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In vitro Studies of Human Cardiac Troponin I Degradation

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Introduction

UPON MYOCARDIAL INFARCTION, cTnI is released from the injured cardiac muscle into the bloodstream. It is a universally recognized early and reliable marker of acute myocardial infarction and its measurements are widely used in clinical practice. It was previously shown that N- and C-terminal parts of cTnI are degraded (upon release from tissue or during circulation in blood) although there remains minimal information available regarding the precise sites of cTnI cleavage. Our goal was to reveal sites of cTnI degradation in model experiments *in vitro*.

Methods

HUMAN CARDIAC TISSUE (postmortem) was homogenized in an extraction buffer and incubated for three hours with gentle shaking at 37°C to initiate the partial proteolysis of cTnI by endogenous proteases. Following centrifugation, tissue extract was used for cTnI analysis.

PROTEINS FROM CARDIAC TISSUE EXTRACT were separated by gel filtration using the ÄKTA pure system on a Superdex 75 16/60 column (GE Healthcare, USA).

FOR THE DETECTION OF CTNI in gel filtration profiles we used several sandwich fluoroimmunoassays (FIA) utilizing anti-cTnI antibodies (Cat.# 4T21, HyTest, Finland) specific to different cTnI epitopes.

N- AND C-TERMINAL CTNI FRAGMENTS were affinity extracted (from corresponding GF fractions) on affinity matrixes utilizing immobilized antibodies that were specific to N-terminal or C-terminal cTnI fragments respectively and then purified by a reverse-phase chromatography.

PURIFIED CTNI TERMINAL FRAGMENTS were analyzed by MALDI-TOF (UltrafleXtreme, BrukerDaltonics, Germany) and identified based on their molecular masses.

Results

DETECTION OF N- OR C-TERMINAL FRAGMENTS FOLLOWING PARTIAL CTNI CLEAVAGE BY ENDOGENOUS PROTEASES

Proteins extracted from myocardial tissue following partial proteolysis by endogenous proteases were separated by gel filtration. cTnI fragments were identified in fractions by two-site combinations of antibodies that were specific to different epitopes located on the N- or C- terminal parts of cTnI. Being aware of the location of the epitopes of antibodies utilized in such two-site combinations meant that we could localize the cleavage sites and identify the most stable part of the cTnI molecule. When epitopes for both MAbs were located at the cleaved fragment of the molecule, two peaks of immunoreactivity corresponding to the untruncated molecule and to the cleaved fragment were detected (see Fig. 1A). When epitopes were located on the parts of the molecule that were separated by the site of proteolysis, untruncated protein only was detected (see Fig. 1B).

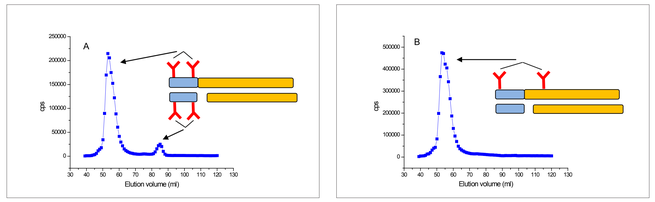

FIGURE 1. Explanation of the approach used for the peptide analysis.

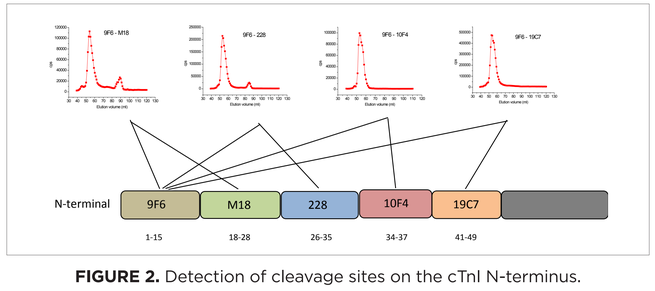

FIGURE 2. Detection of cleavage sites on the cTnI N-terminus.

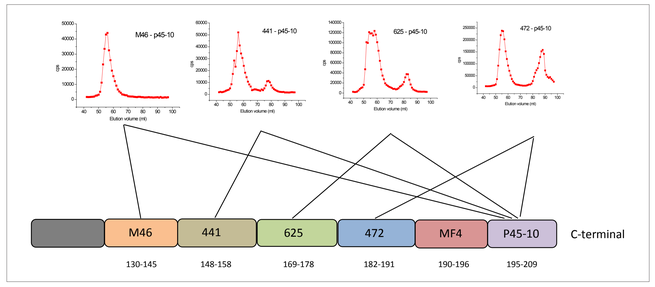

FIGURE 3. Detection of cleavage sites on the cTnI C-terminus.

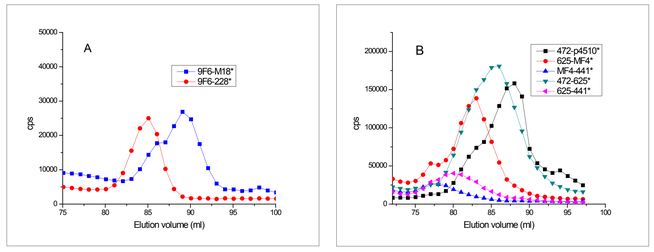

FIGURE 4. cTnI peptides analysis in the gel filtration profile by different two-site MAb combinations.

Using such an approach we have localized the cleavage sites on the N- and C-terminuses of cTnI. According to data that we have obtained, a stable region on the cTnI N-terminus begins from the epitope that is recognized by the MAb 10F4 (34-37 amino acid residues) (Fig. 2). On the C-terminus the stable region ends on the epitope recognized by the MAb M46 (130-145 amino acid residues) (Fig. 3).

The analysis of cTnI peptides in the gel filtration profile by several MAb two-site combinations is shown in Fig. 4. Two distinct immunoreactivity peaks were found by utilizing antibodies specific to the N-terminal part of the cTnI molecule (Fig. 4A) and multiple peptide peaks in cases where C-terminal cTnI peptides were analyzed (Fig. 4B).

IDENTIFICATION OF CTNI TERMINAL PEPTIDES AND CLEAVAGE SITES BY MASS SPECTROMETRY

cTnI terminal peptides were collected, purified and analyzed by mass spectrometry.

The analysis of peptides made it possible for us to identify cleavage sites on the cTnI molecule (Fig. 5). We can conclude that the cTnI fragment susceptible to proteolysis is shorter at the N-terminal end and longer at the C-terminal end. Meanwhile, the C-terminal fragment contains more cleavage sites than the N-terminal part.

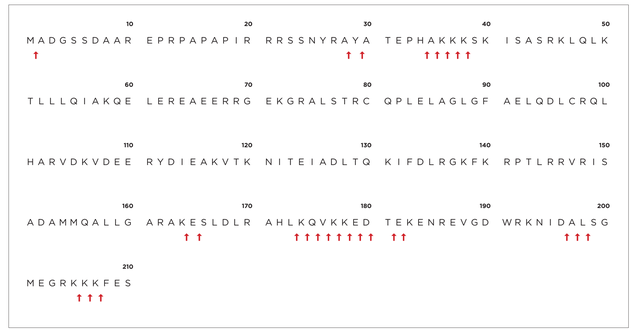

FIGURE 5. Cleavage sites on the cTnI molecule according to the mass spectrometry peptide analysis.

Conclusions

1. cTnI is susceptible to the N- and C-terminal proteolysis
2. The stable part of cTnI begins from approximately the 40th amino acid residue and lasts up until the 145th amino acid residue (approximately).
3. Multiple cleavage sites on the cTnI molecule were identified by the mass spectrometry analysis

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