Site-Directed Methionine Oxidation in Calmodulin

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Introduction

We have examined the oxidation-induced perturbations of calmodulin (CaM) and its interaction with the skeletal muscle ryanodine receptor (RyR1) using site-directed mutagenesis and circular dichroism. The working hypothesis is that the detrimental effects of CaM oxidation on RyR channel regulation can be traced to definable changes in CaM structure and dynamics that result from the modification of specific methionine residues. Oxidative modification disrupts the functional CaM/RyR interaction through changes in structure and dynamics, resulting in reduced SR Ca^{2+} release and incomplete contractile activation.

The accumulation of oxidatively modified proteins has been implicated in the progression of biological aging, muscle degeneration and disease. Oxidative modification of muscle associated proteins has been shown to impair skeletal muscle function *in vivo* and *in vitro* (*1-5*). Oxidative modification can lead to thermodynamic instability, disruption of tertiary structure, and decrease in enzymatic activity.

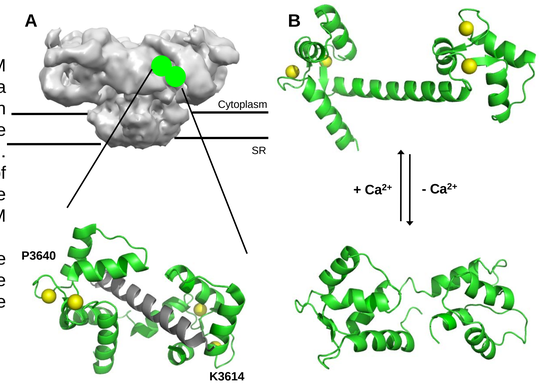

Figure 1. (A) Cryo-EM structure of the 2.3 MDa skeletal muscle RyR shown in grey; CaM binding site on the RyR is highlighted in green. Crystal structure (1BCX) of CaM bound to peptide mimicking the identified CaM binding site on the RyR1 (*5*). (B) Structures of CaM in the presence (1CLL) and absence (1CFC) of Ca^{2+}. Ca ions are shown as yellow spheres.

Site-Directed Mutagenesis of CaM

- Plasmid Preparation and Temperature Cycling (PCR): Template DNA and primers amplifying specific Methionine amino acids and mutated them to Leucine amino acids.
- DPN1 Digest: The mutated PCR product was digested with DPN1.
- Transformation: The DPN1 digest was transformed into XL Gold Competent Cells and grown in LB+Amp media in order to replicate the DNA.
- Mini-Prep: The mutated DNA was isolated by performing a mini-prep and the correct sequence was obtained by analyzing sequencing results.

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Calmodulin Methionine Location and Oxidation

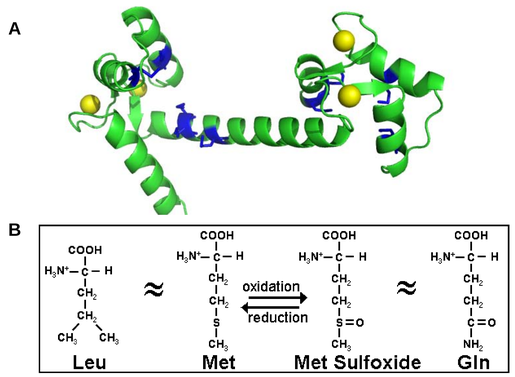
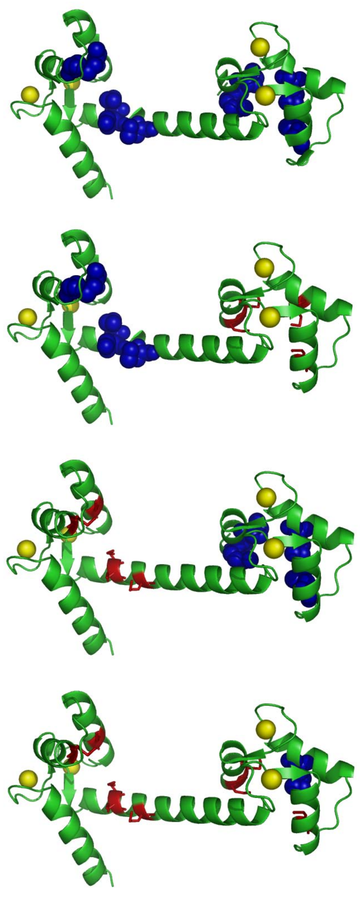


Figure 2. (A) Location of 9 methionine residues (blue) in CaM structure (1CLL). (B) Methionine oxidation to methionine sulfoxide adds oxygen atom to sulfur moiety. The amino acid leucine can be substituted to protect Met residues from oxidation while the amino acid glutamine can be substituted to mimic the effects of oxidation.

CaM Mutants for Functional and Spectroscopic Studies



Wildtype CaM

All nine native Mets present.

N-lobe CaM

All Mets in the C-terminal lobe of CaM mutated to Leu leaving all Mets in N-terminal lobe susceptible to oxidation.

C-lobe CaM

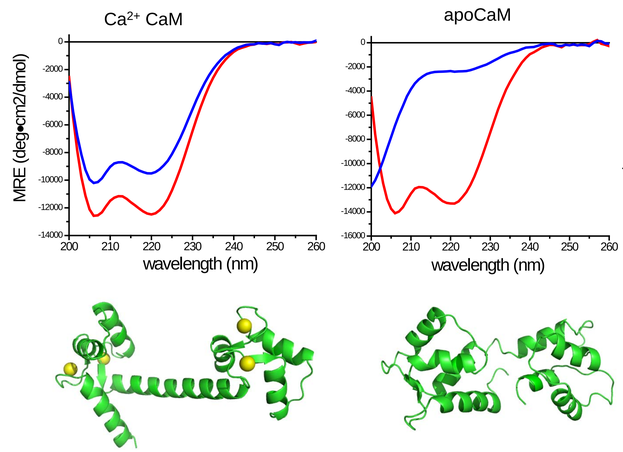
All Mets in the N-terminal lobe of CaM mutated to Leu leaving all Mets in C-terminal lobe susceptible to oxidation.

M124 CaM

All Mets mutated to Leu except Met 124 in the C-terminal lobe. This Met is predicted to be the most important for functional interaction with the RyR.

Figure 3. CaM mutants for functional and spectroscopic studies. Native methionines still present and therefore susceptable to oxidation are shown with blue, space-filling side chains. Methionines mutated to leucine and therefore protected from oxidation are shown in red. Yellow spheres represent bound Ca^{2+}.

Oxidation-Induced Decrease in CaM α-helical Content



% α-helix	Wt	Oxidized
+Ca^{2+}	66%	61%
-Ca^{2+}	61%	26%

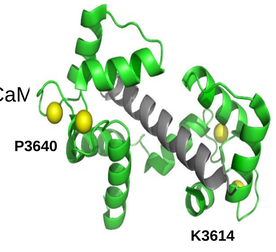
Figure 4. Circular Dichroism spectra before (red) and after (blue) methionine oxidation of wildtype CaM. In the presence of Ca^{2+}, oxidation has minimal effect on CaM secondary structure. In contrast, apoCaM (-Ca^{2+}) shows a significant decrease in α-helical content after oxidation.

Future Direction/Conclusions

(1) Oxidation causes a decrease in CaM α-helical content, with a more pronounced effect in the absence of Ca^{2+}.

(2) Future Direction/Experiments

(1) Expression and purification of N-lobe, C-lobe, and M124 CaM mutants using Site-Directed Mutagenesis.
(2) Circular dichroism of all CaM mutants
  (1)In the presence/absence of Ca2+
  (2)In the presence/absence of RyR peptide
  (3)In the presence of reducing agents
(3)Electron Paramagnetic Resonance (EPR) of all CaM mutants
  (1)In the presence/absence of Ca2+
  (2)In the presence/absence of RyR peptide
  (3)In the presence of reducing agents



3614-KSKKAVWHKLLSKQRRRAVVACFRMTP-3640

Figure 5. Future studies will involved studying CaM in the presence of the RyR peptide, which is residues K3814 through P3840 of the full-length RyR. The crystal structure (1BCX) of CaM bound to the peptide mimicking the identified CaM binding site on the RyR and the amino acid sequence of the RyR peptide are shown.

References

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