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Impact of methionine oxidation on calmodulin structural dynamics

Megan R. McCarthy [a], Andrew R. Thompson [a], Florentin Nitu [a], Rebecca J. Moen [c], Michael J. Olenek [b], Jennifer C. Klein [b,1], David D. Thomas [a,1,*]

[a] Biochemistry, Molecular Biology and Biophysics Department, University of Minnesota, Minneapolis, MN 55455, USA
[b] Biology Department, University of Wisconsin, La Crosse, WI 54601, USA
[c] Chemistry and Geology Department, Minnesota State University, Mankato, MN 56001, USA

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ABSTRACT

We have used electron paramagnetic resonance (EPR) to examine the structural impact of oxidizing specific methionine (M) side chains in calmodulin (CaM). It has been shown that oxidation of either M109 or M124 in CaM diminishes CaM regulation of the muscle calcium release channel, the ryanodine receptor (RyR), and that mutation of M to Q (glutamine) in either case produces functional effects identical to those of oxidation. Here we have used site-directed spin labeling and double electron–electron resonance (DEER), a pulsed EPR technique that measures distances between spin labels, to characterize the structural changes resulting from these mutations. Spin labels were attached to a pair of introduced cysteine residues, one in the C-lobe (T117C) and one in the N-lobe (T34C) of CaM, and DEER was used to determine the distribution of interspin distances. Ca binding induced a large increase in the mean distance, in concert with previous X-ray crystallography and NMR data, showing a closed structure in the absence of Ca and an open structure in the presence of Ca. DEER revealed additional information about CaM's structural heterogeneity in solution: in both the presence and absence of Ca, CaM populates both structural states, one with probes separated by ~4 nm (closed) and another at ~6 nm (open). Ca shifts the structural equilibrium constant toward the open state by a factor of 13. DEER reveals the distribution of interprobe distances, showing that each of these states is itself partially disordered, with the width of each population ranging from 1 to 3 nm. Both mutations (M109Q and M124Q) decrease the effect of Ca on the structure of CaM, primarily by decreasing the closed-to-open equilibrium constant in the presence of Ca. We propose that Met oxidation alters CaM's functional interaction with its target proteins by perturbing this Ca-dependent structural shift.

1. Introduction

1.1. Muscle aging, disease, and methionine oxidation

Reactions that use oxygen to drive cellular respiration create highly reactive oxygen species (ROS) that are potentially damaging to the cell. Biological aging and degenerative disease are strongly influenced by the resulting oxidative stress, causing post-translational modification of DNA, lipids, and proteins. Protein oxidation is strongly associated with loss of strength in both skeletal and cardiac muscle, and is proposed to play a major role in aging [1–3], muscular dystrophy [4], and heart failure [5,6]. Understanding the initiation and progression of muscle aging and disease requires identification and characterization of ROS targets.

The sulfur-containing amino acids, cysteine (Cys) and methionine (Met), are the prime cellular targets of biological oxidants [7–9]. In particular, Met oxidation and subsequent reduction by Met sulfoxide reductase have far-reaching implications in metabolic, cardiovascular, neurological, and immune related dysfunction [10–12]. We have identified specific Met residues in proteins as targets of oxidation in muscle contractile and regulatory proteins [13–15]. Met oxidation has been proposed as a mechanism through which the muscle cell responds to oxidative stress by modulating metabolism and energy utilization [16]. Met oxidation can perturb local secondary structure, induce conformational disorder, and disrupt key hydrophobic interactions [17–19]. However, Met oxidation in the context of protein structure has only been systematically evaluated for a handful of proteins [13,20–22]. Here, we have linked the oxidation of particular functionally sensitive Met residues to discrete and measurable changes in protein structure in order to understand how the oxidation of a single protein

* Corresponding author.
E-mail addresses: jklein@uwlax.edu (J.C. Klein), ddt@umn.edu (D.D. Thomas).
[1] Drs. Klein and Thomas are co-senior authors.

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side chain can contribute to altered regulatory interactions in muscle.

1.2. Methionine oxidation alters CaM regulation of target proteins

We seek a molecular structural explanation for how oxidative modifications impact muscle protein function, focusing on the ubiquitous Ca signaling protein calmodulin (CaM). CaM plays a central role in Ca-mediated regulation of muscle contraction. Among its hundreds of target proteins, CaM acts as a feed-forward activator of calcium pumps, a feed-back inhibitor of calcium channels, and an activator of a multitude of CaM-dependent kinases [23]. CaM has unusually high Met content, including 46% of the hydrophobic residues in the binding pockets, which are crucial for CaM's interactions with over 400 diverse target proteins [24]. CaM containing oxidized Met residues has been isolated from both skeletal muscle and the brain of aged animals [25,26]. Met oxidation impairs CaM's ability to regulate the ryanodine receptor calcium channel (RyR) [27,28], the plasma membrane Ca^{2+} ATPase (PMCA) [17,21,29,30] and numerous other targets [31–33]. As a central node in the calcium signaling network, CaM is in an ideal position to orchestrate redox control of cellular homeostasis.

CaM is a dumbbell-shaped protein, with two globular domains (lobes) connected by a flexible α-helical linker (Fig. 1). CaM's C-terminal lobe (C-lobe) Met residues are particularly susceptible and functionally sensitive to oxidation. Oxidation of Met 144 and Met 145 prevents CaM from fully activating the PMCA [30]. For the RyR, CaM binding is linked to profound changes in the Ca dependence of both activation and inactivation [35,36]. Specific Met residues within the C-lobe are critical for CaM-mediated regulation of the RyR [27,28]. Met-to-Gln (M-to-Q) mutations designed to mimic Met oxidation were used to determine site-specific contributions of C-lobe Met oxidation to changes in CaM regulation of RyR. It was found that M124Q induces a twofold increase in the concentration of CaCaM required for half-maximal inhibition, while M109Q attenuates maximal RyR activation by apoCaM [27,28].

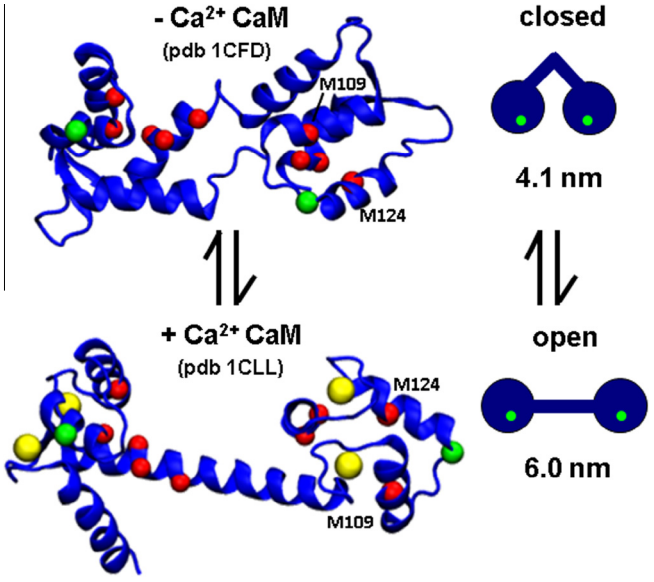

Fig. 1. CaM structural model. The positions of coordinated calcium ions (yellow spheres), T34C and T117C labeling sites (green spheres), and all nine methionine residues (red spheres) are indicated. The two methionine residues of interest are labeled. 1CLL (top, based on NMR in solution) and 1CFD (bottom, from crystallography) were rendered using VMD [34]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

M109Q and M124Q mutations were also found to uniquely block activation of smooth muscle myosin light chain kinase, CaM-dependent protein kinase IIα, and CaM-dependent protein kinase IV [37]. Here we pursue a structural explanation for the observed functional impact of oxidation of M109 and M124, focusing on changes in the structural transition that accompanies Ca binding. We have linked oxidation of specific Met to measurable changes in protein structure by (1) mimicking oxidation of particular amino acids through mutagenesis and (2) using spectroscopic distance measurements to resolve subtle changes in protein structure and dynamics.

1.3. Structural model to be tested

Although CaM is highly dynamic, most crystal and NMR structures can be assigned to one of two broad categories, the "open" or "closed" state (Fig. 1). The open structural state, which is stabilized by Ca binding, is defined by (a) a perpendicular orientation of pairs of EF hand helices, (b) an exposed patch of hydrophobic residues on each lobe, (c) an outward rotation of the lobes that elongates the entire molecule, and (d) a stable α-helical linker connecting the lobes [38]. Exposure of hydrophobic patches is thought to facilitate target protein binding and occurs upon the reorientation of the EF hand helices with Ca binding. The closed structure has been more difficult to characterize because of crystallization problems at low Ca. The solution NMR structure of apoCaM [39,40] (Fig. 1, top) serves as a model for the closed structural state and is characterized by (a) EF hand helices in a tight four-helix bundle, (b) buried hydrophobic patches, (c) inward rotated lobes yielding a compact molecular shape, and (d) a discontinuous α-helical linker connecting the lobes.

The static nature of the models presented in Fig. 1 is an oversimplification. There is probably not a rigid coupling in solution between CaM structural state (open or closed) and biochemical state (low or high Ca), since there is an instance of Ca-loaded CaM crystallized in the closed structural state [41]. There are several lines of evidence suggesting that CaM undergoes conformational exchange in solution, particularly in the linker helix [42] and in the C-lobe [43,44]. Indeed, NMR relaxation measurements and single-molecule FRET studies detected the presence of open and closed CaM states on the millisecond timescale [45,46]. The existence of conformational equilibrium has been proposed as a mechanism through which target protein binding occurs through "selection" of pre-existing CaM conformations [47]. CaM's promiscuity in binding interactions might very well stem from CaM's broad intrinsic dynamics [24].

Here we have used spectroscopic distance measurements to better define the relationship between CaM Ca binding, Met oxidation, and the structural dynamics of the open and closed structural states. We chose to use the increasingly popular pulsed EPR technique DEER, over fluorescence techniques such as FRET, because it allows for the use of smaller, identical probes and provides superior resolution of distinct conformational states, mole fractions, and disorder [48,49]. DEER is also much more effective than NMR in resolving the kind of long-range structural changes and conformational heterogeneity predicted by Fig. 1 [48,49]. The results provide new insight into CaM structural dynamics and function.

2. Materials and methods

2.1. Sample preparation and characterization

Mammalian calmodulin mutants with Cys substitutions for spin-labeling (T34C and T34C.T117C) and Met to Gln substitutions