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Formation of Methionine Sulfoxide during Glycooxidation and Lipoxidation of Ribonuclease A

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Abstract

Chemical modification of proteins by reactive oxygen species affects protein structure, function and turnover during aging and chronic disease. Some of this damage is direct, for example by oxidation of amino acids in protein by peroxide or other reactive oxygen species, but autooxidation of ambient carbohydrates and lipids amplifies both the oxidative and chemical damage to protein and leads to formation of advanced glycooxidation and lipoxidation end-products (AGE/ALEs). In previous work we have observed the oxidation of methionine during glycooxidation and lipoxidation reactions, and in the present work we set out to determine if methionine sulfoxide (MetSO) in protein was a more sensitive indicator of glycooxidative and lipoxidative damage than AGE/ALEs. We also investigated the sites of methionine oxidation in a model protein, ribonuclease A (RNase), in order to determine whether analysis of the site specificity of methionine oxidation in proteins could be used to indicate the source of the oxidative damage, *i.e.* carbohydrate or lipid. We describe here the development of an LC/MS/MS for quantification of methionine oxidation at specific sites in RNase during glycooxidation or lipoxidation by glucose or arachidonate, respectively. Glycooxidized and lipoxidized RNase were analyzed by tryptic digestion, followed by reversed phase HPLC and mass spectrometric analysis to quantify methionine and methionine sulfoxide containing peptides. We observed that: 1) compared to AGE/ALEs, methionine sulfoxide was a more sensitive biomarker of glycooxidative or lipoxidative damage to proteins; 2) regardless of oxidizable substrate, the relative rate of oxidation of methionine residues in RNase was Met²⁹ > Met³⁰ > Met¹³, with Met⁷⁹ being resistant to oxidation; and 3) arachidonate produced a significantly greater yield of MetSO, compared to glucose. The methods developed here should be useful for assessing a protein's overall exposure to oxidative stress from a variety of sources *in vivo*.

Keywords

glycooxidation; lipoxidation; methionine sulfoxide; oxidation; oxidative stress

Introduction

Chemical modification of proteins by carbohydrates and lipids affects the structure, function and turnover of proteins and contributes to the deterioration of biological systems during aging and disease [1]. Maillard or browning reactions are among the major nonenzymatic pathways

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contributing to protein damage, and autooxidation of sugars and lipids during these reactions enhances the chemical modification of proteins by reactive oxygen species (ROS). In addition to direct oxidative damage by ROS, reactive carbonyl intermediates in glycoxidation and lipoxidation reactions also react with protein, forming advanced glycoxidation and advanced lipoxidation end-products (AGE/ALEs) and producing a wide range of chemical modifications and crosslinking of proteins in diabetes and aging [2]. Hyperlipidemia and dyslipidemia are common features of both metabolic syndrome and diabetes [3,4], however the relative importance of carbohydrate versus lipid as a source of chemical damage to proteins *in vivo* is controversial [2]. Indeed, many of the most prominent chemical modifications, such as N^ε-(carboxymethyl)lysine, N^ε-(carboxyethyl)lysine and methylglyoxal hydroimidazolone derivatives of arginine [5] may be formed from both carbohydrates and lipids [2].

In previous work we have observed the rapid oxidation of methionine during glycoxidation and lipoxidation reactions, and in the present work we set out to determine whether the methionine sulfoxide (MetSO) content of a protein would be useful as a more general and sensitive indicator, compared to AGE/ALEs, of overall exposure of a protein to oxidative, glycoxidative and lipoxidative damage. We also investigated the sites of methionine oxidation in protein to determine whether analysis of the site specificity of methionine oxidation would provide an indication of the source of the oxidative damage, *i.e.* carbohydrate or lipid. We describe here the development of an LC/MS/MS for quantification of methionine oxidation at specific sites in the model protein ribonuclease (RNase) during glycoxidation or lipoxidation by glucose or arachidonate, respectively. Control, glycoxidized and lipoxidized RNase were digested with trypsin and analyzed by reversed phase HPLC, electrospray ionization triple quadrupole mass spectrometry (ESI⁺-LC/MS/MS). As shown below, compared to AGE/ALEs, methionine sulfoxide was a more sensitive biomarker of glycoxidative and lipoxidative damage to protein, but, regardless of oxidizable substrate, the relative rate of oxidation of methionine residues in RNase was Met²⁹ > Met³⁰ > Met¹³, with Met79 being resistant to oxidation. In addition, at similar concentrations of glucose and arachidonate, the yield of MetSO was much greater from arachidonate, emphasizing the importance of lipids, compared to carbohydrates, as a source of oxidative damage to proteins *in vivo*. The methods developed in this study should find general applicability for assessing overall exposure of specific proteins to oxidative stress in aging and disease.

Materials and Methods

Materials

Arachidonic acid, chloramine-T, cyanogen bromide (CNBr), diethylenetriamine-pentaacetic acid (DTPA), dithiothreitol (DTT), D-(+)-glucose (ACS grade), 3-(*N*-morpholino)-propanesulfonic acid (MOPS), ribonuclease A (RNase type II-A), trypsin (sequencing grade), urea (Ultra Grade), and 4-vinylpyridine were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Acetonitrile (HPLC grade), methanol (ACS Grade), trifluoroacetic acid (TFA; biochemical grade), and *N,O*-trifluoroacetic anhydride were purchased from Acros Chemicals (Atlanta, GA)

Glycoxidation or Lipoxidation of RNase

RNase (13.7 mg/ml; 1 mM) was incubated with glucose or arachidonic acid in phosphate buffer (200 mM, pH 7.4), as previously described [6,7]. Briefly, the reactions were prepared under sterile conditions and incubated under air in 20 ml glass scintillation vials in a shaking water bath at 37°C. The arachidonate reaction mixture was initially biphasic, but became monophasic between 3 and 5 days of incubation. Sequential aliquots were taken under sterile conditions at various times from the aqueous phase, opening of the vials to allow re-entry of air. Samples

were frozen at -20°C , and RNase was recovered by dialysis against deionised H_2O containing DTPA (1 mM), followed by lyophilization.

Tryptic Digestion

Tryptic digestion of RNase was performed as described previously [8]. Briefly, RNase (1 mg) was suspended in 75 μl of MOPS buffer (100 mM, pH 7.2) containing urea (6 M) and EDTA (1 mM). DTT (6.3 mM) was added followed by incubation at 37°C for 3 hrs. 4-Vinylpyridine (30 mM) was added followed by incubation, in the dark, at room temperature for 1 hr. A two-fold excess of DTT (30 mM) was added to quench the reaction. In preliminary studies, we established that DTT did not reduce MetSO to Met under these conditions. Water was added to dilute the urea to 0.6 M, then trypsin (4.5:100 w/w) was added to the protein followed by incubation at 37°C for 5 hr. Digestions were terminated by freezing at -20°C . Tryptic peptides were characterized and quantified as described below.

Liquid Chromatography-Mass Spectrometry (LC-MS) and LC/MS/MS

Electrospray Ionization liquid chromatography mass spectrometry (ESI^+ -LC/MS) and ESI^+ -LC/MS/MS were performed in the positive ion mode on a Micromass (Manchester, UK) triple quadrupole (Quattro) mass spectrometer, or a Micromass quadrupole time-of-flight (QTOF) mass spectrometer equipped with an Agilent (Palo Alto, CA) series 1100 HPLC system. Chromatography was conducted on an ES Industries (West Berlin, NJ) AquaSep C₁₈ column (250 x 2 mm), using a linear gradient from 0.1% aqueous TFA to 50% acetonitrile over 50 min at a flow rate of 0.2 ml/min. Quantification of peptides was performed with the Quattro set in full scan mode (200 – 1800 amu), and masses of interest were extracted using MassLynx (Micromass) software. Fractional modification of Met-containing peptides was calculated as described previously [8]. Briefly, relative amounts of unmodified peptides were determined by summing the peak areas of all charged forms of the peptide of interest and dividing by the sum of the peak areas of the charged forms of an internal reference peptide in RNase, $\text{H}^{105}\text{-V}^{124}$. Percent oxidation of Met in peptides was determined as the sum of the peak areas of the charged forms of the MetSO peptides, divided by the sum of the peak areas of the non-oxidized (Met) and oxidized (MetSO) peptides. Sequencing of peptides was performed on the QTOF, with the quadrupole set on mass of the peptide of interest and the TOF scanning for daughter ions between 50–3000 amu, using collision energies of 30–40 eV.

Amino Acid Analysis

Quantification of MetSO by amino acid analysis was performed as described previously [9], based on the procedure of Shechter et al. [10]. Briefly, Met residues were converted to homoserine lactone by reaction with cyanogen bromide (CNBr); this procedure was repeated three times to achieve complete oxidation of Met. MetSO, which is resistant to action of CNBr, is reduced to Met during hydrolysis in 6 N HCl and measured as Met by amino acid analysis. Amino acid analysis was performed using a Shimadzu (Columbia, MD) HPLC system, using an LC-10Ai pump equipped with a SIL-10Ai autoinjector, SCL 10A VP injector controller, and SSI Model 505 column oven maintained at 55°C . Samples were fractionated on a Pickering (Pickering Labs, Mountain View, CA) sulfonated divinylbenzene cation-exchange column (250 mm x 3 mm) using Pickering Labs sodium amino acid analysis buffers (buffer A = catalogue Na328; buffer B = catalogue Na740; buffer C = catalogue RG011) at a flow rate of 0.35 ml/min. The gradient was as follows: 0–15 min, 100% A; 15–39 min, linear ramp to 100% B; 39–60 min, hold at 100% B; 60–60.1 min, linear ramp to 100% C; 60.1–62.5 min, hold at 100% C; 62.5–62.6 min, return to 100% A; 62.6–83 min hold at 100% A. Amino Acids were derivatized post-column using *o*-phthaldialdehyde (OPA) and were detected by fluorescence at $\text{Ex} = 375 \text{ nm}$; $\text{Em} = 425 \text{ nm}$.

Results

Characterization of MetSO containing peptides of RNase

The 12 tryptic peptides of RNase are listed in Table 1. Of the four Met residues in the protein, three are located on peptide Q¹¹-M^{13,29,30}-K³¹, while the fourth is located on peptide N⁶⁷-M⁷⁹-R⁸⁵. Figs. 1A and 1B are representative total ion chromatograms (TIC) of tryptic peptides from samples of native and glycosylated RNase, respectively. Figs. 1C and 1D are extracted ion chromatograms (XIC) for the glycosylated sample, showing the +2 charge states of the non-oxidized peptide Q¹¹-M^{13,29,30}-K³¹ (1208 *m/z*) and the oxidized forms of peptide Q¹¹-MetSO^{13,29,30}-K³¹ (1216 *m/z*), respectively. Although a single peak was observed for the native peptide (Fig. 1C), multiple peaks were detected for the oxidized peptide (Fig. 1D). The inset to Fig. 1D is an expanded XIC, showing that there are three well-resolved peaks for the oxidized Q¹¹-MetSO^{13,29,30}-K³¹ peptide, indicating chromatographic resolution of the three MetSO-containing peptides. These peptides, with a molecular mass 16 Da higher than that of the native peptide (8 mass units increase for the +2 charge state), eluted earlier than the native peptide, consistent with oxidation of the methionine residues to a more polar MetSO residue. There was no indication of double oxidation of the Q¹¹-MetSO^{13,29,30}-K³¹, *i.e.* no peptide with a molecular mass of 32 Da higher than the native peptide. The oxidized peptide N⁶⁷-MetSO⁷⁹-R⁷⁹ was not detected in any of the oxidation experiments. This result is consistent with crystallographic data indicating that Met⁷⁹ is buried deeply inside the protein [11]. In contrast, all of the methionines were fully oxidized on exposure of denatured and DTT-reduced protein to chloramine T (data not shown).

To identify which sites of oxidation corresponded with each of the three peaks in Fig. 1, the +2 ions were sequenced by ESI⁺-LC-MS/MS using the QTOF mass spectrometer. To optimize conditions for fragmentation, the native Q¹¹-M^{13,29,30}-K³¹ peak was sequenced first. Fig. 2 shows the entire sequencing spectrum for the native Q¹¹-M^{13,29,30}-K³¹ peak, with all but one *y*-ion (*y*₈) identified, thus confirming the peak assignment. The three peaks corresponding to the oxidized Q¹¹-MetSO^{13,29,30}-K³¹ peptides were also sequenced and the spectra confirmed the identity of these peaks as a Q¹¹-MetSO^{13,29,30}-K³¹ peptide. To identify which peak corresponded to which MetSO residue, the spectra were expanded between 250 and 450 *amu*. This region contains the *y*₂, *y*₃, *b*₂ and *b*₃ ions, which will have mass shifts of 16 *amu*, corresponding to the addition of one oxygen atom at Met¹³, Met²⁹ or Met³⁰. Figs. 3A–D show the expanded sequencing spectra for the native non-oxidized Q¹¹-M^{13,29,30}-K³¹ peptide, as well as the first, second and third Q¹¹-MetSO^{13,29,30}-K³¹ peaks shown in Fig. 1D. The sequencing spectrum of the native peptide at 21.0 min (Fig. 3A) shows *y*₂ and *y*₃ ions at 409 and 278 Da, respectively. The oxidized peptide at 18.9 min (Fig. 3B) shows the *y*₃ ion shifted to a mass of 425 *amu*, but without a change in the *y*₂ ion, indicating oxidation at Met²⁹; this peptide is identified as Q¹¹-MetSO²⁹-K³¹. The sequencing spectrum for the peptide at 19.1 min, (Fig. 3C) shows a shift in mass of both the *y*₃ and *y*₂ ions, to 425 and 294 *amu*, respectively. The shift of 16 *amu* for both ions, indicates oxidation at Met³⁰, so that this peptide is identified as Q¹¹-MetSO³⁰-K³¹. The sequencing spectrum for the peptide at 20.4 min, (Fig. 3D) shows *y*₃ and *y*₂ ions with masses of 409 and 278 *amu*, respectively, which are the same as those seen for the non-oxidized peptide, indicating no oxidation of either Met²⁹ or Met³⁰. However, the *b*₃ ion and the *b*₃-NH₃ ions have masses of 413 and 396 Da, compared to 397 and 380 Da in the non-oxidized peptide, indicating that this peptide is Q¹¹-MetSO¹³-K³¹.

Kinetics of MetSO formation on RNase during glycooxidation and lipoxidation reactions

RNase was incubated with glucose or arachidonate to determine the kinetics and extent of MetSO formation on RNase during glycooxidation and lipoxidation reactions. Both incubations were originally set up at 100 mM substrate (glucose or arachidonate) concentration, representing a 10:1 molar ratio of oxidizable substrate to lysine residues in RNase (10 mol Lys/

mol RNase). However, at 100 mM arachidonate, there was significant loss of native peptides that could not be accounted for by an increase in MetSO peptide, indicating that modification of lysine and/or arginine residues was inhibiting proteolysis. Therefore, the arachidonate concentration was decreased to 50 mM concentration. Fig. 4 shows the loss of Q¹¹-K³¹, compared to the reference peptide H¹⁰⁵-V¹²⁴, during exposure of the protein to glucose or arachidonate. There was a 20% loss of the native Q¹¹-K³¹ by day 3 during the glycooxidation reaction, compared to 80% loss during the lipoxidation reaction, consistent with more rapid oxidation of arachidonate, compared to glucose.

Figs. 5A and 5B show the kinetics of oxidation of Met¹³, Met²⁹ and Met³⁰ during incubation with glucose or arachidonate. The relative increase in MetSO-containing peptides in the two incubations (note differences in vertical scales) was consistent with the relative loss of non-oxidized Q¹¹-K³¹, shown in Fig. 4. For both glucose and arachidonate, the relative rate of oxidation of the Met residues was Met²⁹ > Met³⁰ > Met¹³.

Fig. 6 shows the kinetics of formation of total oxidized peptides Q¹¹-Met^{13,29,30}-K³¹, with values reaching 10 and 80% Met oxidation during incubations with glucose and arachidonate, respectively, consistent with data in Fig. 4. To validate the results of the LC/MS analyses, the MetSO content of modified RNase was measured independently by amino acid analysis. Fig. 7 shows the formation of approximately 0, 0.5, and 3 mol of MetSO per mol of RNase after 7 days incubation in buffer, glucose or arachidonate, respectively. These data agree well with the LC/MS data during glycooxidation by glucose and lipoxidation by arachidonate; 10 and 80 % MetSO formation is equivalent to 0.4 and 3.2 mol MetSO/mol RNase, respectively.

Discussion

Specificity of Met oxidation

In this study we have used ESI⁺-LC/MS analysis to quantify oxidation of specific methionine residues in the model protein, RNase. The method appears robust since MetSO residues are not reduced to Met during work-up with DTT or during alkylation by vinylpyridine; similar results were obtained with iodoacetamide (data not shown). Using this assay (Fig. 5), there was a time-dependent increase in Met oxidation in RNase exposed to oxidant stress, which was confirmed by chemical analysis (Fig. 7). In addition, MetSO formation was site-specific (Fig. 5), with Met²⁹ being most sensitive to oxidation, followed by Met³⁰ ≈ Met¹³, while Met⁷⁹ was completely resistant to oxidation. Met²⁹ and Met³⁰, are near the active site of the enzyme, consistent with the proposal of Levine and Stadtman that methionine residues may serve as sacrificial antioxidants surrounding functionally important regions of proteins [12, 13]. The active site of RNase contains two residues that are especially sensitive to oxidation, His¹² and His¹¹⁹. Oxidation of either of these histidine residues completely inhibits the enzymatic activity of RNase [14]. Further studies, using site-directed mutagenesis to replace the methionine residues, should yield insight into the protective function of Met²⁹ and Met³⁰ in shielding the active site of RNase.

Specificity of Met Oxidation during glycooxidation vs. lipoxidation reactions

There is considerable debate about the role of carbohydrate vs. lipid in oxidative damage to protein *in vivo*, especially in type 2 diabetes [15,16], which is characterized by both hyperglycemia and hyperlipidemia and/or dyslipidemia. For this reason, we asked whether the oxidation of Met residues in RNase would vary with the substrate undergoing autoxidation. If this were the case, it might be possible to determine whether lipids or carbohydrates were the primary source of oxidative damage to proteins in biological systems. However, we observed the same relative specificity of oxidation of Met residues in RNase by both glucose and arachidonic acid, *i.e.* Met²⁹ > Met³⁰ > Met¹³ (Fig. 5). The observation that the same Met

residues are oxidized in a similar order during glycoxidation by glucose and lipoxidation by arachidonate suggests that the site specificity of Met oxidation in RNase is not affected by the primary oxidant, but rather by the surface exposure of the Met residue and/or access by common small-molecule products of glycoxidation and lipoxidation reactions, such as peroxide and superoxide. Now that methods have been developed with RNase, it should be possible to address this issue in greater detail with proteins that have fatty acid binding sites, such as plasma albumin or apolipoprotein-A1 in high-density lipoprotein, in order to probe for differential oxidation of Met residues by different sources of oxidative stress.

Relative Rates of Glycoxidative and Lipoxidative Damage

Despite similarities in the sites of Met oxidation during glycoxidation and lipoxidation reactions, there were significant differences in the rates of Met oxidation during these two oxidative reactions. The extent of Met oxidation during lipoxidation reactions was 5–10 times greater than that during glycoxidation reactions (Figs. 6 & 7), despite the 2-fold higher concentration of glucose, compared to arachidonate. These data indicate that lipoxidative stress (by arachidonate) is more severe than glycoxidative stress (by glucose), undoubtedly because of the greater ease of oxidation of polyunsaturated fatty acids, compared to glucose. In other work, we reported that arachidonate was the most rapidly oxidized fatty acid in plasma [17], suggesting that it may be an important source of oxidative damage and MetSO formation *in vivo* - not only in plasma, but also in cellular membranes.

The yield of MetSO formed during glycoxidation and lipoxidation of RNase (~ 0.5 and 3 mol/mol protein, respectively) was significantly greater than that of AGE/ALEs (< 0.01 mol *N*^ε-(carboxymethyl)lysine (CML) or *N*^ε-(carboxyethyl)lysine (CEL) /mol RNase [6,7], indicating that MetSO is a sensitive and general indicator of oxidative stress during both glycoxidation and lipoxidation reactions. These data are consistent with earlier work from our laboratory in which we reported a strong correlation between levels of MetSO and CML in human skin collagen, both *in vivo* and in glycoxidation reactions *in vitro*. In these studies of both collagen from biopsies and collagen glycoxidized *in vitro*, we observed an approximately 100-fold higher concentration of MetSO, compared to CML (9). In recent work, Thornalley *et al.* [5] also reported that the level of MetSO in plasma proteins was over 30-fold higher than that of CML. Even in red cells and mononuclear leukocytes – cells which have active MetSO reductase pathways – the level of MetSO in proteins was ~25-fold higher than CML. While the correlations between AGE/ALEs and MetSO were not reported in this study, the data suggest that measurement of MetSO in proteins would be as useful as, and more sensitive than, measurement of AGE/ALEs for assessing the status of oxidative stress and chemical damage to proteins in aging and disease.

In summary, we describe a reliable method for measuring the kinetics and specificity of oxidation of methionine residues in RNase. Using this assay, we find that similar Met residues in RNase are oxidized during both glycoxidation and lipoxidation reactions, that oxidative damage is produced more rapidly and to a greater extent in lipoxidation vs. glycoxidation reactions, and that the MetSO content of RNase is an excellent overall integrator of oxidative, glycoxidative and lipoxidative damage to the protein. The methods developed here should be useful in studies of other proteins and for integrating an overall exposure of protein to oxidative stress *in vivo*.

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Abbreviations

AGE

advanced glycoxidation end-product

ALE

advanced lipoxidation end-product

DTT

dithiothreitol

ESI⁺-LC/MS

electrospray, positive ion liquid chromatography/mass spectrometry

Met

methionine

MetSO

methionine sulfoxide

RNase

bovine pancreatic ribonuclease A

TIC

total ion current

XIC

extracted ion current

Fig. 1.

Typical ESI⁺-LC/MS chromatogram of the native and oxidized peptide Q^{11} - K^{31} from 10-day glycated RNase. Total ion chromatogram of A) native and B) glycated RNase tryptic peptides. C) Extracted ion chromatogram of the +2 (1208 m/z) ion of the native Q^{11} - K^{31} peptide. D) Extracted ion chromatogram of the +2 (1216 m/z) ions for the Q^{11} -MetSO^{13,29,30}- K^{31} peptides. *Inset:* Expanded chromatogram (18–21 min) of oxidized Q^{11} -MetSO^{13,29,30}- K^{31} peptides. Assignment of the site of MetSO formation is based on results of peptide sequencing (see text).

Fig. 2.

Representative sequencing spectrum of the doubly charged Q^{11} - K^{31} peptide (806 m/z) from 7-day glycated RNase. All y-ions were detected except y_8 ; b-ions are under-represented, and are not labeled in order to simplify the spectrum. The amino acid sequence of peptide Q^{11} - K^{31} , with theoretical masses of b and y ions is shown below the mass spectrum. $[M+2H]^+$ = doubly charged parent ion of peptide Q^{11} - K^{31} .

Fig. 3.

Expanded sequencing spectra (250–450 m/z) of the doubly charged ions from native and glycosylated RNase. A) native (1208 m/z) Q^{11} - K^{31} peptide eluting at 21.0 min, B) oxidized (1216 m/z) Q^{11} -MetSO²⁹- K^{31} peptide eluting at 18.9 min, C) oxidized Q^{11} -MetSO³⁰- K^{31} peptide eluting at 19.1 min, and D) oxidized Q^{11} -MetSO¹³- K^{31} peptide eluting at 20.4 min. Identities of oxidized Q^{11} - K^{31} peptides were based on the shifts of the y and b-ions resulting from the addition of oxygen (16 m/z) (see text).

Fig. 4.

Kinetics of loss of RNase tryptic peptide Q¹¹-K³¹ during incubation with glucose or arachidonate. RNase was incubated with glucose or arachidonate, dialyzed, digested with trypsin and analyzed by LC/MS, as described in *Materials and Methods*. There was an 18% and 80% loss of Q¹¹-K³¹ during incubation with glucose and arachidonate, respectively. Data are expressed as RA, relative amounts compared to peptide H¹⁰⁵-V¹²⁴ (see *Materials and Methods*). Data points are means $\pm$ range for duplicate incubations.

Fig. 5. Kinetics of Met oxidation on peptide Q¹¹-K³¹ during glycooxidation and lipoxidation reactions. RNase was incubated with A) glucose or B) arachidonate and analyzed as described above.

Fig. 6. Percent Met oxidation on peptide Q¹¹-K³¹ during lipoxidation and glycooxidation reactions. Oxidation of methionine residues on RNase was ~10 times greater during lipoxidation by arachidonate versus glycooxidation by glucose. Data points are means $\pm$ range for duplicate incubations.

Fig. 7. Kinetics of MetSO oxidation by amino acid analysis. RNase incubated with glucose or arachidonate, or in buffer only (control) was treated with CNBr, followed by acid hydrolysis; amino acids were quantified by cation exchange chromatography. Data are from a single, representative experiment.

Table 1

RNase tryptic peptides

Peptide
K ¹ ETAAAK ⁷
F ⁸ ER ¹⁰
Q ¹¹ HM ¹³ DSSTSAASSSNYCNQM ²⁹ M ³⁰ K ³¹
S ³² R ³³
N ³⁴ LTK ³⁷
D ³⁸ R ³⁹
C ⁴⁰ K ⁴¹ PVNTFVHESLADVQAVCSQK ⁶¹
N ⁶² VACK ⁶⁶
N ⁶⁷ GQTNCYQSYSTM ⁷⁹ SITDCR ⁸⁵
E ⁸⁶ TGSSK ⁹¹
Y ⁹² PNCAYK ⁹⁸
T ⁹⁹ TQANK ¹⁰⁴
H ¹⁰⁵ IIVACEWGNPYVPVHFDASV ¹²⁴