Accelerated Publications

S-Methylated Cysteines in Human Lens γS-Crystallins†

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ABSTRACT: The proteins of the eye lens, which do not turn over throughout life, undergo many modifications, some of which lead to senile cataract. We describe a modification, S-methylation of cysteine, that may serve to protect the lens from detrimental modifications. The modification was detected as a +14 Da peak in electrospray ionization mass spectra of human lens γS-crystallins. Derivatization of γS-crystallin with iodoacetamide showed reaction at only six of the seven cysteines, indicating the modification blocked reaction at one cysteine. Further analysis of the modified γS-crystallin as tryptic peptides located the modification primarily at Cys 26, with smaller amounts at Cys 24. Tandem mass spectrometry and exact mass measurements showed that the modification was S-methylation. Methylation of proteins has been documented at several other amino acid residues, but S-methylation of cysteine residues has previously been detected only as part of a methyltransferase DNA repair mechanism or at trace amounts in hemoglobin. The high levels of S-methylated cysteines in lens nuclei and the specificity for Cys 26 and Cys 24 suggest the reaction is enzymatically mediated. This modification is particularly important because it blocks disulfide bonding of γS-crystallins and, thereby, inhibits formation of the high-molecular weight assemblies associated with cataract. Evidence of more S-methylation in soluble than in insoluble γS-crystallins supports the contention that S-methylation of γS-crystallin inhibits protein insolubilization and may offer protection against cataract.

Crystallins, the major structural proteins of the eye lens, undergo little turnover during aging. The lens grows by adding layers of cells at the periphery, accumulating the oldest proteins in the nucleus and the more recently synthesized proteins in the cortex. The crystallins in the nucleus are nearly as old as the organism itself. As the lens ages, the crystallins experience numerous post-translational modifications, many of which are present in young clear lenses (*1*−*3*). With increasing age, concurrent with further modification, an increasing number of the crystallins form high-molecular weight assemblies (*4*, *5*) linked together by both disulfide and nondisulfide bonds (*6*, *7*) and become insoluble.

The association between these high-molecular weight assemblies and cataract is well-established (*7*−*9*). γ-Crystallins, which are particularly rich in cysteines, are the most abundant disulfide-bonded high-molecular weight proteins (*10*). The critical role of disulfide-bonded cysteines was recently illustrated for a type of hereditary, juvenile onset cataract in which a single-point mutation in the γD-crystallin gene resulted in substitution of Cys for Arg 14 (*11*). This cataract has been attributed to Cys 14 forming a disulfide bond with another exposed cysteine, Cys 110, leading to oligomerization (*12*). Inhibition of disulfide bond formation can prevent aggregation (*13*) and, in some animal models, inhibit cataractogenesis (*14*). In our examination of human γS-crystallins, we have identified an in vivo modification of γS-crystallin that may block intermolecular disulfide bonding. This in vivo modification, S-methylation of cysteine residues, has not been previously reported in lens crystallins. The specificity of S-methylation for the most exposed cysteine residues of γS-crystallin, along with the high level of modification, implicates S-methylation of cysteine in preventing formation of intermolecular disulfide cross-links. Through this inhibition, S-methylation of γ-crystallins in human lenses may serve as a form of protection from cataractogenesis.

MATERIALS AND METHODS

Extraction of Crystallins from Human Lenses. Lenses in this study were from the National Disease Research Interchange (Philadelphia, PA) or from the Lions Eye Bank (Omaha, NE). The study included 17 clear lenses from donors ages 11 days to 83 years. The donor histories indicated no disorders known to affect lens clarity. Four additional lenses (ages 60, 64, 65, and 78) had senile type I nuclear cataract (*15*). The lenses were removed within 12 h of death, shipped on ice, and stored at −80 °C until analysis.

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Each lens was divided into two parts, the inner nucleus (average weight, 35 mg) and the remainder of the lens. The inner nucleus was obtained by cutting a cylindrical sample with a 4 mm cork borer and removing 1.0−1.5 mm from each end of the cylinder. The end pieces were processed with the remainder of the lens. Because the 11-day-old lens was so small, no pieces were cut from the ends of the cylinder. The two parts of each lens were homogenized and analyzed separately, using approximately 1 mL of the homogenization buffer [50 mM MES,[1] 0.5 M NaCl, and 1 mM EDTA (pH 6.0)] for each 12 mg of tissue. The tissue was homogenized by strong stirring, under argon, at 0 °C for 1.5 h and centrifuged for 30 min at 33000*g*. The supernatant, which contained the soluble proteins, was used for isolation of soluble γ-crystallins. For cataractous lenses, the pellet was homogenized by mashing it with a glass rod in 3 mL of the buffer. The homogenate was centrifuged, and the supernatant was removed. The extraction step was repeated using the same buffer with 6 M guanidine hydrochloride (GdHCl) to extract the water-insoluble proteins. These proteins were used for isolation of disulfide-bonded γS-crystallins.

To determine whether the results were affected by isolation conditions, inner nuclear portions of five lenses, two that were 19 years old, two that were 43 years old, and one that was 59 years old, were cut in half and the crystallins in each half were extracted using different buffers and components. Data for γS-crystallins isolated in the above MES homogenization buffer were compared with data after homogenization in 50 mM sodium citrate buffer containing 0.5 M NaCl and 1 mM EDTA (pH 6.0), a 50 mM sodium citrate buffer containing 0.5 M NaCl and 1 mM EDTA (pH 4.1), and a 50 mM sodium citrate buffer containing 75 mM NaCl (pH 6.0) with 5 mM *S*-adenosyl-L-methionine (AdoMet), 10 mM betaine, or 3 mM *S*-adenosyl-L-homocysteine (AdoHcy).

Crystallins were also extracted from the outermost cortical layers of three clear lenses, 19, 47, and 60 years old, and two cataractous lenses, 65 and 78 years old, by occasional swirling of the lens in 3 mL of the homogenization buffer for 2.5 h. The solution was removed, centrifuged, and used in further separations.

Isolation of γS-Crystallins. Soluble proteins obtained from the inner nucleus and the portion remaining after removal of the inner nucleus, as well as those obtained by dissolving the outermost cortical layers of the lens, were fractionated by size exclusion chromatography (Superose 12 HR 10/30 column, Pharmacia Biotech) equilibrated with the homogenization buffer. γ-Crystallins were collected and the γS-crystallins isolated by reversed phase HPLC (Vydac 4.6 mm × 150 mm C4 column) equipped with a protein Macrotrap (Michrom BioResources) using a gradient of acetonitrile from 20 to 55% in 0.1% trifluoroacetic acid (TFA) over 35 min (*16*). The γS-crystallins were concentrated to dryness, redissolved in 50% acetonitrile containing 0.3% formic acid, and analyzed by mass spectrometry. Protein concentrations were determined using Coomassie Plus Protein Assay Reagent (Pierce, Rockford, IL).

Insoluble proteins from cataractous lenses were also fractionated by size exclusion chromatography in 50 mM MES buffer containing 6 M GdHCl. The high-molecular weight proteins were collected and reduced with 250 mM DTT, and the γS-crystallins, which had been disulfide-bonded, were isolated as previously described (*10*).

Derivatization of Sulfhydryl Groups of γS-Crystallins. γS-Crystallins were redissolved in 400 μL of 250 mM Tris-HCl buffer containing 6 M GdHCl, 1 mM EDTA, and 5 mM DTT (pH 8.5). The sulfhydryl groups were derivatized by reaction with 15 mM iodoacetamide or iodoacetic acid for 30 min or with 25 mM 4-vinylpyridine for 90 min. The reactions were quenched by adding excess DTT. The derivatized proteins were desalted by reversed phase HPLC and analyzed by ESIMS. In this report, the term "derivatization" refers to in vitro reactions used in the analysis, while the term "modification" refers to in vivo post-translational reactions.

Enzymatic Digestions of γS-Crystallins. γS-Crystallins derivatized with iodoacetamide were digested with trypsin or chymotrypsin in 100 mM ammonium bicarbonate at 37 °C for 8 h at an enzyme:protein ratio of 1:50. The digests were freeze-dried and dissolved in 0.1% TFA for mass spectrometric analysis.

Electrospray Ionization Mass Spectrometric Analysis of γS-Crystallins. Intact γS-crystallins were dissolved in 50% acetonitrile and 0.3% formic acid and injected directly into a Q-Tof mass spectrometer (Micromass) using a solvent flow rate of 5 μL/min. The typical uncertainty in protein mass determinations was 0.005%. Peptides produced by enzymatic digestions were analyzed by on-line reversed phase HPLC−ESIMS using an ion trap mass spectrometer (Finnigan MAT LCQ, San Jose, CA). For peptide mapping, the mass spectrometer was routinely operated in the full scan MS mode with the most abundant ion of each scan analyzed by MS/MS. The uncertainty in the peptide mass determinations was ±0.2 Da over the mass range of 100−2000 Da.

Selective ion monitoring was used to determine the relative abundance of modified peptides. For detection of peptide 19−35 in a tryptic digest of γS-crystallin derivatized by iodoacetamide, the doubly charged ions at m/z 1106.0 ± 1.5 (the modified peptide) and m/z 1127.5 ± 1.5 (the unmodified peptide) were monitored. Similarly, modified and unmodified peptides 21−32 from a chymotryptic digest were monitored at m/z 768.50 ± 1.5 and 790.0 ± 1.5 as doubly charged ions. Also, the modified peptide 19−35 from a tryptic digestion of nonderivatized γS-crystallin was monitored at m/z 1049.0 ± 1.5. Amino acid sequences of peptides detected by selective ion monitoring were confirmed by MS/MS analyses. A collision energy of 35% was typically used for MS/MS analyses.

Exact Mass Measurements of Modified Peptides. The chymotryptic peptide 19−32 of γS-crystallin was used to establish the elemental composition of the in vivo modification. Exact masses of both the modified and unmodified peptides were determined using a Q-Tof Ultima mass spectrometer (Micromass) according to the manufacturer's manual. After the instrument had been calibrated, two different peptide mixtures were used for the mass determination. The first one included [Glu[1]]fibrinopeptide B (GFP) and unmodified peptide 19−32. The second one contained GFP and modified peptide 19−32. The doubly charged ions

[1] Abbreviations: MES, 2-(*N*-morpholino)ethanesulfonic acid; GdHCl, guanidine hydrochloride; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; AdoMet, adenosyl-L-methionine; AdoHcy, *S*-adenosyl-L-homocysteine; HPLC, high-pressure liquid chromatography; BHMT, betaine-homocysteine methyltransferase; MS/MS, tandem mass spectrometry.

were analyzed. GFP (*m*/*z* 785.8424) was used as the lock mass in these measurements.

Quantification of Methylation of γS-Crystallin. The extent of methylation of γS-crystallins was determined from mass spectra of γS-crystallins derivatized with 4-vinylpyridine using the following equation:

$$\text{fraction methylated} = \frac{I(M_r = 21\,562)}{I(M_r = 21\,653) + I(M_r = 21\,562)}$$

where $I(M_r = 21\,562)$ and $I(M_r = 21\,653)$ are the relative intensities due to the monomethylated and nonmethylated species, respectively. The dimethylated species ($M_r = 21\,471$) contributed less than 2% to the total methylation, and therefore was not included in the calculations.

Accessibility Calculations. To calculate the surface accessibilities of the cysteine residues in γS-crystallin, a model of human γS-crystallin was made in which the C-terminal domain of human γS-crystallin (*17*) was superimposed onto the C-terminal domain of complete bovine γB-crystallin (*18*) to obtain the orientation of the N-terminal domain relative to the C-terminal domain. Nonidentical residues in the N-terminal domain were then mutated to match the human γS-crystallin sequence. C-Terminal accessibilities were calculated from the dimer found in the single-domain crystal lattice (*17*), and N-terminal accessibilities were calculated from the model structure with the program GETAREA 1.1 (http://www.scsb.utmb.edu/cgi-gin/get_a_form.tcl) developed at The Sealy Center for Structural Biology (University of Texas Medical Branch, Galveston, TX). A probe with a diameter of 1.4 Å, the same radius as water, was rolled around the van der Waals surface of the macromolecule. The calculation makes successive thin slices through the three-dimensional molecular volume to calculate the accessible surface of individual atoms.

RESULTS

ESIMS of Derivatized γS-Crystallins. Mass spectra of soluble γS-crystallins from the inner nucleus of a 19-year-old lens are presented in Figure 1. The major peak is close to the expected mass of γS-crystallin ($M_r = 20\,918$), while a second peak showing a protein with an M_r of 20 932 is due to a modified form of γS-crystallin (Figure 1A). This second peak had previously been observed in ESIMS spectra of insoluble γS-crystallins from nuclear cataracts (*10*), but was thought to be due to oxidation at methionines, a mass increase of 16 Da. However, analysis of tryptic peptides did not yield any masses corresponding to methionine-containing peptides with 16 Da. A clue to the nature of the modification can be found in the mass spectrum of γS-crystallin derivatized with iodoacetamide, which adds 57 Da at each Cys (Figure 1B). γS-Crystallin modified at all seven cysteine residues gives a peak at 21 317 Da. If the modified form contained an oxidized methionine, one would expect an additional peak at 21 333 Da. Instead, a peak at 21 274 Da was observed (Figure 1B). This mass implies that derivatization with iodoacetamide is blocked at one of the cysteines by a 14 Da modification. Similar results were obtained after derivatization with iodoacetic acid (58 Da/Cys) or 4-vinylpyridine (105 Da/Cys) (Figure 1C).

The ESI mass spectra of γS-crystallins isolated in 50 mM sodium citrate buffer (pH 6.0 or 4.1) or in the presence of

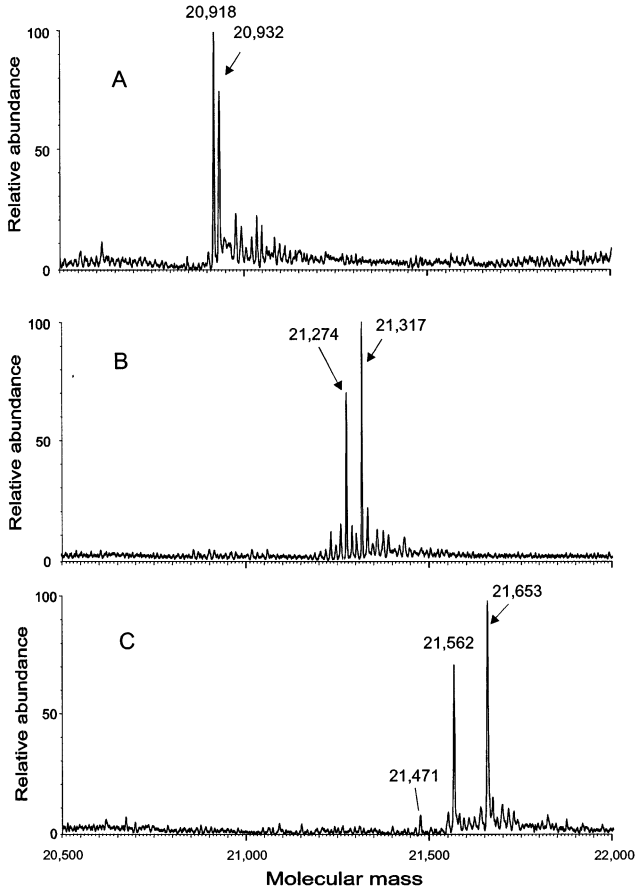

FIGURE 1: Reconstructed ESI mass spectra of nuclear γS-crystallins isolated from a 19-year-old lens: (A) not derivatized, (B) derivatized by iodoacetamide, and (C) derivatized by 4-vinylpyridine.

AdoMet, betaine, or AdoHcy exhibited the same masses and abundance of modified γS-crystallin as samples extracted in the MES homogenization buffer (data not shown). These data indicated that the modification had occurred in vivo and was not an artifact of protein isolation. These data were also evidence that the methyl donors, AdoMe and betaine, did not enhance S-methylation of cysteine within the incubation period.

ESIMS of Peptides from Enzymatically Digested γS-Crystallins. For a more accurate determination of the mass of the modification and to locate the exact sites of modification, peptides from tryptic and chymotryptic digests of γS-crystallins were analyzed by HPLC−ESIMS. The tryptic digest of γS-crystallin derivatized by iodoacetamide included a peak for a peptide of 2252.9 Da, the expected mass for peptide 19−35, and another at 2209.9 Da, 43 Da lower. Similarly, the chymotryptic digest showed peptide 21−32 with masses of 1577.4 and 1534.5 Da. These peptides include Cys 22, Cys 24, and Cys 26. Selective monitoring of the chymotryptic peptides as doubly charged ions at *m*/*z* 790.0 (unmodified) and 768.5 (modified) showed the unmodified peptide eluting in one peak (Figure 2A) and the modified peptide eluting in two peaks (Figure 2B). The MS/MS spectrum of the unmodified peptide (Figure 3A) is consistent with the fragmentation pattern expected for this peptide (see the top of Figure 2 for the sequence). The MS/MS spectra of the first (Figure 3B) and second (Figure 3C) peaks of the modified peptide indicated modification at Cys 24 and Cys 26, respectively. No evidence for modification at Cys 22 was

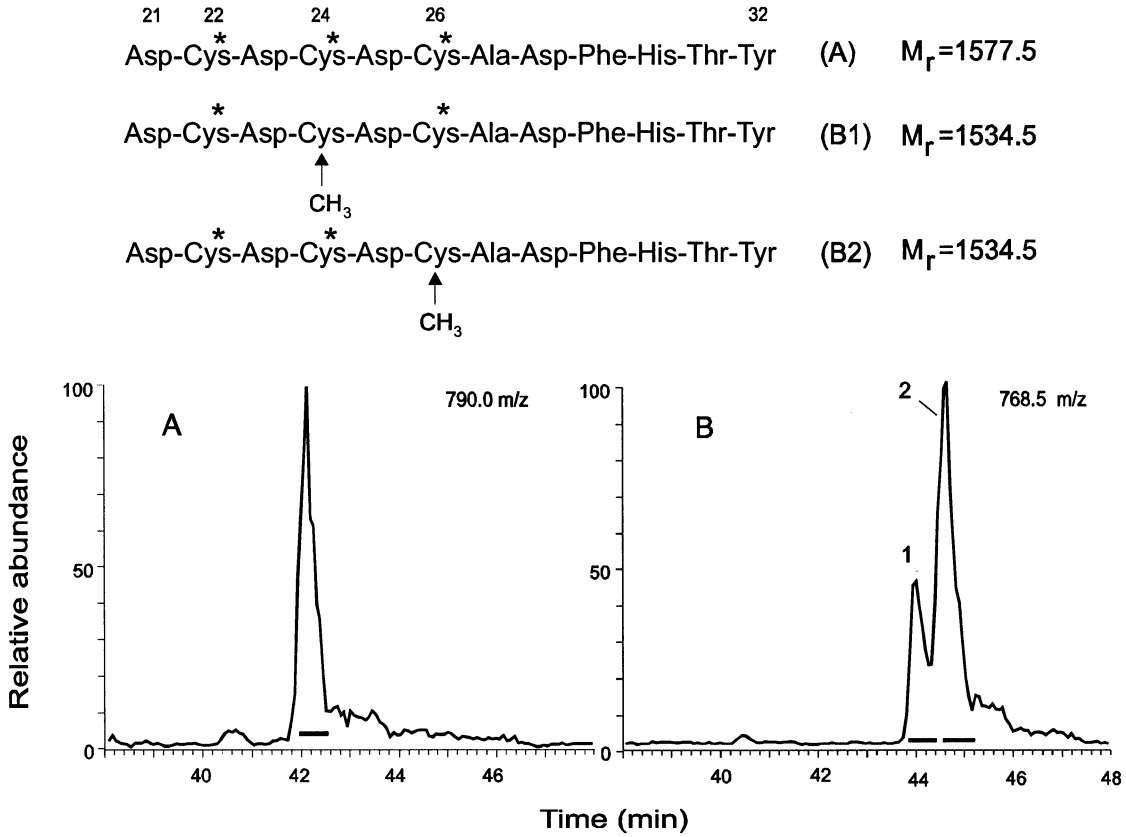

FIGURE 2: Selective ion monitoring of peptide 21−32 from a chymotryptic digest of iodoacetamide-treated nuclear γS-crystallin containing (A) no methylated cysteines (*m*/*z* 790.0) or (B) one methylated Cys residue (*m*/*z* 768.5). The amino acid sequence of the peptide and molecular masses of the different forms are shown at the top. *Cys is carboxyamidomethylcysteine.

detected. A small amount of peptide modified at both Cys 26 and Cys 24 was detected and confirmed by MS/MS analysis (data not shown). Identification of this peptide is in agreement with the small peak at 21 471 Da in Figure 1C. This mass fits γS-crystallin derivatized at five cysteines with 4-vinylpyridine (105 Da/Cys) and the 14 Da modification at two cysteines.

Exact Mass Determination of Modified Peptides. The increase of 14 Da could be due to several combinations of elements. To distinguish among these possibilities, the exact masses of both the modified and unmodified forms of chymotryptic peptide 21−32 were determined. The molecular mass of peptide 21−32 with three cysteines derivatized by iodoacetamide was 1577.4849 ± 0.0012 Da (theoretical value of 1577.4855 Da). The mass of the corresponding modified peptide was 1534.4790 ± 0.0014 Da, indicating the modification that prevented reaction with iodoacetamide had a mass of 14.0155 Da. This mass, along with the small uncertainty in the mass determination, fits addition of CH_2 and excludes all other combinations of likely elements. The presence of *S*-methylcysteine was also confirmed by MS/MS analysis of the peptide after oxidation with H_2O_2 to the sulfoxide derivative (data not shown).

Specificity of Methylation. Only peptides containing Cys 26 and Cys 24 were detected with the modification, with 2−2.5 times more methylation at Cys 26 than at Cys 24 (Figure 2B). The analysis of peptides from tryptic, chymotryptic, and Asp-N digests of γS-crystallin permitted detection of methylation of 2% at any one site. This specificity appeared to be related to the exposure of the cysteine residues. Calculation of surface accessibility of the seven cysteines of γS-crystallin showed five cysteines with very low accessibility, less than 6 Å^2, and Cys 24 and Cys 26 with surface exposures of 66 and 44 Å^2, respectively. It is interesting to note that these exposed cysteine residues are located in an acidic cluster (Figure 2). The specificity of methylation did not vary with the age of the lens.

Methylation of Cysteine in γS-Crystallins from Lenses of Different Ages. To determine the extent of methylation of the protein isolated from different parts of the lens, the relative abundances of the peaks of modified and unmodified forms of γS-crystallins derivatized with 4-vinylpyridine were measured. Derivatization with 4-vinylpyridine was chosen because the peak at +14 Da in spectra of underivatized γS-crystallin could include contributions from oxidized γS (16 Da), while the masses of oxidized γS and methylated γS after derivatization with 4-vinylpyridine were well-separated. The ratio of γS to γC and γD increases through childhood and adolescence with γS becoming the major γ-crystallin in adult lenses. In adult nuclei, γS comprises ~15% of the total soluble protein. Among the nuclear γS-crystallins, the extent of methylation increased from 6 to 40% for lenses from 11 days old to 19 years old, but did not change much for older lenses (Table 1, column 4). Because crystallins in the inner nucleus are nearly as old as the donor, this increase during childhood and adolescence with a plateau for adults suggests that methylation of γS is primarily a phenomenon in young lenses. The portion remaining after removal of the inner nucleus showed a similar increase in methylation during childhood and adolescence, but the extent of methylation was lower, ranging from 4 to 26% (Table 1, column 7). This portion, which was a large fraction of the lens, included some

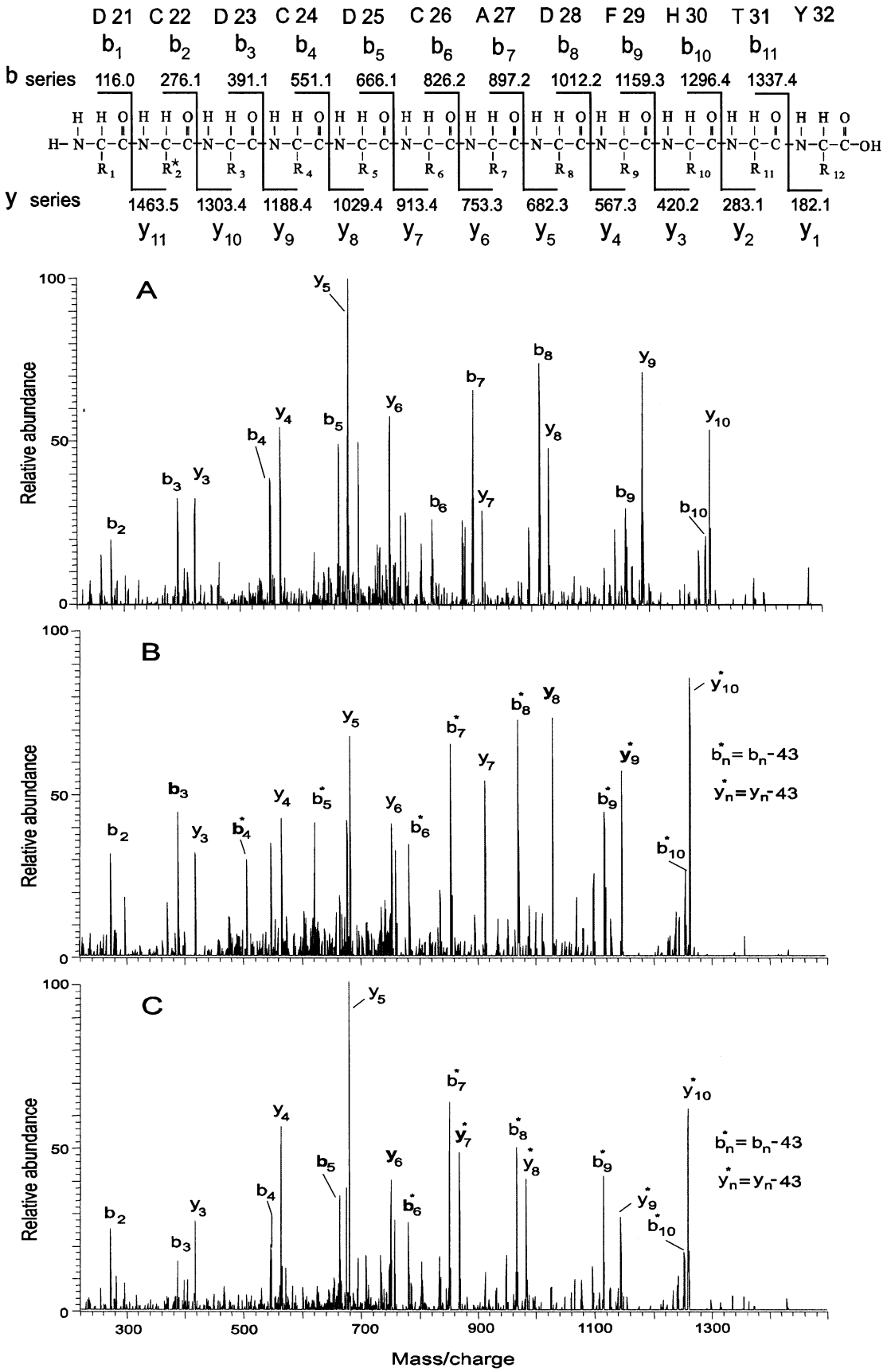

FIGURE 3: MS/MS spectra of peptide 21−32 containing (A) no methylated cysteines, (B) methylated Cys 24, and (C) methylated Cys 26. The y- and b-fragments critical for identification of the methylation sites are shown in bold. Because the methylated Cys residue could not be derivatized by iodoacetamide (57 Da), all fragments that included the methylated cysteine (14 Da) exhibited a net loss of 43 Da. The expected y- and b-fragments for the peptide with three carboxyamidomethylated cysteines are given at the top.

Table 1: Methylation of Soluble γS-Crystallins from Human Lenses of Different Ages[a]

	nucleus			remainder of lens		
lens age	weight (mg)	percent soluble proteins	percent methylated	weight (mg)	percent soluble proteins	percent methylated
11 days	35	98	6.1 ± 0.7	25	98	4.2 ± 0.7
11 years	38	95	31.4 ± 1.2	84	95	14.7 ± 0.9
19 years	33 ± 3	≥93	40.4 ± 2.2	109 ± 7	>93	26.0 ± 2.4
43 years	32 ± 3	≥73	49.0 ± 2.6	192 ± 4	≥84	30.9 ± 1.8
50 years	34 ± 2	≥71	51.5 ± 2.1	188 ± 7	≥80	30.7 ± 1.2
59 years	33	66	52.0 ± 1.4	185	81	31.3 ± 0.7
72 years	33	62	39.0 ± 1.4	181	79	29.9 ± 0.9
83 years	31	54	37.1 ± 1.1	178	72	28.6 ± 1.1
60 years (cataract)	34	48	soluble, 51.6 ± 1.4; insoluble,[b] 37.0	182	74	32.6 ± 1.4
64 years (cataract)	36	50	soluble, 50.1 ± 2.3; insoluble,[b] 34.6	179	74	33.0 ± 1.6

[a] Two independent analyses were performed on each lens extract. Data are from the following lenses: one each for 11 day-old and 11-, 59-, 72-, and 83-year-old clear lenses, two each for 43- and 50-year-old clear lenses, three for 19-year-old lenses, and one each for 60- and 64-year-old cataractous lenses. [b] γS-Crystallins obtained from insoluble high-molecular weight aggregates after DTT reduction of disulfide bonds.

nuclear crystallins along with the cortical crystallins. The methylated crystallins probably reflected the fraction of nuclear crystallins. Methylation as primarily a reaction in the nucleus was further supported by the lower levels of methylation, less than 15%, in the outermost cortical layers of lenses of all ages, including cataractous lenses (data not shown).

The extent of methylation was also determined for γS-crystallins isolated from insoluble high-molecular weight aggregates from nuclear cataracts. These aggregates are complex mixtures of proteins with γS-crystallin as the major disulfide-bonded component (*10*). Reduction of the disulfide bonds in the aggregates permits isolation of γS-crystallins. The extent of methylation among these previously disulfide bonded γS-crystallins was approximately 15% lower than among the soluble γS-crystallins from the same nuclear cataracts (Table 1).

DISCUSSION

Methylation is a common post-translational modification of DNA, RNA, and proteins. In proteins, in vivo methylation of some N-terminal residues, methylation of side chains of lysine and arginine residues, and O-methylesterification of glutamic and aspartic acids are well-known (*19*). Methylation of the lysine and arginine residues of histones involved in chromatin function is a mechanism for regulating gene activity (*20*). In addition, methylation of arginine residues is implicated in numerous functions from signaling and activation of hormone-responsive genes to protein sorting (*21*). A coordinated interaction of methylation−demethylation reactions at specific glutamate and glutamine residues of a receptor with a histidine−aspartate phosphorelay signal transduction system provides the basic mechanism of chemotaxis that allows bacteria to sense gradients of attractants and repellents (*22*). The importance of some other methylation reactions is yet to be established. Methylation at the imidazole ring of histidine in the contractile proteins, actin and myosin, was discovered long ago (*23*); however, the function of this rare post-translational modification is still unknown.

Sulfhydryl groups of cysteine residues, which are among the most reactive groups in proteins, can be modified in vitro by a number of methylating agents (*24*−*26*). Despite the reactivity of cysteines, naturally occurring *S*-methylcysteine residues in proteins have been detected only at trace levels (∼0.02%) in hemoglobin (*27*), following exposure to methylating agents (*28*), or as a product of methyltransferase reactions that repair DNA damage caused by alkylating agents (*29*). In *Escherichia coli*, the Ada protein is a critical component of the adaptive response, an inducible resistance system that is involved in the repair of O^6-methylguanine in DNA (*30*). DNA adducts at the O(6) position of guanine are the major factor in carcinogenic and mutagenic actions of methylating agents. Ada repairs O^6-methylguanine via direct irreversible transfer of the methyl group to Cys 321 on the protein itself (*31*), and therefore, it may be considered a sacrificial reagent for DNA repair. Direct transfer of methyl groups from DNA to another cysteine residue, Cys 69, illustrates another function of methylation. Ada methylated at Cys 69 activates transcription of its own gene, resulting in induction of Ada (*32*).

S-Methylation of γS-crystallin, which is specific for Cys 26 and Cys 24, is one of the most abundant in vivo post-translational modifications of human lens crystallins. The experiments with different isolation conditions clearly showed that methylation had not occurred during crystallin preparation. The concentration of methylated γS is low in outer cortical layers of lenses of all ages, even old cataractous lenses, and is highest in inner nuclei. The extent of methylation also varied with the age of the lens. Unlike some post-translational modifications which continue to increase as the lens ages, the level of methylation reaches 40% by ∼19 years of age, increases further to 50%, and then decreases slightly in older lenses.

Although the direct action of a methylating agent in the lens may occur, S-methylation of γS-crystallin is most likely an enzymatic reaction. In addition to being highly specific and occurring principally in very young lenses, there is other evidence that S-methylation of γS-crystallin occurs via a highly specific enzymatic reaction. The lens contains *S*-adenosylmethionine and its synthesizing enzyme, methionine adenosyltransferase, indicating that enzymatic methylation reactions may take place in the lens (*33*). The presence of methylating enzymes in the lens is further supported by

recent observations of methylation of several lens crystallins at lysine and arginine residues (*34*). Also, catechol *O*-methyltransferase (COMT), a member of the *O*-methyltransferase family, was identified in rat lenses (*35*), and there is evidence that COMT activity is also present in human lenses (J. Sanderson, personal communication).

The most intriguing evidence supporting an enzymatic reaction is the presence of betaine-homocysteine methyltransferase (BHMT) (*36*). BHMT is particularly abundant in rhesus monkey lenses, comprising up to 10% of the protein in the lens nucleus (*36*). The enzyme, which catalyzes methylation of homocysteine, is strongly concentrated in the central region of the lens, but is not detected in the epithelium or in peripheral layers of the cortex. It was suggested that BHMT is developmentally downregulated, resulting in a higher concentration of this enzyme in the nuclear part of the lens, in the cells produced during embryonic and fetal development (*36*). Although not found in nonprimate lenses, BHMT activity is also present in human lenses and is much higher in the nucleus than the cortex (*36*). The spatial distribution of S-methylated γS-crystallin in human lenses and its variation with age are strikingly similar to the pattern for BHMT in monkey lenses. We speculate that γS-crystallin may be a substrate for BHMT or a similar enzyme in human lenses. Our preliminary data indicate that S-methylation in human lenses is not limited to γS-crystallin; γC- and γD-crystallins are also methylated at cysteine residues.

The high level of methylation at Cys 26 in γS-crystallin from young lenses suggests that this modification does not negatively impact protein function but may, on the contrary, be beneficial. Calculation of the accessibilities of the cysteines of γS-crystallin shows only Cys 24 and Cys 26 among seven cysteine residues of human γS-crystallin are accessible. Consequently, these exposed cysteines are the most likely to form intermolecular disulfide bonds, and when they are methylated, oligomerization is blocked. Methylation of the accessible cysteines may also prevent participation in other reactions leading to non-disulfide cross-links (*37*, *38*). The substantially lower level of methylation found in γS-crystallins isolated from insoluble high-molecular weight aggregates suggests that Cys 26 and Cys 24 are key residues in intermolecular bonding and methylation of exposed cysteine residues may be an important in vivo protective mechanism.

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