Selective Degradation of Oxidized Calmodulin by the 20 S Proteasome*

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Deborah A. Ferrington§§, Hongye Sun¶, Kathryn K. Murray¶¶, Jessica Costa¶¶, Todd D. Williams§§, Diana J. Bigelow§§, and Thomas C. Squier¶¶

From the ‡Department of Ophthalmology, University of Minnesota, Minneapolis, Minnesota 55455 and §Biochemistry and Biophysics Section, Department of Molecular Biosciences and ¶Mass Spectrometry Laboratory, University of Kansas, Lawrence, Kansas 66045-2106.

We have investigated the mechanisms that target oxidized calmodulin for degradation by the proteasome. After methionine oxidation within calmodulin, rates of degradation by the 20 S proteasome are substantially enhanced. Mass spectrometry was used to identify the time course of the proteolytic fragments released from the proteasome. Oxidized calmodulin is initially degraded into large proteolytic fragments that are released from the proteasome and subsequently degraded into small peptides that vary in size from 6 to 12 amino acids. To investigate the molecular determinants that result in the selective degradation of oxidized calmodulin, we used circular dichroism and fluorescence spectroscopy to assess oxidant-induced structural changes. There is a linear correlation between decreases in secondary structure and the rate of degradation. Calcium binding or the repair of oxidized calmodulin by methionine sulfoxide reductase induces comparable changes in α-helical content and rates of degradation. In contrast, alterations in the surface hydrophobicity of oxidized calmodulin do not alter the rate of degradation by the proteasome, indicating that changes in surface hydrophobicity do not necessarily lead to enhanced proteolytic susceptibility. These results suggest that decreases in secondary structure expose proteolytically sensitive sites in oxidized calmodulin that are cleaved by the proteasome in a nonprocessive manner.

Critical to the ability of a cell to reestablish cellular homeostasis after a range of different environmental stresses is the removal of oxidatively modified proteins by the proteasome (1, 2). The proteasome represents approximately 1% of the total cellular protein and is present in the cytosol and nuclei of all mammalian cells in two major forms (i.e. the 20 S and 26 S proteasomes) (3). The 20 S proteasome is a 700-kDa complex with a 10–20-Å-diameter opening into an internal cavity that provides a sequestered environment for proteolysis. The 26 S proteasome is a 2000-kDa complex containing two 19 S regulatory complexes bound to the 20 S multicatalytic core. The 26 S proteasome is responsible for the degradation of the majority of cellular proteins through an ATP-dependent and ubiquitin-mediated pathway (4–6). In contrast, the 20 S proteasome core selectively degrades a range of different oxidized proteins in an ATP-independent manner and has been suggested to represent the primary mechanism in the rapid removal of oxidized proteins after oxidative stress (7–11). The signal for recognition and degradation of oxidized proteins by the 20 S proteasome is unknown but has been suggested to involve (i) exposure of hydrophobic surfaces after oxidative modification, (ii) recognition of molecular “markers” associated with the oxidative modification of specific amino acid side chains, and (iii) increases in the conformational flexibility of oxidized proteins (12–16).

To distinguish which signals are involved in targeting an oxidized protein for degradation by the 20 S proteasome, we have investigated the mechanisms involving the recognition and cleavage of oxidized calmodulin (CaM).1 CaM was chosen because of its key role in intracellular signaling and its functional sensitivity to conditions of oxidative stress (17, 18). After oxidative modification of multiple methionines to their corresponding sulfoxides, CaM is unable to activate a range of different target proteins involved in intracellular signaling (19, 20). These results suggest that CaM oxidation has the potential to result in large changes in cellular function as a result of changes in both calcium signaling and energy metabolism (21). Consistent with this suggestion, CaM isolated from senescent brain contains multiple methionine sulfoxides that may be partially responsible for observed alterations in calcium signaling associated with a range of age-related diseases (17). Although intracellular enzymes exist that can reduce (i.e. repair) oxidized methionines and partially restore CaM function (22, 23), it is essential that rapid turnover mechanisms exist to clear oxidatively modified CaM (CaMox) so as to maintain normal calcium signaling. In this respect normal CaM turnover is slow (t1⁄2 ~ 18 h) in comparison with many transcription factors and other cellular regulatory molecules (24, 25). However, it has been shown that the proteasome selectively degrades posttranslationally modified CaM after deamidation of selected asparagines through a ubiquitin-independent cellular degradation mechanism (16). It is therefore of interest to determine whether the oxidation of methionines in CaM facilitates recognition and degradation by the 20 S proteasome and, if so, identify the molecular determinants that are responsible for the degradation of CaM after methionine oxidation.

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§ To whom correspondence should be addressed. Tel.: 612-624-8267; Fax: 612-626-0781; E-mail: ferri013@tc.umn.edu.

1 The abbreviations used are: CaM, calmodulin; CaMox, oxidized CaM; ANS, 1-anilinonaphthalene-8-sulfonate; CD, circular dichroism; ESI-MS, electrospray ionization mass spectrometry; HPLC, high performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; HOMOPIPES, homopiperazine-1,4-bis-(2-ethane-sulfonic acid).
To identify the mechanisms underlying the degradation of oxidized CaM by the 20 S proteasome, in vitro conditions were used to selectively oxidize variable numbers of methionines in CaM (18, 19). Under these conditions no amino acids other than methionine are oxidized, and the pattern of methionine oxidation is similar to that found in CaM isolated from senescent brain (17, 18, 21). We assessed the ability of the 20 S proteasome to degrade CaM$_{\text{ox}}$ and identified recognition elements within CaM$_{\text{ox}}$ that promote degradation. Our results demonstrate that the 20 S proteasome selectively degrades oxidized CaM in preference to native (unoxidized) CaM. Although neither hydrophobicity nor the presence of methionine sulfoxide is a signal for the degradation of oxidized CaM, the rate of degradation correlates with the extent of secondary structural loss.

**EXPERIMENTAL PROCEDURES**

**Materials**—Fluorescamine, fluorogenic peptides (Ala-Ala-Phe-7-amido-4-methylcoumarin (AAF-AMC), Leu-Leu-Glu-β-naphthylamide (LLE-Na), N-t-Boc-Leu-Ser-Thr-Arg-7-amido-4-methylcoumarin (LSTR-AMC)), 7-amino-4-methylcoumarin, β-naphthylamine, and 5-bromo-4-chloro-3-indolyl phosphate were obtained from Sigma. 1-Anilino-naphthalene-8-sulfonic acid (ANS) was purchased from Molecular Probes (Eugene, OR). Polyclonal antibodies to the α and β subunits of the 20 S proteasome were purchased from Zymed Laboratories Inc. (San Francisco, CA). Immobilon-P polyvinylidene difluoride membrane (0.45 μm) for Western immunoblotting was from Millipore (Bedford, MA). All reagents for sodium dodecyl sulfate polyacrylamide gel electrophoresis (PAGE) were supplied by Bio-Rad. Benchmark prestained protein ladder weight markers were from Life Technologies, Inc.

**Purification of 20 S Proteasome**—The 20 S proteasome was purified from frozen livers of 4–6-month-old Fischer 344 rats essentially as described previously (26). The final purified proteasome was made to a concentration of approximately 1 mg/ml in 50 mM potassium phosphate (pH 7.0), 0.1 mM KCl and stored at –70 °C. The purity of the proteasome, assessed by SDS-PAGE and Western immunoblotting, was similar to that previously reported (26). The maximal catalytic activity of the chymotrypsin-like, trypsin-like, and peptidylglutamyl peptide hydrolase activities of the proteasome were, respectively, 0.3, 0.1, and 0.1 μmol/min/mg as assayed using the fluorogenic peptides Ala-Ala-Phe-7-amido-4-methylcoumarin (AAF-AMC), Leu-Leu-Glu-β-naphthylamide (LLE-Na), and N-t-Boc-Leu-Ser-Thr-Arg-7-amido-4-methylcoumarin (LSTR-AMC). These catalytic activities were independent of the free calcium concentration (measured in reaction buffers containing 0.1 mM EGTA or after additions of either 100 and 500 μM calcium) and were comparable with values previously reported (27). Protein concentrations were determined using the bicinchoninic acid (BCA) protein assay reagent obtained from Pierce, using bovine serum albumin as the standard.

**Expression and Purification of Methionine Sulfoxide Reductase**—A clone encoding the bovine liver isozyme of methionine sulfoxide reductase, kindly provided by Drs. Bret and Moskovitz, was expressed in *Escherichia coli* as a fusion protein with glutathione S-transferase and purified as described previously using a glutathione-Sepharose 4B affinity column (28).

**Expression and Purification of CaM**—A single isoform of CaM corresponding to the cDNA encoding vertebrate CaM provided by Professor Sam George (Duke University) was subcloned into the expression vector pALTER-Ex1, overexpressed in *E. coli* strain JM109, and purified essentially as described previously using phenyl-Sepharose CL-4B and weak anion exchange HiPLC (29, 30). Protein concentration was determined using a micro-BCA protein assay reagent kit (Pierce) using desalted CaM as the protein standard. The concentration of CaM standard was determined using the published molar extinction coefficient (ε$_{\text{327 nm}}$ = 3029 M$^{-1}$ cm$^{-1}$) for calcium-saturated CaM (29, 30).

**CaM Oxidation**—Methionines in CaM were oxidized essentially as described previously (18) by incubating 60 μM CaM (1 mg/ml) in 50 mM HOMOPIPES (pH 5.0), 0.1 mM KCl, 1 mM MgCl$_2$, and 0.1 mM CaCl$_2$ with 50 mM H$_2$O$_2$ at 25 °C for times ranging from 1 h to 24 h. Hydrogen peroxide concentration was determined by using the published extinction coefficient, ε$_{\text{234 nm}}$ = 39.4 ± 0.2 M$^{-1}$ cm$^{-1}$ (31). The reaction was stopped by dialyzing the sample overnight at 4 °C against multiple changes of distilled water (5 × 1 liter) buffered with ammonium bicarbonate (pH 7.7).

**CaM Proteolysis by the 20 S Proteasome**—Rates of proteolysis using oxidized CaM as a substrate for the proteasome were determined using two different assays that involved 1) monitoring the disappearance of the integrated intensity of CaM bands on SDS-polyacrylamide gels and 2) measurement of the initial release of peptides generated by proteasome cleavage using fluorogenic peptides (Fig. 1). To address the selectivity and structural requirements of the proteasome in the degradation of oxidized CaM (CaM$_{\text{ox}}$), we first used ESI-MS to identify the extent of oxidation and distribution of oxidized CaM molecules that could be used as potential substrates. Before oxidative modification, CaM exhibits a single major ESI-MS peak corresponding to a mass of 16,707 ± 3 Da (Fig. 1A), in close agreement with the theoretical average mass of vertebrate CaM (16, 705.4). In vitro oxidative modification of CaM (see “Experimental Procedures”) for increasing periods of time resulted in the appearance of additional peaks that differ in mass by 16 atomic mass units, which correspond to the nine possible CaM oxiforms. After correction for the charge-induced dissociation product generated in the mass spectrometer (32), the area of each peak in the ESI-MS spectra provided an estimate of the relative abundance of each CaM species. In this manner the average number of oxygens incorporated into each CaM sample after in vitro oxidation was calculated (Fig. 1B).

An alternative resolution of CaM oxiforms was obtained by electrophoretic separation on SDS-polyacrylamide gels, taking advantage of differences in the electrophoretic mobilities of CaM oxiforms. Using a single band with the greatest electrophoretic mobility (Fig. 2A). After oxidative modification, multiple protein bands were resolved whose relative mobilities decreased after increasing amounts of oxidative modification. It should be noted that the large shifts in the relative mobility of CaM oxiforms are the result of methionine oxidation, as no
For mass spectrometry analysis, 30 μg of CaM in 5 mM ammonium bicarbonate (pH 7.1) and 0.1 mM EGTA were trapped, desalted, and then directly infused (on line) into an Autospec EQ mass spectrometer as described previously (32). Masses of native and oxidized CaM were resolved to within 3 atomic mass units, where the spectrometer was shown before (lane 6) and after enzymatic repair by methionine sulfoxide reductase (lane 7), which, respectively, contain 7.7 ± 0.5 and 4.2 ± 0.3 methionine sulfoxide/CaM. B, the time course is shown for the degradation of CaMox (2.6 methionine sulfoxides/CaM) by 20 S proteasome after incubation at 37 °C in 50 mM HEPES (pH 7.5), 0.1 M KCl, 10 mM MgCl2, and 0.1 mM CaCl2 for 0, 3, and 6 h, where the respective concentrations of CaM and the 20 S proteasome were 12.5 μM and 95 nm. The average extent of CaM oxidation was determined using ESI-MS, as described in the legend to Fig. 1. Each lane represents 5 μg (A) or 3 μg (B) of protein applied to a 15% (m/v) polyacrylamide SDS gel (61). Mobilities of 15- and 20-kDa molecular mass markers are indicated on the left side of each panel. MCP, 20 S proteasome.

FIG. 1. Electrospray ionization mass spectra of native and oxidized CaM after deconvolution of multiply charged ions. Spectra corresponding to native CaM (A) or subsequent to exposure with 50 mM H2O2 for increasing amounts of time results in average methionine sulfoxide concentrations of 3.6 ± 0.2 (B), 6.1 ± 0.4 (C), or 7.7 ± 0.5 (D) mol/mol of CaM. For mass spectrometry analysis, 30 μg of CaM in 5 mM ammonium bicarbonate (pH 7.1) and 0.1 mM EGTA were trapped, desalted, and then directly infused (on line) into an Autospec EQ mass spectrometer as described previously (32). Masses of native and oxidized CaM were resolved to within 3 atomic mass units, where the spectrometer was shown before (lane 6) and after enzymatic repair by methionine sulfoxide reductase (lane 7), which, respectively, contain 7.7 ± 0.5 and 4.2 ± 0.3 methionine sulfoxide/CaM. B, the time course is shown for the degradation of CaMox (2.6 methionine sulfoxides/CaM) by 20 S proteasome after incubation at 37 °C in 50 mM HEPES (pH 7.5), 0.1 M KCl, 10 mM MgCl2, and 0.1 mM CaCl2 for 0, 3, and 6 h, where the respective concentrations of CaM and the 20 S proteasome were 12.5 μM and 95 nm. The average extent of CaM oxidation was determined using ESI-MS, as described in the legend to Fig. 1. Each lane represents 5 μg (A) or 3 μg (B) of protein applied to a 15% (m/v) polyacrylamide SDS gel (61). Mobilities of 15- and 20-kDa molecular mass markers are indicated on the left side of each panel. MCP, 20 S proteasome.

To investigate the recognition signals and mechanism of degradation of extensively oxidized CaM (8.2 ± 0.6 methionine sulfoxides/CaM) by the 20 S proteasome, we used mass spectrometry to measure the masses of the peptides released from the proteasome. After exposure of CaMox to the proteasome, CaMox and released peptides were separated from the proteasome using a 100-kDa molecular weight cutoff filter. Their respective masses were identified by ESI-MS. After 1 h of degradation, approximately 10% of CaMox was degraded by the proteasome, and the released peptides were in large molar excess relative to the proteasome.

Thus, the majority of these peptides represent products released from the proteasome before separation. At this time point, masses for 26 peptides were identified whose size distribution varied between 1188 and 4977 Da, which corresponds to peptide lengths between 10 and 39 amino acids. Of the resolved masses, 15 peptides were uniquely identified that varied in mass between 1187.8 and 3589.9 Da (Table I; Fig. 3A). From these data, it is apparent that cleavage occurs preferentially on the carboxyl-terminal side of Glu, Leu, Asp, Phe, Lys, and Ala (Fig. 4). The preferential cleavage of these sites in CaMox agrees with earlier results using other protein substrates, suggesting that specific amino acid side chains are preferentially recognized for cleavage (33, 36). In contrast, after 24 h of CaM exposure to proteasome, 25 peptides were identified in the mass spectra. The mass distribution for these peptides varied between 672 and 1430 Da, which corresponds to peptide lengths between 10 and 39 amino acids. Of these peptides, eight were uniquely identified (Table I). Although these peptides also involved the preferential cleavage of the peptide bond on the carboxyl-terminal side of side chains previously implicated to enhance the proteolytic susceptibility of protein substrates, it is

other protein modifications are present under these experimen-
apparent that the majority of these peptides involve new cleavage sites. In conjunction with the large decrease in the average size of the released peptides after incubation of CaMox with the proteasome for long times, these results suggest that CaMox is initially cleaved into large pieces that are subsequently further degraded by the proteasome.

Rate of CaMox Degradation Correlates with Decreases in Secondary Structure—Methionine oxidation has previously been shown to result in a large reduction in the α-helical content of CaM, indicating the methionines function to stabilize the native conformation of CaM (22). To determine whether these structural changes may predispose CaMox to degradation by the proteasome, we investigated whether changes in the secondary structure of CaM resulting from methionine oxidation correlate with the sensitivity of degradation by the proteasome. CD spectroscopy was used to estimate changes in the α-helical content of CaM resulting from methionine oxidation. From the CD spectra, it is apparent that a progressive increase in the molar ellipticity ([θ]) is observed, indicating a loss of secondary structure that correlates with the extent of oxidation (Fig. 5).

The α-helical content was calculated using a non-linear least squares-fitting algorithm (34). After the oxidation of all nine methionines, approximately one-half of the native α-helical content of CaM is lost. These results confirm that methionine oxidation disrupts the secondary structure of CaM and that the decrease in α-helical content depends on the extent of methionine oxidation. Furthermore, observed changes in the molar ellipticity correlate with the rates of degradation by the proteasome, irrespective of whether the initial release of peptides is measured using the fluorescamine assay or if slower rate of disappearance of protein bands is detected using SDS-PAGE (Fig. 6).

Decreased Degradation of CaMox after Partial Repair by Methionine Sulfoxide Reductase—Methionine sulfoxide reductase has previously been shown to reduce (i.e. repair) methionine sulfoxides in CaMox to induce partial refolding and to restore the function of oxidized CaM (22). After repair, an average of 4.2 ± 0.3 methionine sulfoxides remain in each CaM, and the distribution of oxidized methionines is very different from that initially obtained after in vitro oxidation by H2O2 (22). There are corresponding differences in the relative mobility of CaMox after repair compared with that observed after in vitro oxidation (Fig. 2A). Therefore, since this physiological repair system has the potential to modulate the rate of degradation of CaMox by the proteasome and because of differences in the pattern of methionine oxidation, it is of interest to investigate the sensitivity of repaired CaM to degradation by the proteasome. After the repair of fully oxidized CaM by methionine sulfoxide reductase there is a 30% reduction in the rate of degradation by the proteasome accompanied by a corresponding decrease in the molar ellipticity, which indicates an increase in α-helical content (Fig. 6).

Calcium-dependent Changes in Secondary Structure of CaM Correlate with Degradation Rate—To distinguish whether the increased degradation rate of CaMox by the proteasome reflects a preferential recognition of methionine sulfoxide or, rather, to the resulting loss of CaM secondary structure, we took advantage of the fact that the α-helical content of extensively oxidized CaM increases dramatically upon calcium binding, so that calcium-saturated CaMox assumes a native-like structure (22, 32). Accordingly, we have compared the calcium dependence of the degradation rate of extensively oxidized CaM containing 8.2 ± 0.5 methionine sulfoxides with changes in the secondary structure, as measured by the molar ellipticity at 222 nm. Upon increasing the calcium concentration, the rate of degradation by the proteasome decreases by approximately 75% with corresponding decreases in the molar ellipticity (Fig. 7). Since the range of calcium concentrations used in this experiment did not alter the catalytic efficiency of the proteasome, as assayed independently with fluorescent peptides (data not shown), these calcium-dependent differences in the rate of degradation of CaMox reflect calcium-induced conformational changes. Furthermore, since calcium binding results in an increased solvent exposure of methionine side chains in CaM, the rate of CaM degradation depends on the loss of native structure rather than solvent exposure of methionine sulfoxide (Fig. 6).

Hydrophobicity and CaMox Degradation—The oxidant-in-
duced exposure of hydrophobic regions within proteins has been suggested to uncover a universal recognition motif that targets proteins for degradation by the 20 S proteasome (13–15). However, since calcium activation results in the exposure of methionine-rich (hydrophobic) binding pockets in CaM, the previous results showing that calcium binding diminishes the rate of proteasome-mediated degradation argues against this suggestion. Therefore, it is of interest to directly determine the relationship between hydrophobicity and the degradation of CaMox by the proteasome. These measurements have taken advantage of the large increase in the fluorescence intensity and changes in the emission maximum of ANS associated with binding to surface-exposed hydrophobic sequences (35). In comparison to apo-CaM, ANS binding to calcium-activated CaM results in a large fluorescence increase and a blue-shift in the emission maximum of ANS (data not shown), consistent with the calcium-dependent exposure of hydrophobic pockets within each of the opposing globular domains of CaM (37–39).
initial differences in ANS fluorescence associated with calcium binding to native CaM are diminished as a result of methionine oxidation, so that upon oxidation of the majority of the nine methionines in CaM essentially no differences in the ANS fluorescence to either apo- or calcium-activated CaM are retained. Under these conditions there is a 4-fold difference in the rate of degradation of CaMox by the proteasome (Fig. 7). Thus, these results suggest that the exposure of hydrophobic residues have little or no effect with respect to the recognition and degradation of CaMox by the proteasome.

DISCUSSION

Protein Degradation by the Proteasome—Post-translational modifications decrease the half-life of a range of different proteins by enhancing their rates of degradation, suggesting that they mark proteins for degradation (12). It is generally believed that the degradative mechanisms that enhance protein turnover are important to the maintenance of cellular function. However, although a range of different protein modifications have been identified that correlate with enhanced rates of degradation by cellular proteases, relatively little is known regarding how site-specific protein modifications enhance their rates of degradation by the proteasome. In this respect, the oxidative modification of a number of proteins has previously been shown to correlate with the exposure of hydrophobic surfaces, resulting in enhanced rates of proteolysis by the 20 S proteasome (7, 9, 10, 13, 15, 40–42). Results from these studies have been interpreted to suggest that a universal recognition signal for target protein degradation by the 20 S proteasome involves partial protein unfolding and the exposure of hydrophobic surfaces. However, a simple interpretation of this previous data has been complicated by the oligomeric structure of the proteins studied, and their tendency to undergo self-association after oxidative modification. In addition, the significance of the in vitro oxidative modifications to cellular physiology is unclear, as neither the products of their in vitro oxidation nor evidence that these proteins are oxidized in cells under conditions of physiological significance have been presented. It is, therefore, important to identify the degradative mechanisms of oxidatively modified proteins that (i) have been shown to be present in cells, (ii) have the potential to modulate cellular metabolism, and (iii) are normally degraded by the proteasome. With respect to the first two criteria, previous measurements have demonstrated that multiple methionines are oxidatively modified to their corresponding sulfoxides in the calcium-signaling protein CaM, isolated from senescent brain, resulting in the inability to fully activate a range of different target proteins (17, 19, 20, 43). Furthermore, post-translational modifications in CaM involving the deamidation of asparagines induced by in vitro storage result in its selective degradation by the proteasome relative to other proteins (16, 44, 45).

Ubiquitin-calmodulin derivatized at Lys12 has been identified in cells; a corresponding ubiquitin-calmodulin ligase is likely to mediate this derivatization (46, 47). However, ubiquitinylation does not result in the degradation of ubiquitinated CaM by the 26 S proteasome (16, 46). Rather, ubiquitinylation of CaM appears to regulate the binding and activation of CaM to target proteins (e.g. glycogen phosphorylase kinase) and is not coupled to the mechanism of protein degradation by the proteasome. These results are consistent with the suggestion that ubiquitin conjugation can have consequences other than direct targeting to the proteasome (48) and suggest the physiological relevance of measuring the mechanisms underlying the degradation of CaM by the 20 S proteasome. CaM is a relatively long-lived signaling protein (t½ ~ 18 h) in comparison to the short half-lives of many transcription factors and other cellular regulatory molecules (24, 25). Thus, cellular regulatory mechanisms that stringently maintain constant levels of functional CaM, such as the degradation of excess or non-functional CaM, are important in the maintenance of cellular homeostasis (49).

Mechanism of Degradation—The observation of the nonprocessive degradation of CaMox by the proteasome is consistent with earlier observations in which the degradation of many other protein substrates has been observed to involve the initial cleavage into large proteolytic fragments at early times of digestion that can dissociate and then rebind and be further digested by the proteasome (50–54). The dissociation of large proteolytic fragments from the proteasome suggests the opportunity for other cellular protease systems to also be involved in the degradation of cellular proteins (12, 54). However, in a number of cases the proteasome has been shown to degrade proteins in a processive manner, involving the release of relatively homogeneous population of small peptides (55–57). Although the underlying reasons for observed differences in the mechanisms of protein degradation are unclear, it is significant that the majority of these earlier studies have involved chemically modified and unfolded proteins that are not likely to be present in the cellular milieu. In contrast, it is likely that oxidized CaM containing multiple methionine sulfoxides is a physiological substrate of the proteasome (17).

The lack of a functional correlation between changes in the substrate hydrophobicity and CaMox degradation by the proteasome suggests that hydrophobic sequences do not function in the recognition of CaMox by the 20 S proteasome. Rather, the best correlation is observed between protein degradation rates and decreased α-helical content induced by either CaM oxidation or calcium binding. The nonprocessive digestion of CaMox into a limited number of large fragments that are released and further digested suggests that partial protein unfolding is sufficient to initiate recognition and cleavage by the 20 S proteasome, without the need for global protein unfolding to allow access of substrate into the internal cavity of the proteasome.

Conclusions and Future Directions—The oxidative modification of methionines in CaM results in a loss of secondary structure and the preferential degradation by the 20 S proteasome into multiple large fragments that are subsequently degraded into small peptides with an average length of about eight amino acids. The preferential degradation of oxidized CaM is consistent with the observation that methionine oxidation destabilizes the α-helical structural elements (58–60) and suggests that conformationally disordered structures may serve as recognition elements for the proteasome. Future experiments aimed at identifying the mechanism of degradation will require direct structural measurements of protein intermediates bound to the proteasome, which will permit the identification of specific structural motifs that facilitate binding and degradation.

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