective folding of the bacterially made troponin proteins (most likely cardiac TnC since the TnC protein was in excess and only a small portion needed to be correctly folded to saturate the co-expressed cardiac TnI). It has been demonstrated by us and many other laboratories that TnI and TnC purified from bacterial expression both have biochemical activities and can form functional TnI–TnC binary complex and tertiary troponin complex [3,7,10]. These results indicate that denaturing (urea buffer) and renaturing (final dialysis) steps during the purification of bacterial made TnI are necessary for the yield of biologically active proteins.

Effect of improving overall codon usage on the expression of human cardiac TnI in E. coli

The low versus high expressions of cardiac TnI and TnC proteins from a bi-cistronic mRNA indicates a posttranscriptional restriction of cardiac TnI expression. As discussed above, identical Shan-Dalgaro ribosomal binding
sequences were used for the two translational units for similar efficiency in translational initiation. Therefore, the effect of translational efficiency of human cardiac TnI mRNA on its expression in *E. coli* needs to be considered. It is well established that certain codons (AGG, AGA, CUA, AUA, CGA or CCC) that are frequently used in eukaryotic mRNA have low usages in prokaryotic cells and they may hinder the expression of eukaryotic proteins in *E. coli* [24]. To investigate whether improving the overall usage of human cardiac TnI codons in the host *E. coli* cell would improve the protein expression, we compared the use of regular BL21(DE3)pLysS cell containing only *E. coli* tRNAs and BL21 CodonPlus(DE3)-RP cell (Stratagene) that has been genetically modified by the addition of tRNAs recognizing eukaryotic codons AGA, AGG, and CCC. The results in Fig. 3d and Table 1 show that human
cardiac TnI was expressed at higher levels in BL21 CodonPlus(DE3)-RP cells than that in BL21(DE3)pLysS cells. This observation supports the notion that low usage of these codons in the human cardiac TnI mRNA may be a limiting factor during protein expression in E. coli.

Similar to the expression of cardiac TnI alone, the use of tRNA-enhanced E. coli host cell also produced better expression by the bi-cistronic vector than that of using BL21(DE3)pLysS cell (Fig. 3d and Table 1). This observation suggests that in the presence of TnC that may moderately reduce the toxic effect of cardiac TnI, the initial production of cardiac TnI protein remains a dominant limiting factor during bacterial expression.

**Modification of the 5’-regional codons did not reproduce the beneficial effect of tRNA enhancement on cardiac TnI expression**

It has been demonstrated that codon usage in the very 5’-region of the mRNA [8,9] may be critical to the optimal expression of eukaryotic proteins in E. coli. There are two codons (Gly4, GGG and Arg10, AGG) in the 5’-region of human cardiac TnI mRNA that are predicted to have low usage in bacterial cells and may have contributed to the codon usage limitation to the protein expression. To investigate this hypothesis, we replaced these two codons in cloned human cardiac TnI cDNA with ones frequently used in E. coli [24] (Fig. 2). Although a previous study saw improved expression of human cardiac TnI in E. coli by modifying codons Ala2 and Gly4 [25], our modification of Gly4 and Arg10 codons did not reproduce the improvement in cardiac TnI expression as that seen in the tRNA-enhanced E. coli cells (Fig. 3d and Table 1). The results indicate that although overall codon usage is an important factor in the expression efficiency of cardiac TnI in E. coli (Fig. 3d), the 5’-regional codon usage alone does not have a determining role.

**Significantly increased expression of cardiac TnI after deleting the N-terminal 28 amino acids**

We previously observed that the core structure of turkey cardiac TnI without the N-terminal segment expressed in E. coli at a much higher level than that of intact turkey cardiac TnI [6]. Such improvement was also seen by others [25]. To quantitatively investigate the incompatibility effects of cardiac TnI N-terminal region on bacterial expression, we studied the expression of an N-terminal truncated mouse cardiac TnI lacking amino acids 1–28. The results in Fig. 3f and Table 1 demonstrated that deletion of the N-terminal 28 amino acids of mouse cardiac TnI significantly improved the level of protein expression in E. coli. This effect was much larger than that by using the codon-enhanced E. coli host cell (Table 1). The use of codon-enhanced host cell did not further improve the expression level of N-terminal truncated cardiac TnI (Fig. 3f and Table 1).

The expression of N-terminal truncated cardiac TnI involves alterations in both the mRNA and the protein produced. It has been observed that the mRNA structure may affect bacterial expression of eukaryotic proteins [26]. The 5’-structure may affect the stability of the mRNA and reduce the level of protein expression. However, the very high level TnC synthetized from the bi-cistronic mRNA indicates a significant amount of the mRNA (Fig. 3) and precludes the role of mRNA instability in causing the low level bacterial expression of intact cardiac TnI.

The N-terminal segment of cardiac TnI polypeptide is a unique structure that is not present in fast or slow skeletal muscle TnI and may be proteolytically removed in the cardiac muscle during physiological adaptations [23]. The significantly improved expression of N-terminal truncated cardiac TnI in E. coli suggests that the N-terminal segment has a negative effect on bacterial expression. Deletion of this segment (Fig. 4) does not disrupt the core structure of cardiac TnI.
TN [6,23] and does not alter the overall positive charge of the protein (the isoelectric points of intact and N-terminal truncated mouse cardiac TN are 9.57 and 9.59, respectively). Therefore, the possible mechanisms may include its direct toxicity to the host metabolism and/or its effects on the stability and accumulation of the exogenous protein synthesized.

It is worth noting that the N-terminal domain of cardiac TN contains the protein kinase A phosphorylation site (Ser23/Ser24 in the mouse sequence, Fig. 4) that is a crucial site for physiological regulation of cardiac thin filament function [5]. Absent in skeletal muscle TN, this region does not contain any known binding sites for other myofilament proteins [3]. Deletion of this segment from cardiac TN resulted in dominant effects on cardiac muscle function in transgenic mice [16]. Therefore, the N-terminal segment of cardiac TN may serve as a conformational modulator for the core structure and this conformational effect may also be a mechanism for the incompatibility of intact cardiac TN in bacterial cells.

Effects of early and late inductions on the expression of intact cardiac TN

The incompatibility of the N-terminal peptide of cardiac TN in the host bacteria may reduce the proportion of the positively transformed cells as a decreased growth rate was observed in E. coli cultures after IPTG induction of intact cardiac TN expression (Fig. 5). Due to the negative selection pressure from the N-terminal segment of cardiac TN in the host bacteria, even a very low level leaked expression of intact cardiac TN may rapidly reduce the positive cell population during culture. Consistently, we have found that the use of freshly transformed bacteria is critical to a successful preparative expression (data not shown). Starting from a predominantly positive cell population, the induction of cardiac TN expression at a proper time point during the course of culture is also critical to the level of protein expression. Earlier induction would ensure that most cells in the culture are still positive. However, since a restricted duration of induction is also crucial to avoid the over growth of negative cells, earlier induction would yield less cells harvested per unit volume of the culture and lower the total yield of protein. To establish proper induction conditions for a maximum yield, we examined the induction of intact cardiac TN expression at a series of cell densities. The results in Fig. 5 showed that when intact cardiac TN was expressed alone, the expression level was decreased when induced at O.D.600nm > 0.3. Co-expression of cardiac TN with TnC produced higher levels of expression with a higher optimal induction O.D.600nm (0.4).

Fig. 4. The N-terminal segment of cardiac TN. 5’-truncated cDNA was used to express cardiac TN lacking the N-terminal 28 amino acids (underlined on the lower sequence). The protein kinase A phosphorylation site Ser23/Ser24 is indicated in bold letters. A Met codon was added in the construct as the translation initiation codon for the expression of N-terminal truncated cardiac TN (underlined on the upper sequence).

Fig. 5. Effects of early and late inductions on the level of cardiac TN expression. BL21(DE3)pLysS E. coli transformed with wild type cardiac TN-pAED4 or cardiac TN-cardiac TnC-pAED4 expression plasmid was cultured in 2×TY media at 37 °C with vigorous shaking and induced by 0.4 mM IPTG at a series of cell densities as monitored by O.D.600nm. The induced bacteria were harvested by centrifugation and lysed in SDS-PAGE sample buffer for SDS-PAGE and Western blot analysis using anti-TnI mAb TnI-1. Cardiac TN expression detected by the Western blots was quantified by two-dimensional densitometry and normalized against the total bacterial protein determined by Coomassie brilliant blue R250 staining of parallel gels. The values are means ± standard error. The results showed that when cardiac TN was expressed alone, the expression level was decreased when induced at O.D.600nm > 0.3. Co-expression of cardiac TN with TnC produced higher levels of expression with a higher optimal induction O.D.600nm (0.4).

In summary, difficulties in bacterial expression of cardiac TN represent a challenging but intriguing model system for understanding the compatibility of eukaryotic proteins to the bacterial host during preparative expression. The present study demonstrates that removal of an incompatible submolecular structure of a foreign protein may overcome the low codon usage limitation that universally exists during bacterial expression of eukaryotic proteins. The finding that the N-terminal domain of
cardiac TnI is a dominant factor for the incompatibility in bacterial cells supports its role in modulating the global conformation.

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