Free Rad. Res., Vol. 26, pp. 103–111
Reprints available directly from the publisher
Photocopying permitted by license only

Identification of Hydrogen Peroxide Oxidation Sites of αA- and αB-Crystallins

JEAN B. SMITH*[,a], XIANGYU JIANG[a] and E.C. ABRAHAM[b]

[a]*Department of Chemistry, University of Nebraska, Lincoln, NE 68588-0304 and* [b]*Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA 30912-2100, USA*

Accepted by Prof B. Halliwell

(Received 24 June 1996; In revised form 17 July 1996)

The α-crystallins are the most abundant structural proteins of the lens and, because of their chaperone activity, contribute to the solubility of the other crystallins. With aging, the lens crystallins undergo a variety of modifications which correlate with a loss of solubility and the development of cataract. A recent study demonstrating that α-crystallins exposed *in vitro* to $FeCl_3$ and H_2O_2 exhibit decreased chaperone activity, implicates metal catalyzed oxidations of α-crystallins in this loss of solubility. The present study has determined that α-crystallins incubated with $FeCl_3$ and H_2O_2 are modified by the nearly complete oxidation of all methionine residues to methionine sulfoxide, with no other detectable reaction products. The modifications were identified from the molecular weights of peptides formed by enzymatic digestion of the α-crystallins and located by tandem mass spectrometric analysis of the fragmentation pattern of the modified peptides. A dominant pattern in the mass spectra of the fragments from peptides with oxidized methionine is loss of 64 Da, which corresponds to loss of CH_3SOH from the methionine sulfoxide. These fragments are useful in identifying peptides that include oxidized methionine residues.

Keywords: Methionine sulfoxide, cataract, lens crystallins, mass spectrometry

INTRODUCTION

Oxidative modifications of the lens crystallins have been implicated in numerous studies of aging and cataract. Because the components required for metal-catalyzed oxidation are present in the lens, the hypothesis that metal-catalyzed oxidation is a major contributor to age related changes and cataract has received wide support.[1] The aqueous humor and the lens contain relatively high levels (20–50 μM) of H_2O_2.[2,3] Both H_2O_2 itself, and the hydroxyl radicals produced by the reaction of H_2O_2 with trace concentrations of metals, known as the Fenton reaction, are damaging to lens proteins.[4,5] In the vertebrate lens, α-crystallin is the first protein to appear during early development and constitutes about 35% of the total soluble protein. α-Crystallin, which is composed of two major closely related subunits, αA and αB,[6] is a major structural element in the highly ordered and concentrated protein matrix essential to the

*Corresponding author. Fax: 402 472-9862. E-mail: jbsmith@unlinfo.unl.edu.

transparency and refractive properties of the lens. Recent studies have shown that, in addition to its structural function, α-crystallin has chaperone-like activity,[7] contributing to the solubility of the β- and γ-crystallins. During aging, as lens α-crystallins undergo extensive post-translational modification, they demonstrate decreased chaperone activity,[8,9] Recently, the chaperone activities of α-crystallins modified by several *in vitro* reactions that may occur as the lens ages were compared.[9] Of the reactions examined, treatment of the α-crystallins with H_2O_2 and $FeCl_3$ caused the greatest reduction in chaperone activity. The amino acid residues of proteins most likely to be modified by metal catalyzed oxidation systems have been reported to be His, Arg, Lys, Pro, Cys and Met.[10,11] In the present study, oxidation of methionine was the only modification detected following incubation of α-crystallin in 1mM H_2O_2 and 0.1 mM $FeCl_3$, the same oxidation system that was effective in reducing chaperone activity. Each of the two methionines in each subunit of α-crystallin was converted, almost completely, to methionine sulfoxide.

MATERIALS AND METHODS

Preparation of Calf α-Crystallins Methods described previously[9] were used with minor modification for the preparation of α-crystallin. Calf lenses were homogenized in 50 mM Tris/HCl, pH 8.0 containing 50 mM sodium bisulfite, 20 mM EDTA and 0.02% NaN_3. After centrifugation at 10,000 g for 1 hr at 4°C, supernatant water-soluble fractions were collected. The α-crystallins (30 mg) were isolated by size exclusion chromatography (100 × 1.5 cm Sephacryl S-300-HR column) using the homogenizing buffer as the mobile phase. The purity of the α_L fraction was confirmed by SDS-PAGE[12] after concentration by ultrafiltration.

Oxidation of α-Crystallin with $H2O_2$ A solution of 5 mg/ml of the protein from the α_L fraction was prepared in 50 mM phosphate, 0.1% NaN_3, pH 7.0, sterile filtered through 0.2 μm filters and incubated for 24 hr with 1 mM H_2O_2 containing 0.1 mM $FeCl_3$.[5,9] There was no visible precipitation of $Fe(OH)_3$ in these solutions. After incubation, the proteins were examined by SDS-PAGE for evidence of cross-linking.

Reversed-Phase Separation of α-Crystallins Oxidized α-crystallins were fractionated by reversed-phase HPLC (0.46 × 15 cm, 300 Å, 5 μ particle size Vydac C4 column, Separations Group, Hesperia, CA) using a gradient HPLC system (Rainin Instrument Co., Woburn, MA). Solvent A was water, solvent B was acetonitrile, both with 0.1% trifluoroacetic acid. The gradient was 10–35% solvent B in 5 min., followed by 35–60% solvent B in 25 min. The UV absorbance was monitored at 280 nm. Fractions were dried and stored at –20 °C until analyzed. A control sample of bovine α-crystallins was similarly fractionated.

Protein Molecular Weight Determinations The molecular weights of the oxidized and control samples of α-crystallins were determined by electrospray ionization mass spectrometry (ESIMS) using a Micromass Platform II quadrupole mass spectrometer with Mass Lynx software (Micromass, Manchester, UK). The instrument was calibrated over the range of 700–1800 mass/charge (m/z) with the multiply charged peaks of horse heart myoglobin. The sample was introduced at a flow of 5 μl/min of 50:50 acetonitrile: water. Molecular weights of the proteins were calculated from the multiply charged peaks in the spectra with an accuracy of ±2 Da.

Proteolytic Digestions The oxidized αA- and αB-crystallins were enzymatically digested into peptides. Proteins (10 nmoles) dissolved in 0.1M ammonium carbonate buffer, pH 7.6, were digested either with trypsin, (Sigma Chemical Co., St. Louis, MO) with a 1:50 ratio of enzyme:substrate, or with endoproteinase Asp-N (Boehringer Mannheim, Indianapolis, IN) with a 1:100 ratio of enzyme:substrate. The protein was

digested at 37 °C for 4 hr for trypsin or 7 hr for Asp-N. The chromatogram for the HPLC fractionation indicated that no protein remained undigested.

Peptide Molecular Weight Determinations Peptides formed by proteolytic digestion of the proteins were fractionated by reversed phase HPLC online to the Micromass Platform II mass spectrometer as described previously.[13] The flow rate through the column (1 × 50 mm C8) was 50 µl/min. A post-column splitter was used to direct 5 µl/min to the mass spectrometer and 45 µl/min to a UV detector and subsequent collection for further analysis. The mass spectrometer was calibrated over the range 300–2000 Da with NaI. Molecular weights of the peptides present in each of the fractions separated by HPLC were determined with an accuracy of ±0.3 Da. The fractions collected after UV monitoring were dried and stored at –20°C until further analysis by tandem mass spectrometry.

Tandem Mass Spectrometric (MS/MS) Analysis of the Fragmentation Patterns of Oxidized Peptides Tandem mass spectrometry, also called MS/MS, consists of one mass spectrometer which isolates the peptide of interest, a chamber where the peptide is fragmented by collision with a gas such as xenon, and a second mass spectrometer which is used to determine the masses of the resulting fragments.[14] Following collisional excitation, peptide ions fragment along the backbone of the peptide and at some side chains. From the fragmentation pattern produced by collisional activation of the peptide, the peptide identity can be confirmed and the exact location of a modification can be determined. The tandem mass spectrometer used in this investigation (Micromass Autospec oa-TOF) consisted of a conventional magnetic sector instrument as the first mass spectrometer and an orthogonal acceleration time-of-flight analyzer as the second mass spectrometer.[15] These analyses were performed in the fast atom bombardment mode of ionization and used Xe as the collision gas.

RESULTS

SDS-PAGE Analysis SDS-PAGE examination of the α-crystallins after H_2O_2 oxidation (Fig. 1) showed evidence of much less of the high molecular weight cross-linked proteins than had been observed in investigations using longer or more vigorous oxidations by UV light[16] or solutions containing EDTA.[5,17,18] The lower amount of cross-linking indicates that the modifications studied here are initial oxidation products.

Protein Molecular Weight Determinations The ESIMS determined molecular weights of the principal components of the oxidized α-crystallins were 19,864 Da for αA, and 20,110 Da for αB, each 32 Da higher than the calculated molecular weights of the respective unmodified proteins.[19,20] The reconstructed mass spectrum for oxidized αA-crystallin (Fig. 2) shows the major component with an additional 32 Da, a smaller component (19,847) with an additional 17 Da (which is within experimental error for addition of one oxygen atom), and very minor components representing phosphorylated αA (19,912

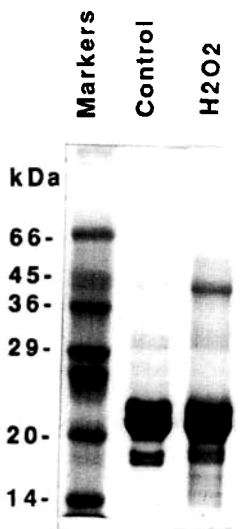

FIGURE 1 SDS-PAGE analysis of the α-crystallins under reducing conditions before and after oxidation by H_2O_2 in the presence of $FeCl_3$ for 24 hr.

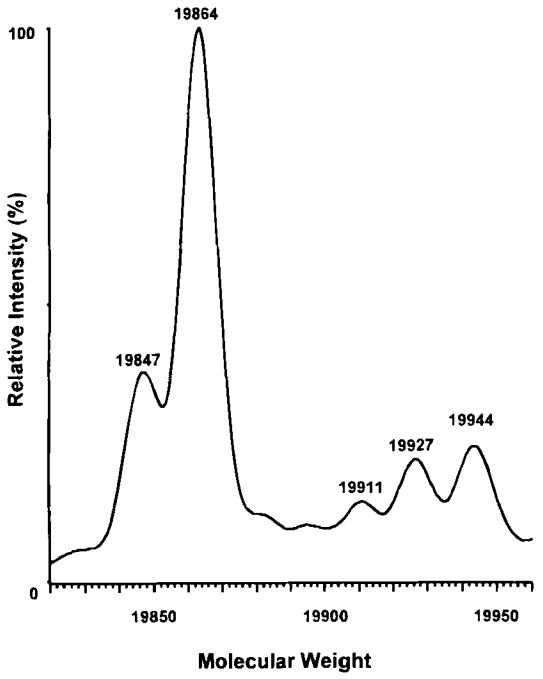

FIGURE 2 *Electrospray* ionization mass spectrum for αA-crystallin treated with $FeCl_3$ and H_2O_2. The molecular weight of the principal component, 19,864, is 32 Da higher than the molecular weight calculated from the sequence of αA, suggesting the addition of two oxygen atoms. Other components have molecular weights corresponding to the expected molecular weight plus one oxygen atom (19,848), a phosphorylated form (19,912) and the phosphorylated form plus one and two oxygen atoms (19,928 and 19,944, respectively).

Da), also with an additional +16 (19,927 Da) and +32 (19,944 Da). The peak heights suggest that the oxidation is more extensive for non-phosphorylated αA than for phosphorylated αA-crystallin. A similar, but not quite as dramatic, decrease in the ratio of oxidized:unoxidized forms was evident also for phosphorylated αB-crystallin (data not shown).

Molecular Weight Determination of Peptides in Proteolytic Digests After digestion with trypsin, which cleaves C-terminally to Lys and Arg, the oxidized and unoxidized αA- and αB-crystallins were each fractionated by reversed phase HPLC, and the molecular weights of the peptides in each of the fractions were determined by mass spectrometry. Peptides with molecular weights corresponding to all expected peptides in tryptic digests of unmodified αA- and αB-crystallins were found, and agreed with the calculated molecular weights to within 0.2%. The molecular weights of the peptides in the tryptic digests of the oxidized αA- and αB-crystallin were the same as those in the unoxidized crystallins except for two peptides in each digest. The digest of oxidized αB was missing peptides 1–11 (MH^+ 1430.7) and 57–69 (MH^+ 1462.7) (Table I); the digest of oxidized αA was missing peptides 1–11 (MH^+ 1426.7) and 120–145 (MH^+ 2626.0) (Table II). Comparision of the HPLC chromatograms of the unoxidized (Fig. 3A) and oxidized αB-crystallin (Fig. 3B) showed that the elution times of two fractions had changed. The peaks at 35 and 36 minutes (T4 and T1, respectively) which contained peptides matching residues 1–11 and residues 57–69 of the unoxidized αB-crystallin had shifted to 31 and 32 minutes in the chromatogram of the oxidized protein and contained masses matching the same residues with an additional 16 Da (MH^+ 1446.8 and MH^+ 1478.8). Finding an additional 16 Da in the masses of two peptides is consistent with the observation of an additional 32 Da in the molecular weight of the intact protein. Although peptide mapping showed oxidation within peptides 1–11 and 57–69, the exact site of modification was not indicated. Both of these peptides contain several possible sites of oxidation. For example, peptide 1–11 contains Met-1, His-6, His-7 and Trp-9; peptide 57–69 contains Trp-60 and Met-68.

A similar analysis of the tryptic digest of αA-crystallin (Table II) indicated that an additional 16 Da were present on peptide 1–11, which contains Met-1, His-7 and Trp-9. However, a second peptide with an extra 16 Da was not found in the tryptic digest. Since the αA peptide corresponding to residues 120–145 was not seen, it seemed likely that the missing peptide included the oxidation. Digestion of oxidized αA-crystallin with Asp-N, an enzyme that cleaves N-terminally to aspartic acid residues, yielded a peptide with MH^+ 1521.7, corresponding to residues 136–150 plus 16 Da.

TABLE I Peptides found in a tryptic digests of oxidized αB-crystallins

peptide	residues	sequence	calculated molecular weight	observed molecular weight
T1	1–11	MDIAIHHPWIR	1429.7	1445.8
T2	12–22	RPFFPFHSPSR	1373.7	1373.8
T3	23–56	LFDQFFGEHLLESDLFPAS TSLSPFYLRPPSFLR	3975.6	3975.2[a]
T4	57–69	APSWIDTGLSEMR	1461.7	1477.8
T5	70–72	LEK	388.2	388.3
T6	73–74	DR	289.1	—
T7	75–82	FSVNLDVK	920.5	920.4
T8	83–90	HFSPEELK	985.5	985.6
T9	91–92	VK	245.2	—
T10	93–103	VLGDVIEVHGK	1164.7	1164.8
T11	104–107	HEER	569.3	569.3
T12	108–116	QDEHGFISR	1087.5	1087.5
T13	117–120	EFHR	587.3	587.3
T14	121	K	146.1	—
T15	122–123	YR	337.2	337.5
T16	124–149	IPADVDPLAITSSLSSDGV LTVNGPR	2593.4	2594.6
T17&T18	150–157	KQASGPER	871.5	871.4
T19	158–163	TIPITR	699.4	699.4
T20	164–174	EEKPAVTAAPK	1139.6	1139.7
T21	175	K	146.1	—

[a]Also observed in the phosphorylated (+80 Da) form.

TABLE II Peptides found in a tryptic digests of oxidized αA-crystallins

peptide	residues	sequence	calculated molecular weight	observed molecular weight
T1	1–11	MDIAIQHPWFK	1426.7	1442.8
T2	12	R	174.1	
T3	13–21	TLGPFYPSR	1036.5	1036.6
T4	22–49	LFDQFFGEGLFEYDLLPFL SSTISPYYR	3365.8	3365.0
T5	50–54	QSLFR	649.4	649.3
T6	55–65	TVLDSGISEVR	1174.6	1174.6
T7&T8	66–70	SDRDK	619.2	619.4
T9	71–78	FVIFLDVK	979.6	979.7
T10	79–88	HFSPEDLTVK	1171.6	1171.8
T11	89–99	VQEDFVEIHGK	1299.6	1299.8
T12	100–103	HNER	554.3	554.4
T13	104–112	QDDHGYISR	1089.5	1089.6
T14	113–116	EFHR	587.3	587.3
T15	117	R	174.1	
T16	118–119	YR	337.2	337.2
T17	120–145	LPSNVDQSALSCSLSADG MLTFSGPK	2626.0	—
T18	146–157	IPSGVDAGHSER	1223.6	1223.7
T19	158–163	AIPVSR	641.4	641.4
T20	164–173	EEKPSSAPSS	1017.5	1017.6

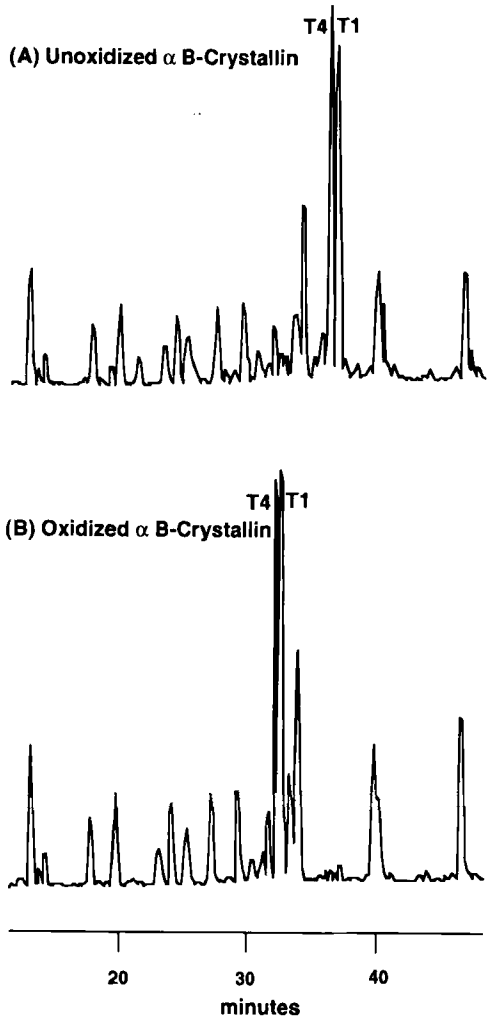

FIGURE 3 Chromatogram showing the reversed phase fractionation of tryptic digest of αB-crystallin A) unoxidized and B) oxidized by treatment with $FeCl_3$ and H_2O_2. Note that the chromatograms are the same except that two peaks in the chromatogram of the oxidized protein elute earlier than the corresponding peaks of the unoxidized protein.

Possible sites of oxidation in this peptide, DGMLTFSGPKIPSG, are Met-138 and Phe-141.

MS/MS Analysis of Oxidized Peptides The masses of the peptides established the specific segments in which oxidation had occurred, but, because there were several possible oxidation sites in each of the peptides, further analysis was required to determine which residue in each segment was oxidized. MS/MS analysis of the fragments produced by collisional activation of a peptide can give unambiguous identification of the site of post-translational modification.[19,21,22] In the first stage of MS/MS analysis, the peptide of interest is selected; in the second stage, collision with xenon causes cleavage, primarily along the backbone of the peptide. When the charge on the peptide stays with the N-terminus, fragments from the a, b, c series are seen; when the charge stays with the C-terminus, fragments from the x, y, z series are seen (insert of Fig. 4). Determination of the masses of these fragments indicates exactly which residue is modified. This technique has been used previously to determine sites of phosphorylation,[19] carbamylation,[23] glycation[24] and oxidation[13,25] in human and bovine lens crystallins. For the four peptides that had an additional 16 Da, the MS/MS analysis of the fragments formed by collisional activation showed that the methionines, and only the methionines, were modified. The MS/MS spectrum for αB (1–11) is shown in Fig. 4. The masses of fragments expected from cleavage along the backbone that were found are indicated at the top of the figure.[14,26] For the peptides shown in Fig 4, the dominant fragments are from the b and y″ series. The masses of all the b-series fragments, 190, 305, 418, 489, 739, 848, 876 and 1132, are 16 Da higher than would be expected for fragments from this sequence if it were unmodified. The only oxidation site consistent with modification of all of the b-series ions is the N-terminal amino acid; i.e., the additional 16 Da is at the methionine. If, for exammple the oxidation had been at Trp-9, only those fragments including R_9 would exhibit the additional 16 Da. The masses of the y-series fragments gave further support for the conclusion that Met-1 is oxidized. The y-series fragments, which do not include the methionine, have masses corresponding to unmodified fragments from this sequence. If the oxidation had been at Trp-9, y-series fragments including R1 through R9 would have been 16 Da larger. Oxidation at any amino

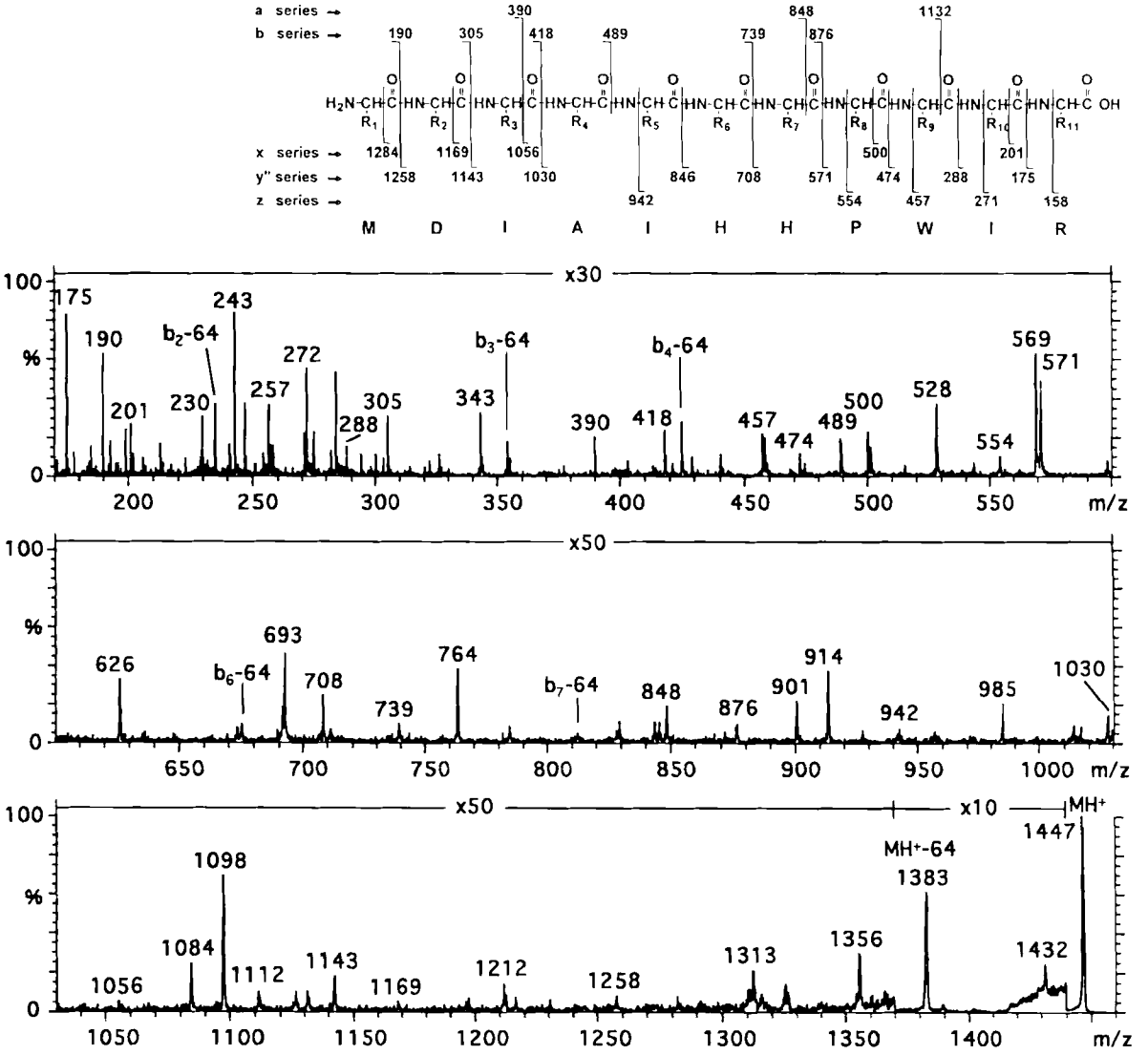

FIGURE 4 The MS/MS spectrum showing the fragmentation pattern of αB-crystallin peptide 1–11. The fragments are consistent with the presence of an additional 16 Da on Met-1.

acid other than the N-terminal methionine would yield fragments with the masses different from those in Fig. 4.

In addition to the expected fragments, fragments containing an oxidized Met also yielded a peak 64 Da lower which can be attributed to loss of neutral methanesulfenic acid, CH_3SOH, from the side chain of methionine. Production of CH_3SOH by collisional activation of methionine sulfoxide parallels the well characterized thermal degradation of organic sulfoxides to produce methanesulfenic acid.[27,28] Because the methionine is the N-terminus of αB peptide 1–11, loss of 64 Da is most evident in the b-series. A series of fragments showing loss of 64 Da is diagnostic for the presence of methionine sulfoxide in peptides.[29] Similar analysis of the MS/MS spectra of each of the modified pep-

tides showed conclusively that all four Met residues, and no other residues of the α-crystallins were oxidized. These modifications account for the additional 32 Da in the molecular weight of each of the proteins and agree with the absence of any other detectable modifications.

DISCUSSION

The possibility that oxidative damage contributes to the modifications associated with lens aging and cataract has much supporting evidence. *In vitro* studies have shown that post-translational modifications leading to cataract might be caused by ultraviolet radiation,[30] H_2O_2 oxidation[3] and/or glycation.[31,32] The hypothesis that oxidation is due to metal catalyzed oxidation, involving the reaction of a metal with H_2O_2 to form hydroxyl radicals is particularly appealing because H_2O_2 is found in high concentrations in normal human lens and aqueous fluid and is significantly elevated in some cataract patients.[1,3] Moreover, H_2O_2 was shown to produce cataract in cultured rat lenses,[33] and the damage produced by hydroxyl radicals closely mimicked modifications that occur with maturity onset cataracts.[5] Although in studies of other proteins and oxidation systems, several residues have been reported to be susceptible to oxidation by H_2O_2, the present study clearly shows that only the methionines of αA- and αB-crystallins have been oxidized after incubation with 1mM H_2O_2 and 0.1 mM $FeCl_3$. Thus, the cause of the decrease in α-crystallin chaperone-like function due to oxidation by H_2O_2 and $FeCl_3$[9] must be the modification of these methionine residues to methionine sulfoxides. Whether the reduction in chaperone activity requires all or part of the methionines to be modified is not known. Both the N-terminal and C-terminal regions of α-crystallin have been implicated in α-crystallin recognition of the chaperone target protein binding site.[34] The N-terminal residues of both αA- and αB-crystallin are methionines; the other methionine of αA is at residue 138, the other of αB is at residue 59. Oxidation of methionines may also be responsible for the recent observation that α-crystallin oxidized with H_2O_2 loses its ability to efficiently bind partially denatured β-crystallin (Cherian and Abraham, unpublished). The formation of only slight amounts of the high molecular weight cross-linked protein (demonstrated by the SDS-PAGE analysis in Fig. 1) is consistent with the fact that oxidation of methionine does not lead to cross-links. The small amount of cross-linking, less than 5% modification, may be due to oxidation at other amino acids. Zigler *et al.*[5] also observed little cross-linking with similar incubation conditions, using $FeSO_4$ and H_2O_2; however, when EDTA was added, extensive cross-linking occurred due to a more efficient production of OH radicals.[35]

The present finding that H_2O_2 oxidizes all four methionines in α-crystallin raises the question whether a similar oxidation of methionines occurs *in vivo*. Takemoto *et al*[25] have identified a peptide containing an oxidized N-terminal methionine in a tryptic digest of bovine αA-crystallin. In α-crystallins isolated from the water-insoluble protein of a 45 year old human lens, 5–10% of all the methionines of αA- and αB-crystallin were found as methionine sulfoxides[13] with no evidence of oxidation of other residues. Because oxidation is observed exclusively at methionine in both the normal lens and in the *in vitro* reactions of the lens with H_2O_2, it is likely that the mechanisms for the two reactions are similar. Although an increase in oxidized methionines with increasing age of the lens has not yet been shown, oxidation of methionine may contribute to the decline in chaperone activity associated with aged α-crystallin.[8,9]

Acknowledgments

This investigation was supported by NIH grants EY 07394 (ECA), EY 11352 (ECA) and EY 07609 (JBS).

References

[1] D. Garland (1990). Role of site-specific, metal-catalyzed oxidation in lens aging and cataract: a hypothesis. *Experimental Eye Research*, **50**, 677–682.

[2] F. J. Giblin, J. P. McCready, T. Kodama and V. N. Reddy (1984). A direct correlation between the levels of ascorbic acid and H_2O_2 in aqueous humor. *Experimental Eye Research*, **38**, 87–93.

[3] A. Spector and W. H. Garner (1981). Hydrogen peroxide and human cataract. *Experimental Eye Research*, **33**, 673–681.

[4] J. S. Zigler, H. M. Jernigan, D. Garland and V. N. Reddy (1985). The effect of oxygen radicals generated in the medium on lens organ culture. Inhibition of damage by chelated iron. *Archives of Biochemistry and Biophysics*, **241**, 163–172.

[5] J. S. Zigler, Jr., Q.-L. Huang and X.-Y. Du (1989). Oxidative modification of lens crystallins by H_2O_2 and chelated iron. *Free Radical Biology and Medicare*, **7**, 499–505.

[6] H. Bloemendal (1981). *Molecular and Cellular Biology of the Eye Lens*. John Wiley and Sons, New York.

[7] J. Horwitz (1992). α-crystallin can function as a molecular chaperone. *Proceedings of the National Academy of Sciences, USA*, **89**, 10449–10453.

[8] J. Horwitz, T. Emmons and L. Takemoto (1992). The ability of lens α-crystallin to protect against heat-induced aggregation is age-dependent. *Current Eye Research*, **8**, 817–822.

[9] M. Cherian and E. C. Abraham (1995). Decreased molecular chaperone property of α-crystallins due to posttranslational modifications. *Biochemical and Biophysical Research Communications*, **208**, 675–679.

[10] A. Amici, R. L. Levine, L. Tsai and E. R. Stadtman (1989). Conversion of amino acid residues in proteins and amino acid homopolymers to carbonyl derivatives by metal-catalyzed oxidation reactions. *Journal of Biological Chemistry*, **264**, 3341–3346.

[11] E. R. Stadtman (1993). Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. *Annual Review of Biochemistry*, **62**, 797–821.

[12] U. K. Laemmli (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)*, **277**, 680–685.

[13] A. L. Lund, J. B. Smith and D. L. Smith (1996). Modifications of the water-insoluble human lens α-crystallins. *Experimental Eye Research* (in press).

[14] K. Biemann (1990). Sequencing of peptides by tandem mass spectrometry and high-energy collision-induced dissociation. In Methods in Enzymology (ed. McCloskey, J. A.), Academic Press, San Diego, CA, pp. 455–479.

[15] K. F. Medzihradszky, G. W. Adams, A. L. Burlingame, R. H. Bateman and M. R. Green (1996). Peptide sequence determination by matrix-assisted laser desorption ionization employing a tandem double focusing magnetic-orthogonal acceleration time-of-flight mass spectrometer. *Journal of the American Society of Mass Spectrometry*, **7**, 1–10.

[16] R. F. Borkman (1995). The molecular chaperone function of α-crystallin is impaired by UV photolysis. *Photochemistry and Photobiology*, **62**, 1046–1051.

[17] M. J. McDermott, R. Chiesa and A. Spector (1988). Fe^{2+} oxidation of α-crystallin produces a 43,000 Da aggregate composed of A and B chains cross-linked by non-reducible covalent bonds. *Biochemical Biophysical Research Communications*, **157**, 626–631.

[18] M. J. McDermott, R. Chiesa and A. Spector (1989). Purification of a 43,000 Dalton aggregate generated from α-crystallin. *Current Eye Research*, **8**, 771–779.

[19] J. B. Smith, G. Thevenon-Emeric, D. L. Smith and B. Green (1991). Elucidation of the primary structures of proteins by mass spectrometry. *Analytical Biochemistry*, **193**, 118–124.

[20] J. B. Smith, Y. Sun, D. L. Smith and B. Green (1992). Identification of the posttranslational modifications of bovine lens αB-crystallins by mass spectrometry. *Protein Science*, **1**, 601–608.

[21] K. Biemann and H. A. Scoble (1987). Characterization by tandem mass spectrometry of structural modifications in proteins. *Science*, **237**, 992–998.

[22] I. A. Papayannopoulos (1995). The interpretation of collision-induced dissociation tandem mass spectra of peptides. *Mass Spectrometry Reviews*, **14**, 49–73.

[23] W. Qin, J. B. Smith and D. L. Smith (1993). Reaction of aspirin with cysteinyl residues of lensγ-crystallins: a mechanism for the proposed anti-cataract effect of aspirin. *Biochemica Biophysica Acta*, **1181**, 103–110.

[24] J. B. Smith, S. R. A. Hanson, R. L. Cerney, H.-R. Zhao and E. C. Abraham (1996). Identification of the glycation site of lens γB-crystallin by fast atom bombardment tandem mass spectrometry. *Analytical Biochemistry* (accepted)

[25] L. Takemoto, J. Horwitz and T. Emmons (1992). Oxidation of the N-terminal methionine of lens alpha-A crystallin. *Current Eye Research*, **11**, 651–655.

[26] P. Roepstorff and J. Fohlman (1984). Proposal for a common nomenclature for sequence ions in mass spectra of peptides. *Biomedical Mass Spectrometry*, **11**, 601.

[27] B. M. Trost, T. N. Salzmann and K. Hiroi (1976). New synthetic reactions. sulfenylations and dehydrosulfenylations of esters and ketones. *Journal of the American Chemical Society*, **98**, 4887–4902.

[28] F. Turecek, D. E. Drinkwater and F. W. McLafferty (1989). Gas-phase chemistry of CH_3SOH, $-CH_2{}^+SHOH$, CH_3SO, and CH_3SOH by neutralization-reionization mass spectrometry. *Journal of the American Chemical Society*, **111**, 7696–7701.

[29] X. Jiang, J. B. Smith and E. C. Abraham (1996). Identification of a MS/MS fragment diagnostic for methionine sulfoxide. *Journal of Mass Spectrometry* (in press)

[30] S. Lerman, J. M. Megaw and M. N. Moran (1985). Further studies on the effects of UV radiation on the human lens. *Ophthalmic Research*, **17**, 354–361.

[31] M. S. Swamy, K. Green and E. C. Abraham (1996). Inhibition of cataracts in moderately diabetic rats by aminoguanidine. *Experimental Eye Research*, **62**, 505–510.

[32] N. J. Unakar, M. Johnson, J. Tsue, M. Cherian and E. C. Abraham (1995). Effect of germanium-132 on galactose cataracts and glycation in rats. *Experimental Eye Research*, **61**, 155–164.

[33] A. Spector, G.-M. Wang, R.-R. Wang, W. H. Garner and H. Moll (1993). The prevention of cataract caused by oxidative stress in cultured rat lenses. I. H_2O_2 and photochemically induced cataract. *Current Eye Research*, **12**, 163–179.

[34] D. Boyle and L. Takemoto (1994). Immunolocalization of the C-terminal and N-terminal regions of αA- and αB-crystallins. *Current Eye Research*, **13**, 497–504.

[35] J. B. Smith, J. C. Cusumano and C. F. Babbs (1990). Quantitative effects of iron chelators on hydroxyl radical production by the superoxide-driven Fenton reaction. *Free Radical Research Communications*, **8**, 101–106.