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ARTICLE

Mass Spectrometric Characterization and Redox Instability of Turkey and Chicken Myoglobins As Induced by Unsaturated Aldehydes

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Turkey and chicken myoglobins (Mbs) were isolated, purified, and characterized using electrospray ionization mass spectrometry (ESI-MS), and the effect of unsaturated aldehydes (nonenal and hexenal) on their redox stability was investigated in vitro. The deconvoluted spectra from ESI-MS exhibited a molecular mass of 17291 Da for both turkey and chicken Mbs. Significant homogeneity in the fragmentation pattern of both Mbs was indicated by ESI-MS/MS. Both turkey and chicken oxymyoglobins (OxyMbs) were more prone to oxidation at pH 5.8 than at pH 7.4. Metmyoglobin formation was greater in the presence of unsaturated aldehydes than controls ($P < 0.05$). The results demonstrated that both turkey and chicken Mbs have identical molecular mass and that the effects of α,β-unsaturated aldehydes on their redox stability are consistent with those of mammalian livestock Mbs.

KEYWORDS: Myoglobin; chicken; turkey meat; ESI-MS; unsaturated aldehydes; 4-hydroxynonenal; lipid oxidation

INTRODUCTION

Color is an important quality attribute that influences consumer acceptance of many food products, including poultry meat. Consumers base their estimates of poultry freshness on flavor, odor, and color. Color variation is a major problem in poultry meat because consumers reject poultry meats with noticeable color differences (*1*). Meat color is primarily determined by the concentration and chemical state of myoglobin (Mb), a globular heme protein localized in muscle fibers (*2*). Most discolorations in poultry meat appear to be related to Mb (*3*). Although color variations and their related problems occur in the poultry industry, they tend to be inconsistent in severity and are often not well characterized. A thorough understanding of poultry Mb is a logical first step in reducing the potential negative impact of meat color variation.

Recent investigations have utilized mass spectrometry (MS) to accurately measure the molecular weight of Mb for potential meat species differentiation (*4*) and to understand the basic mechanism of interaction of this heme protein with other macro- and micromolecules (*5*−*7*). Although mass spectrometry has evolved as an effective tool for characterizing proteins, relatively little work has utilized MS for poultry Mb studies. Differences in the amino acid sequences of Mb proteins between different species may produce different fragmentation patterns that could be used as a tool for species differentiation by performing an electrospray ionization collision-induced dissociation MS/MS (ESI-CID-MS/MS) analysis (*4*). The amino acid sequence of turkey Mb is not available, and its MS/MS fragmentation pattern could provide an indication of the degree of similarity between turkey and chicken Mbs.

Froning (*8*) attributed some of the discoloration in turkey meat to more pronounced Mb oxidation. Turkey meat is particularly prone to lipid oxidation (*9*) because of its high polyunsaturated fatty acid content within membrane phospholipids and its poor ability to store vitamin E compared to other species (*10*). Many authors have suggested that lipid and pigment oxidation are closely related in beef (*6*), pork (*7*), and lamb (*11*). Lipid oxidation generates a wide range of secondary reactive products including *n*-alkanals, *trans*-2-alkenals, 4-hydroxy-*trans*-2-alkenals, and malonaldehyde (*12*). These aldehyde-based lipid oxidation products can alter Mb redox stability (*13*). Heme proteins are capable of binding to cellular membranes and fatty acids, and their proximity to lipid rich membranes would enhance the likelihood of interaction with products released as a consequence of lipid oxidation (*14*). Haurowitz et al. (*15*) were among the first investigators to demonstrate that lipid oxidation products could be deleterious toward heme protein stability. Covalent modification of equine, bovine, porcine, and tuna myoglobins by 4-hydroxynonenal and other aldehydes has been demonstrated (*5*−*7*, *16*). Of the aldehydes tested, the α,β-unsaturated aldehydes nonenal and heptenal were found to be especially pro-oxidative in equine myoglobin (*5*). They further reported that increased carbon chain length from six to nine carbons increased the pro-oxidative nature of these aldehydes.

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Even though researchers have noted greater rates of autoxidation in turkey and chicken Mbs than in beef Mb (*3*, *17*), no results have been reported on the relationship between redox stability of myoglobin proteins and lipid oxidation in these poultry meats. Hence, this work was undertaken to characterize turkey and chicken Mbs using MS-based tools and to investigate the redox destabilizing effect of two unsaturated aldehydes of different carbon chain lengths, nonenal and hexenal, on turkey and chicken Mbs with the objective to better explain color defects in fresh poultry meat.

MATERIALS AND METHODS

Materials. *trans*-2-Nonenal, *trans*-2-hexenal, Sephacryl S-200 HR, sodium bicarbonate, sodium citrate, sodium hydrosulfite, ethanol, bicinchoninic acid (BCA) protein assay kit, and horse heart myoglobin were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). 4-Hydroxynonenal was obtained from Cayman Chemical (Ann Arbor, MI). HiTrap DEAE FF columns were obtained from Amersham Biosciences (Uppsala, Sweden). Kits for SDS-PAGE were obtained from Bio-Rad (Hercules, CA). PD-10 columns were from Pharmacia Inc. (Piscataway, NJ). All chemicals were of reagent grade or greater purity.

Isolation and Purification of Turkey and Chicken Myoglobins. Beef hearts, turkey hearts, and spent layer chicken meat were obtained locally. Turkey Mb was isolated from cardiac muscles, whereas chicken Mb was purified from skeletal muscles of spent chicken thighs and drumsticks, according to the method of Faustman and Phillips (*18*) with several modifications. Because of the relatively low concentration of myoglobin in turkey meat, hearts were used as a source of myoglobin. Because of the small size of chicken hearts, thigh and drumstick muscles from aged, spent layer hens were used. Meat samples were trimmed of fat, homogenized with 20 mM ammonium bicarbonate buffer (pH 9.0, 4 °C) at a 1:1 ratio, and centrifuged at 5000*g* for 10 min at 4 °C using a RC-5B centrifuge (Sorvall, Norwalk, CT). The reported isoelectric pH of turkey Mb (7.99) (*3*) is higher than that of bovine Mb (7.1) (*19*). Therefore, homogenization buffer with a higher pH (9.0) was chosen, after trial and error, for Mb extraction rather than Tris-EDTA buffer with pH 8.0 as described by Faustman and Phillips (*18*). The supernatant was brought to 50% ammonium sulfate saturation rather than 70% (*18*) and centrifuged at 18000*g* for 20 min at 4 °C. After the precipitate had been discarded, the supernatant was brought to 100% ammonium sulfate saturation and centrifuged at 20000*g* for 1 h at 4 °C. The precipitate was resuspended in 20 mM ammonium bicarbonate buffer (pH 9.0, 4 °C) and dialyzed against 10 volumes of 10 mM ammonium bicarbonate buffer (pH 9.0) for 24 h, with buffer changes every 8 h. All isolation and purification steps were carried out at 4 °C. Ten milliliters of dialysate with a protein concentration of approximately 10 mg/mL was applied to a Sephacryl S-200 HR gel filtration column (2.5 × 100 cm) with an elution buffer of 20 mM ammonium bicarbonate, pH 9.0, 4 °C, instead of 5 mM Tris-HCl−EDTA, pH 8.0, as suggested by Faustman and Phillips (*18*), at a flow rate of 60 mL/h. Fractions (5 mL) were collected, and the absorbance was monitored at 280 and 540 nm using a Shimadzu UV-2101 PC spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD).

Diethylaminoethyl (DEAE) Cellulose Chromatography. The gel-filtered Mb was purified further using DEAE-cellulose anion-exchange chromatography on HiTrap DEAE-FF columns (Amersham Biosciences). The pH of the gel-filtered Mb sample was adjusted to 8.4 by passage through PD-10 columns calibrated against starting buffer (10 mM Tris buffer, pH 8.4). Approximately 8−10 mL of Mb sample was loaded onto each column previously equilibrated with 10 volumes of starting buffer. Mb was eluted in the same buffer followed by elution of hemoglobin and other proteins using 50 mM Tris buffer with 0.2 M sodium chloride, pH 8.6. Fractions of 3 mL were collected, and absorbance was measured at 280 nm to identify the presence of any colorless proteins and at 540 nm for colored proteins.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE analyses of turkey and chicken fractions from different steps of purification were performed under reducing conditions with a 4% stacking gel and a 12% separating gel (*20*) using a Mini Protean II unit (Bio-Rad Laboratories, Inc., Richmond, CA). The current for each gel was maintained at 10 mA. After separation, the proteins were stained with 0.1% (w/v) Coomassie brilliant blue R-250 in 50% (v/v) methanol and 10% (v/v) acetic acid and destained with 40% methanol (v/v) and 10% (v/v) acetic acid.

ESI-MS and ESI-CID-MS/MS Analyses. Horse, beef, chicken, and turkey Mb samples (20 μM) were prepared in 1:1 aqueous methanol/distilled water with 0.1% acetic acid to enhance protonation. Samples (100−200 μL) were analyzed on an electrospray ionization−triple-quadrupole (ESI-Q-TOF) mass spectrometer (Applied Biosystems/MDS SCIEX, Ontario, Canada). The ESI-MS raw spectra were obtained in the first quadrupole by reading 10−15 scans over the scan range from m/z 700 to 1500 at 10 s per scan. The molecular mass was determined by transformation of the ESI-MS raw data into a true mass scale between 10000 and 60000 Da and obtained through the instrumentation software.

The ESI−collision-induced dissociation (CID)-MS/MS of intact Mbs was achieved by selecting the most abundant precursor ion in the first stage using a mass range of ±0.5 amu of the nominal mass (*4*). Fragmentation was then achieved by transferring the selected ions into the collision cell using nitrogen as the collision gas (collision energy = 35 V). The same conditions were applied to both chicken and turkey Mb samples until a suitable fragmentation pattern was obtained. The product ions were then transported into the third quadrupole, which separated them according to their mass-to-charge ratio with the scan range from 700 to 1500 at 10 s per scan. The software detected the mass and charge state of each fragment present in the acquired spectra, and the fragmentation pattern was compared manually for any similarities between chicken and turkey Mbs.

Preparation of Oxymyoglobin. Oxymyoglobin (OxyMb) was prepared by hydrosulfite-mediated reduction of purified chicken and turkey MetMbs (*21*) accompanied by flushing with air. Residual hydrosulfite was removed by passage through PD-10 columns previously calibrated with 50 mM sodium citrate, pH 5.8, buffer.

Reaction with Aldehydes and MetMb Formation. Turkey (0.05 mM) and chicken (0.015 mM) OxyMbs were each combined with 0.35 and 0.1 mM *trans*-2-nonenal and *trans*-2-hexenal, respectively, at 4, 25, and 37 °C, pH 5.8, the typical pH in post-mortem poultry muscle (*22*). These Mb concentrations were selected according to the method of Tang et al. (*23*) as they reflect the average Mb levels found in turkey and chicken meats (*24*, *25*). The molar ratio of turkey/chicken Mb/aldehyde (nonenal, hexenal) was maintained at 1:7 for all reactions (*5*). Controls consisted of OxyMb and a volume of ethanol equivalent to that used to deliver the aldehydes to the treatment mixture. During incubation, the control (OxyMb + ethanol) and treatment (OxyMb + aldehydes) solutions were scanned spectrophotometrically from 650 to 450 nm using a Shimadzu UV-2101 PC spectrophotometer. The blank contained only buffer. MetMb formation was calculated according to the method of Tang et al. (*23*) using absorbance values at 503, 557, and 582 nm.

Preparation and ESI-Q-TOF-MS of Turkey and Chicken Mb−4-Hydroxynonenal Adducts. Turkey (0.05 mM) and chicken (0.015 mM) OxyMbs were each combined with 0.35 and 0.1 mM 4-hydroxynonenal, respectively, and incubated at 25 °C, pH 5.8. After 5 h of incubation, 1 mL of the reaction solution was passed over a PD-10 desalting column calibrated with distilled water to remove unreacted 4-hydroxynonenal. Turkey and chicken Mb−4-hydroxynonenal adducts were detected using ESI-Q-TOF mass spectrometry as explained earlier.

Statistical Analysis. Data for turkey and chicken myoglobin oxidation were analyzed separately. The experimental design was a completely randomized design with repeated measures. Fixed effects included time and three treatments consisting of control, hexenal, and nonenal. The data for different temperatures (4, 25, and 37 °C) within a species were analyzed separately. Each experiment was replicated three times (n = 3). Data were analyzed using the Mixed procedure of SAS (version 9.1, SAS Institute, Inc., Cary, NC) with the Repeated statement to assess the repeated measures (i.e., multiple measurements taken at different time points of incubation). Least-squares means were generated for significant F tests ($P < 0.05$) and separated using least significant differences.

RESULTS AND DISCUSSION

Isolation and Purification of Myoglobins from Turkey and Chicken. Isolation and purification of beef, pork, sheep, turkey,

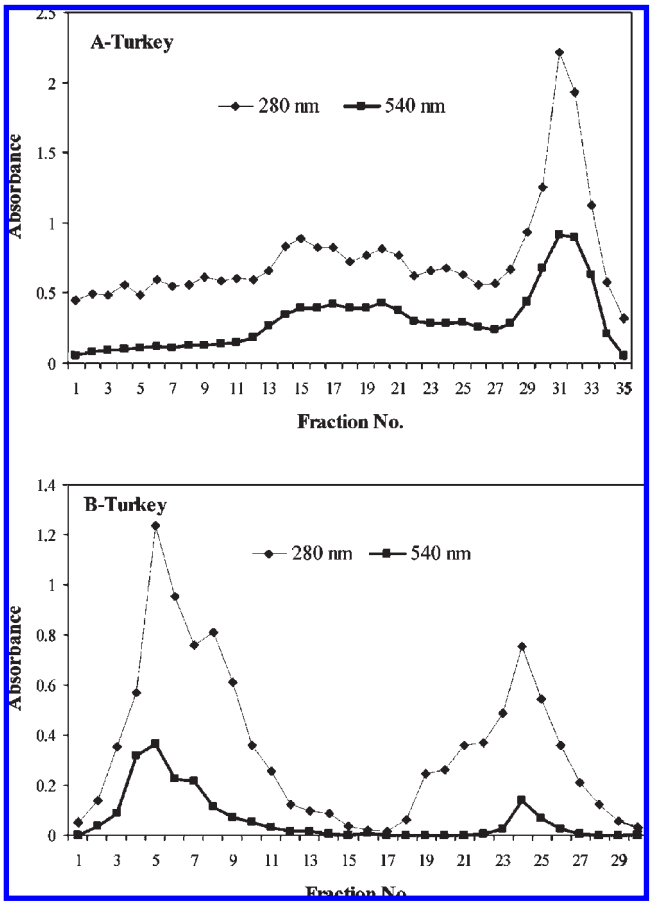

Figure 1. Elution profile of turkey myoglobin from (**A**) Sephacryl S-200 HR gel filtration chromatography and (**B**) Hitrap DEAE-FF ion-exchange chromatography.

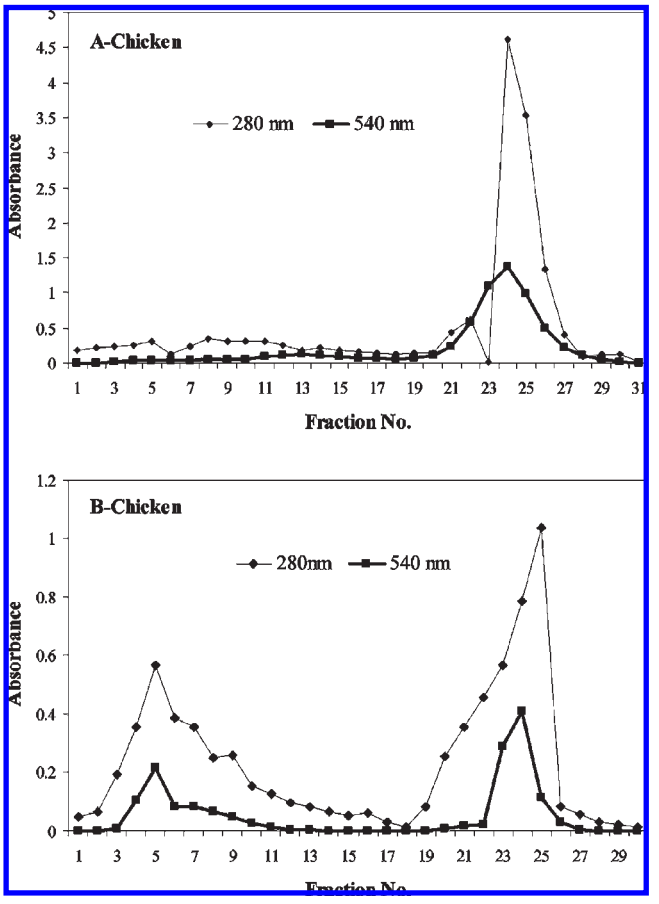

Figure 2. Elution profile of chicken myoglobin from (**A**) Sephacryl S-200 HR gel filtration chromatography and (**B**) Hitrap DEAE-FF ion-exchange chromatography.

and chicken myoglobins (Mbs) from either skeletal, heart, or smooth (gizzard) muscles using ammonium sulfate precipitation and gel filtration have been reported (*3*, *18*, *26*, *27*). Initially, we attempted to purify the turkey and chicken Mbs using the procedure of Cornish and Froning (*3*) but discovered that unlike beef or pork Mb, the chicken and turkey Mbs began salting out just above 50% ammonium sulfate saturation and continued to do so until complete saturation (100%) was obtained. The application of the crude Mb extract obtained from ammonium sulfate precipitation directly and solely to gel filtration chromatography or DEAE-cellulose or carboxymethyl (CM)-cellulose ion-exchange chromatography (*3*) did not yield a single protein band on SDS-PAGE, but rather consistently exhibited two or more bands. Purification of ammonium sulfate-precipitated and dialyzed Mb with Sephacryl S-200 HR gel filtration revealed one minor and one major peak at A_{540} for turkey (**Figure 1A**) and one peak for chicken (**Figure 2A**) preparations. For turkey Mb isolation, the first peak (pooled from fractions 13−23) was attributed to hemoglobin and the second peak (pooled from fractions 27−33) was attributed to Mb. This was based on absorbance at both 280 and 540 nm and behavior of Mb and hemoglobin (Hb) standards applied to the column. SDS-PAGE of pooled fractions (27−33 for turkey; 21−27 for chicken) from gel filtration consistently revealed the presence of two bands (lane 4) at approximately 14 and 17 kDa in both turkey (**Figure 3A**) and chicken (**Figure 3B**) samples. SDS-PAGE would result in the dissociation of Hb quaternary structure and the appearance of constituent α and β chains.

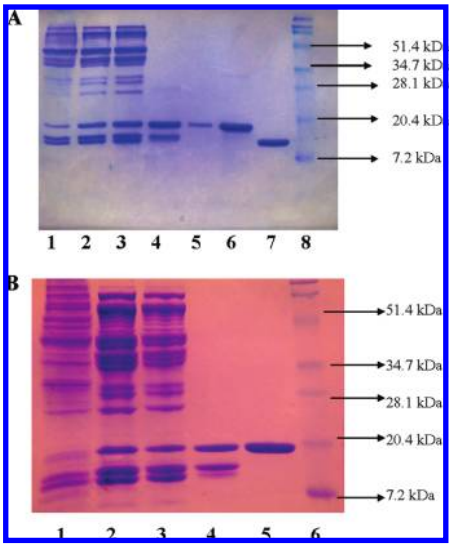

Figure 3. SDS-PAGE pattern of samples obtained during purification of (**A**) turkey myoglobin [lane 1, crude extract; lane 2, ammonium sulfate precipitated fraction; lane 3, dialyzed fraction; lane 4, gel filtered myoglobin (pooled from fractions 27−33) ; lane 5, ion-exchange filtered myoglobin (pooled from fractions 2−9); lane 6, horse heart myoglobin; lane 7, horse cytochrome *c*; lane 8, molecular weight standard] and (**B**) chicken myoglobin [lane 1, crude extract; lane 2, ammonium sulfate precipitated fraction; lane 3, dialyzed fraction; lane 4, gel filtered myoglobin (pooled from fractions 21−27); lane 5, ion-exchange filtered myoglobin (pooled from fractions 3−7); lane 6, molecular weight standard].

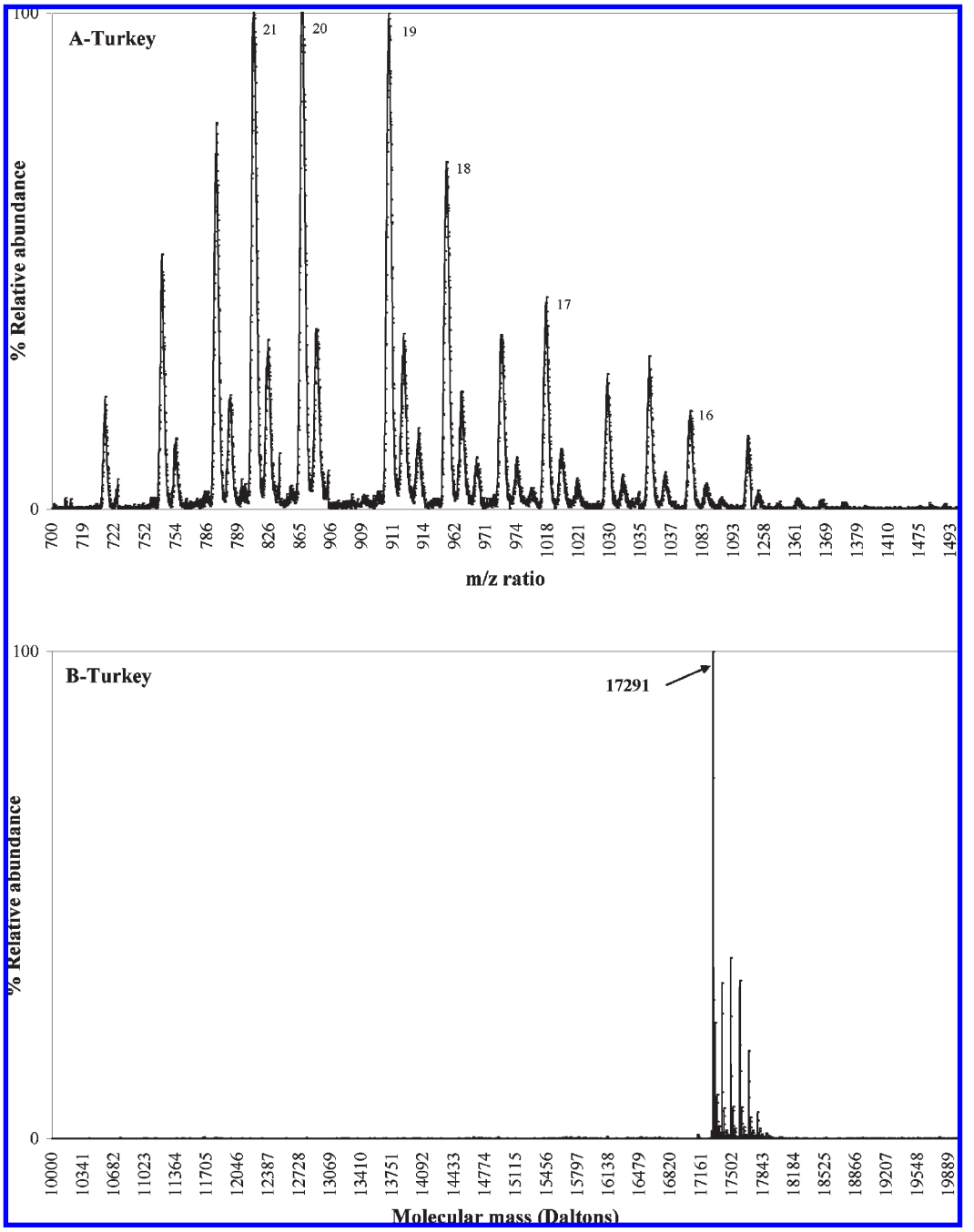

Figure 4. ESI-MS spectra of turkey myoglobin after purification on DEAE-cellulose: (**A**) raw spectra; (**B**) deconvoluted spectra.

Cornish and Froning (*3*) purified crude turkey Mb using Sephadex G-75 and reported possibly four major peaks. Therefore, we adopted the extraction and gel filtration procedure of Faustman and Phillips (*18*) with several modifications followed by DEAE-cellulose ion-exchange chromatography as suggested by Cornish and Froning (*3*). CM-cellulose (cation-exchange) chromatography failed to resolve the gel-filtered Mb fractions (data not shown), but DEAE-cellulose chromatography revealed the presence of two peaks in both turkey (**Figure 1B**) and chicken (**Figure 2B**) samples. We hypothesized that the p*I* of 8.0 for both turkey and chicken Mbs (*3*, *27*) resulted in relatively low negative charges at pH 8.4, resulting in immediate elution of Mb. This elution pattern is in agreement with the findings of Brown (*28*), who reported that whale carboxyMb was eluted in a first peak with 0.02 M Tris buffer, pH 8.6, followed by the elution of carboxyhemoglobin (Hb) in the second peak with 0.02 M Tris buffer, pH 7.3. He further reported that Mb always eluted in the buffer with which the column was equilibrated; Hb and other soluble proteins were eluted by a change to buffers of lower pH or higher ionic strength. Our results are in contrast to earlier findings for turkey (*3*), sheep (*26*), and chicken Mb (*27*). These workers reported the elution of cytochrome *c* and other nonheme proteins in the first peak followed by elution of Mb in the second peak. However, a major procedural difference was that in these previous studies, ammonium sulfate precipitated Mb preparation was applied to a DEAE column directly without prior gel filtration, and samples were not dialyzed before loading onto ion-exchange columns. SDS-PAGE of pooled fractions (2−9 for turkey; 3−7 for chicken) from DEAE-cellulose chromatography consistently revealed a single band (lane 5) at approximately 17 kDa in both turkey (**Figure 3A**) and chicken (**Figure 3B**) preparations. Hence, the extraction procedure of

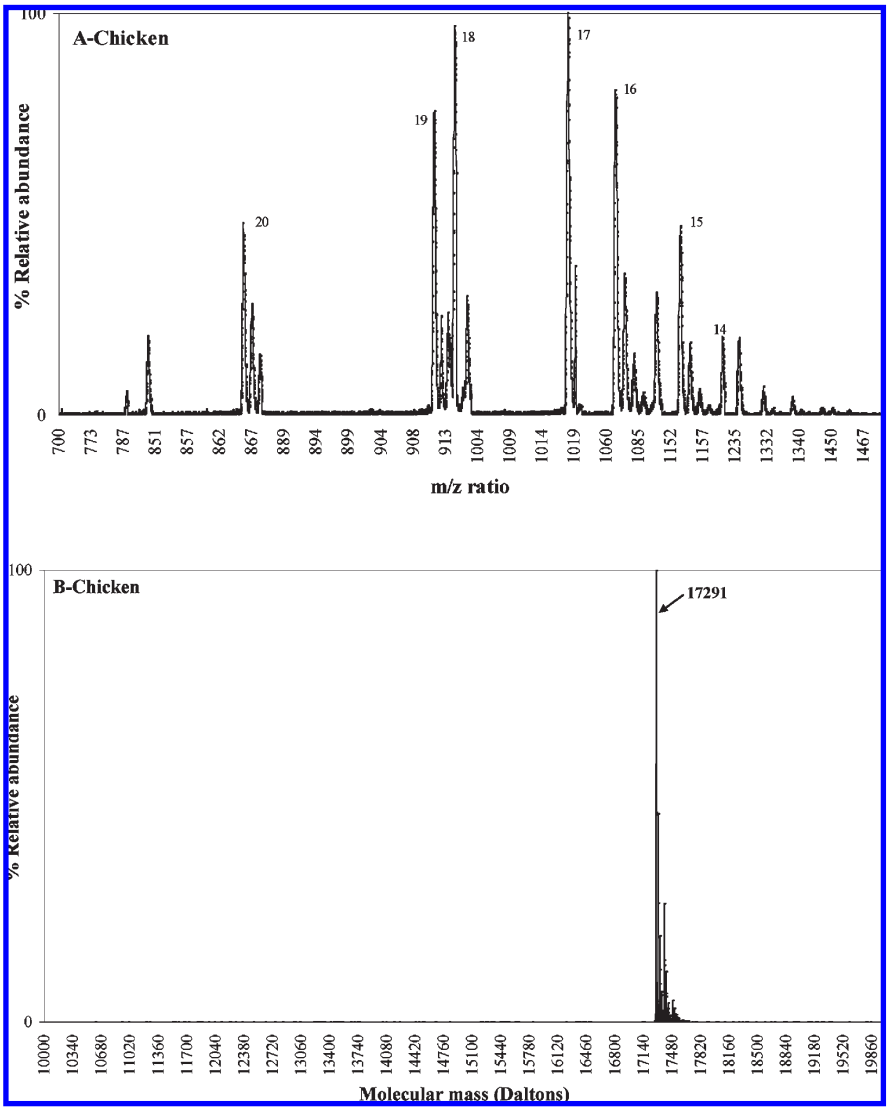

Figure 5. ESI-MS spectra of chicken myoglobin after purification on DEAE-cellulose: (**A**) raw spectra; (**B**) deconvoluted spectra.

Faustman and Phillips (*18*) with suitable modifications was used to isolate Mb from turkey and chicken for mass spectrometry studies.

Mass Spectrometric Characterization. The ESI-MS of turkey (**Figure 4A**) and chicken (**Figure 5A**) Mbs each yielded a series of multiply charged peptide ions typically carrying 10−20 charges with a mass-to-charge ratio (m/z) of 700−1500. Molecular masses were determined by transformation of the ESI-MS raw data into a true molecular mass scale. The deconvoluted spectra showed a molecular mass of 17291 Da for both turkey (**Figure 4B**) and chicken (**Figure 5B**). To check the accuracy of our results, commercially available horse heart Mb and beef heart Mb prepared in our laboratory were also analyzed using the same ESI-MS equipment. Excellent agreement between literature and measured values for horse and beef Mb was obtained (**Table 1**). However, discrepancies were observed for turkey and chicken Mbs when compared to literature values, wherein molecular masses of 18199 and 18026 Da were reported for turkey and chicken Mbs, respectively (*3*, *27*). These latter molecular masses were calculated on the basis of amino acid composition, and they were 735−908 Da greater than our findings. However, the molecular mass observed in our study for turkey Mb is very close to that reported by Joseph et al. (personal communication) (i.e., 17295 Da) using MALDI-TOF-MS.

The most abundant precursor ion in both turkey and chicken showed an m/z of 911. The resultant ESI-MS/MS spectra (**Figure 6**) from both Mb species were compared manually. Because the sequence for the turkey Mb is not known, the fragment ions were not marked into b and y ions. The fragmentation patterns for both turkey and chicken were similar and demonstrated relatively intense peaks with m/z values of 902, 910, 949, 952, and 962. The similarity in fragmentation pattern of chicken and turkey Mb indicated substantial homogeneity between these two species, although this was not confirmed with data-mining algorithms. Different researchers have reported microheterogeneity of Mb in numerous meat animal species.

Table 1. Molecular Masses of Myoglobins from Different Species

species	ESI-MS data (present study)	muscle type	reported mass (Da) (*ref*)	muscle type
turkey	17291	cardiac	18199 (*3*)	smooth
			17295[a]	cardiac
chicken	17291	skeletal	18026 (*27*)	skeletal
			17422 (*17*)	smooth
horse	16951	cardiac	16951 (*4*)	skeletal
beef	16946	cardiac	16946 (*4*)	skeletal
sheep			16923 (*4*)	skeletal
pork			16953 (*4*)	skeletal

[a] Joseph et al., 2009, personal communication.

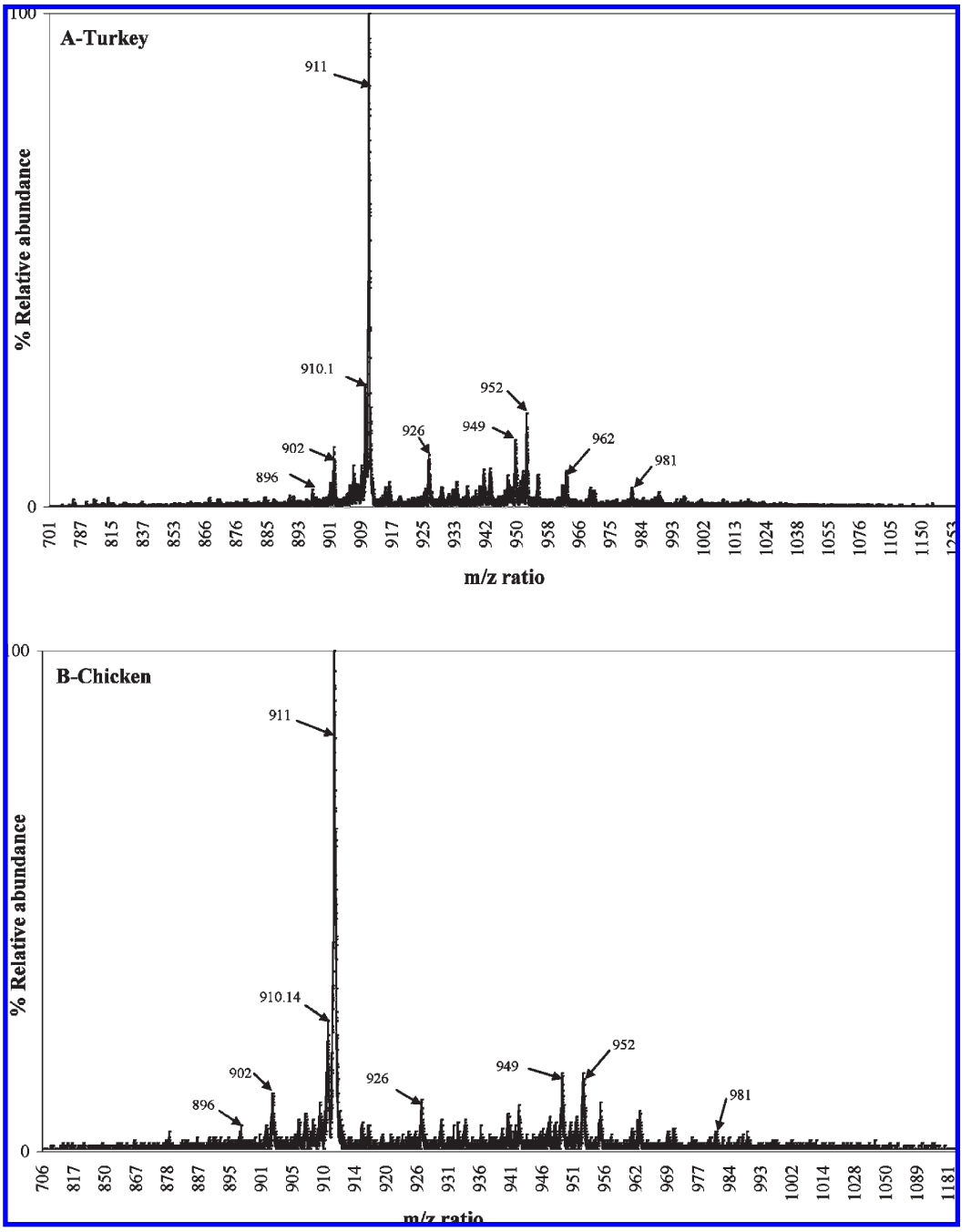

Figure 6. ESI-CID-MS/MS spectra of *m/z* 911 ion from (**A**) turkey and (**B**) chicken myoglobin.

Satterlee and Snyder (*19*) demonstrated the occurrence of microheterogeneity in ovine and porcine Mbs on the basis of their isoelectric point and amino acid composition. Goldbloom and Brown (*27*) demonstrated microheterogeneity in control and dystrophic chicken muscle Mb using disk-gel electrophoresis. Cornish and Froning (*3*) reported that amino acid residues were similar in both turkey and chicken Mbs, except for minor differences in the contents of glutamic acid, methionine, serine, proline, alanine, phenylalanine, and histidine; these avian Mbs were different from their mammalian counterparts (*26*, *29*) relative to amino acid composition as well as molecular mass. Goldbloom and Brown (*27*) suggested that avian Mb ranks between fish and mammalian Mbs on the basis of the number of amino acids in the primary sequence.

Myoglobin Redox Stability. The effects of nonenal and hexenal on turkey and chicken Mb redox stability are presented in **Figures 7** and **8**, respectively. When unsaturated aldehydes were added into the OxyMb solutions, MetMb formation was slightly but consistently greater ($P < 0.05$) than controls at all three temperatures for both turkey and chicken. MetMb accumulation as affected by preincubation with aldehydes followed the order nonenal > hexenal > control ($P < 0.05$), demonstrating that the pro-oxidative behavior of unsaturated aldehydes was, as expected, greater with increased chain length (*5*). The redox instability of OxyMb was likely due to covalent binding of aldehydes to OxyMb (*13*). It has been reported that α,β-unsaturated aldehydes are very reactive toward protein (*5*). Turkey and chicken OxyMbs appeared to be more prone to autoxidation as evidenced by the relatively high MetMb percentages even in controls when compared to equine (*5*), beef (*6*), and pork (*7*) Mbs under similar pH and temperature conditions.

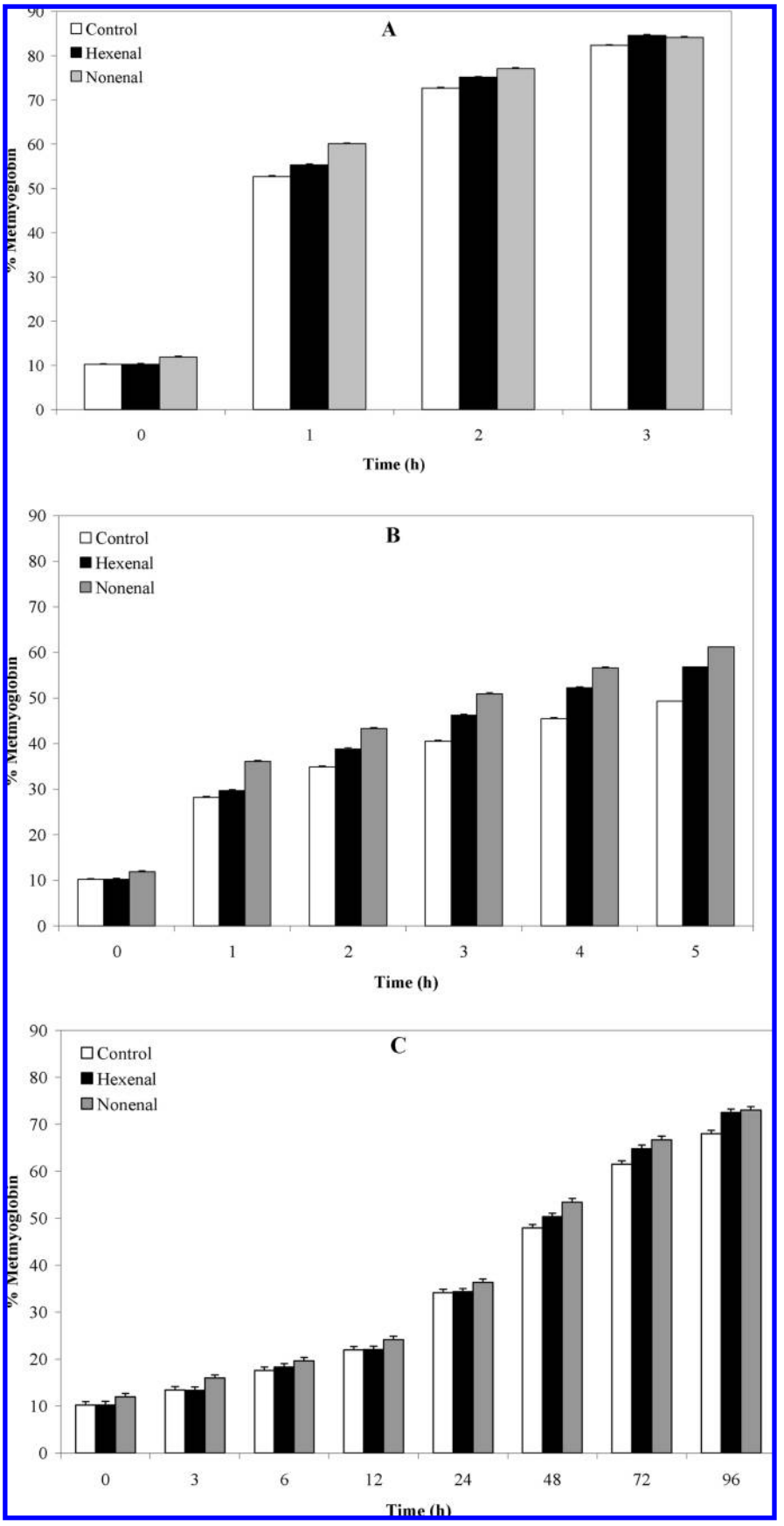

Figure 7. Metmyoglobin formation during the reaction of turkey oxymyoglobin (0.05 mM) with *trans*-2-hexenal and *trans*-2-nonenal (0.35 mM) at pH 5.8 and (**A**) 37 °C, (**B**) 25 °C, and (**C**) 4 °C. Standard error bars are indicated.

Froning (*8*) reported that meat color problems associated with Mb oxidation were more pronounced in turkey than in beef because of its greater rate constant (0.60) for autoxidation when compared to beef (0.22). Both turkey and chicken meats contain

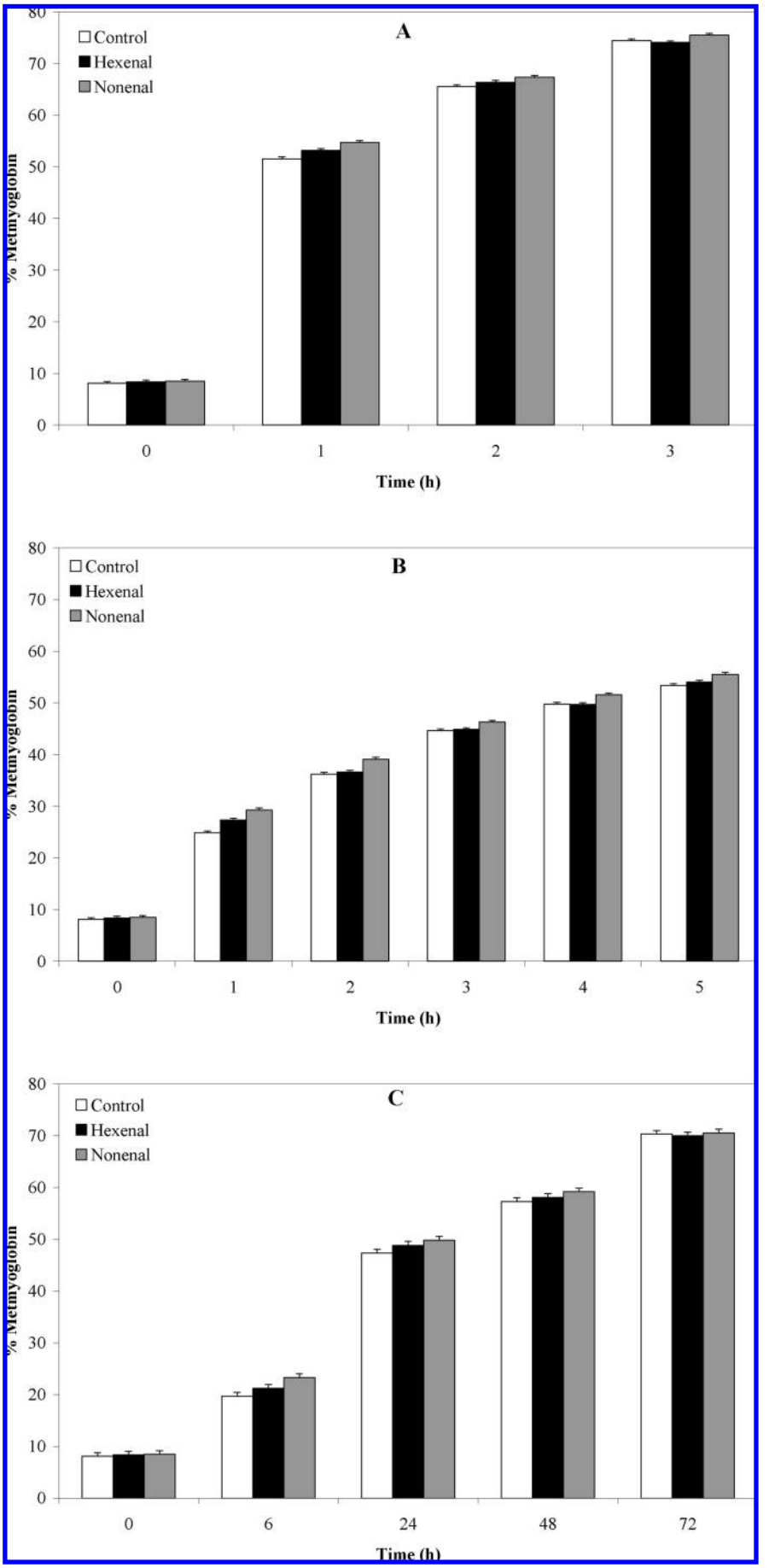

Figure 8. Metmyoglobin formation during the reaction of chicken oxymyoglobin (0.015 mM) with *trans*-2-hexenal and *trans*-2-nonenal (0.1 mM) at pH 5.8 and (**A**) 37 °C, (**B**) 25 °C, and (**C**) 4 °C. Standard error bars are indicated.

greater concentrations of readily oxidizable polyunsaturated fatty acids (PUFA) than beef (*30*). Turkey meat appears to be particularly susceptible to oxidation due to its weak ability to absorb and/or store dietary α-tocopherol in cell membrane phospholipids (*30*). Matsuoka et al. (*17*) reported the greatest autoxidation rate for chicken gizzard OxyMb followed by human, bovine, and sperm whale Mbs in 0.1 M phosphate buffer, pH 7.2, at 25 °C.

Turkey and Chicken Mbs Adduction with 4-Hydroxynonenal. To understand the potential basis for the pro-oxidative effect of the α,β-unsaturated aldehydes, 4-hydroxynonenal was incubated with turkey and chicken OxyMbs for 5 h at 25 °C and pH 5.8. ESI-Q-TOF-MS revealed that 4-hydroxynonenal adducts of turkey and chicken Mbs were present as indicated by small peaks corresponding to 17447 and 17446 Da, respectively. A major peak corresponding to 17290 Da was present and attributed to native (unadducted) Mb. The smaller adduct peaks were 155−156 Da (a mass shift consistent with the molecular mass of 4-hydroxynonenal) greater than the mass of native Mbs and suggested that monoadducts of 4-hydroxynonenal were formed with turkey and chicken Mbs. The appearance of peaks representing monoadducts of 4-hydroxynonenal with Mb is consistent with previous reports in other species (*5*−*7*, *16*), but the relative abundance observed in the current work was low. Adduction of OxyMb with 4-hydroxynonenal would be expected to alter the protein's tertiary structure and predispose it to greater oxidation (*5*). The specific site(s) of 4-hydroxynonenal adduction in turkey and chicken Mbs was (were) not investigated in this study.

ABBREVIATIONS USED

Mb, myoglobin; OxyMb, oxymyoglobin; MetMb, metmyoglobin; Hb, hemoglobin; MS, mass spectrometry; ESI-MS, electrospray ionization mass spectrometry; ESI-Q-TOF, electrospray ionization-triple quadrupole; CID-MS/MS, collision-induced dissociation−tandem mass spectrometry; BCA, bicinchoninic acid; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; DEAE, diethylaminoethyl; CM, carboxymethyl; MALDI-TOF-MS, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry; *m*/*z*, mass-to-charge ratio.

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