51st International Congress of Meat Science and Technology
August 7-12, 2005 – Baltimore, Maryland USA

COMPARISON OF HYDROXYNONENAL-INDUCED REDOX INSTABILITY OF PORCINE AND BOVINE MYOGLOBINS

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Key Words: Myoglobin; Lipid oxidation; HNE

Introduction

Lipid oxidation generates a variety of secondary oxidation products, for example – aldehydes, which attack other biological molecules in the cellular systems. These secondary products are responsible for off-flavors and off-odors associated with rancidity in muscle foods. The aldehyde products, when compared to free radicals, are more stable and readily diffuse into the cellular environment where they can exert toxicological effects (Esterbauer et al., 1991). Several aldehydes are formed as a result of meat lipid oxidation, and these can enhance oxidation of oxymyoglobin (OxyMb) (Chan et al., 1997). 4-Hydroxynonenal (HNE), an α, β-unsaturated aldehyde formed by oxidation of ω-6 unsaturated fatty acids (Schneider et al., 2001), is reactive towards proteins (Sakai et al., 1995) and has substantial toxicological and biological activity (Esterbauer et al., 1991). HNE has been detected in meat and was suggested to be used as a reliable marker to assess the quality of muscle foods containing higher amount of polyunsaturated fatty acids (Sakai et al., 1995). The concentration of HNE in beef and pork was reported to be approximately 0.15 mM (Sakai et al., 1995). HNE accelerated equine OxyMb oxidation in vitro, and covalent modification of equine Mb by HNE was demonstrated by Faustman et al. (1999).

Lipid oxidation and subsequent discoloration of meat can be minimized by use of antioxidants in animal feeds. Specifically, vitamin E (α-tocopherol) protects highly oxidizable polyunsaturated fatty acids from peroxidation by reactive oxygen species and free radicals (Burtiss & Diplock, 1988). Supranutritional supplementation of α-tocopherol in the finishing diet of cattle increased color and lipid stability of beef obtained from these animals (Faustman et al., 1989). However, in vitamin E supplemented pork even though lipid oxidation was reduced significantly, a color stabilizing effect was not readily observable (Lanari et al., 1995; Cannon et al., 1996; Jensen et al., 1997; Houben et al., 1998). Interestingly, porcine muscle generally has a greater proportion of unsaturated fatty acids than beef and it would be reasonable to hypothesize that pork lipids would undergo oxidation more readily and produce more secondary products than beef. In turn, this would be expected to result in decreased OxyMb (color) stability in pork, which would be prevented by α-tocopherol. However, findings that pork did not demonstrate improved Mb redox stability from α-tocopherol supplementation led us to consider the

possibility that pork Mb differs from beef Mb relative to its susceptibility to lipid oxidation.

Objectives

The present study was carried out to compare HNE-induced redox instability in porcine and bovine Mb at storage temperatures and pH values typical of meat.

Methodology

Oxymyoglobin Preparation: Myoglobin was isolated from fresh cardiac muscle obtained from a local slaughterhouse, and OxyMb was prepared as outlined by Faustman and Phillips (2001). Briefly, a tissue-free supernatant was obtained from homogenized cardiac muscle, filtered through cheesecloth and subjected to ammonium sulfate fractionation. Following dialysis to remove ammonium sulfate, gel permeation chromatography was used to further purify Mb. To ensure 100% starting OxyMb the protein was treated by dithionite-mediated reduction and residual dithionite was removed by dialysis (3 x 40 volumes) against 50 mM sodium citrate (pH 5.6) buffer.

Reaction with HNE: OxyMb was incubated with HNE (Porcine OxyMb 0.075 mM + HNE 0.5 mM; Bovine OxyMb 0.15 mM + HNE 1.0 mM) at 4°C and pH 5.6. For each species, the concentrations of OxyMb were adjusted to simulate those in beef and pork muscles. However the molar ratio of Mb: HNE was maintained at 1:7 for both species. Controls consisted of OxyMb plus a volume of ethanol equivalent to that used to deliver the aldehyde to treatment mixtures. Samples (0.5 mL) were removed from the reaction assays at 0, 24 and 48 h; passed through a PD-10 desalting column to remove unreacted HNE and frozen at −80 °C for subsequent analysis.

Matrix Assisted Laser Desorption Ionization–Time Of Flight Mass Spectrometry (MALDI-TOF MS) of Mb: HNE adducts: Native and HNE treated myoglobins were analyzed by MALDI-TOF MS to detect changes in the total mass resulting from HNE adduction. Briefly, 1μL sample was mixed with 1μL freshly prepared 1% solution of sinapinic acid in acetonitrile: 0.1% aqueous trifluoroacetic acid (60:40 vol / vol); 0.5μL mixture was spotted on the MALDI target plate, spread uniformly and allowed to dry. The spots were treated with 1μL 0.1% aqueous trifluoroacetic acid to remove buffer salts, prior to MALDI-TOF MS analysis. Protein molecular ions were analyzed in a linear, positive ion mode using a Voyager-DE STR MALDI-TOF mass spectrometer (Applied Biosystems, Foster City, CA) set at an acceleration voltage of 25 KV. Each spot was analyzed a minimum of 3 times, accumulating spectra composed of 250 laser shots per analysis. The resulting spectra were analyzed by Data Explorer (Applied Biosystems, Foster City, CA), noise-smoothed, baseline-corrected and mass-calibrated using an external standard of equine apomyoglobin.

Results & Discussion

MALDI-TOF analysis of the HNE adducted myoglobins revealed that prior to incubation the primary peaks present were equivalent to the mass of porcine Mb (16954 Da) and bovine Mb (16940). However, following 48 h incubation with HNE, a relatively small peak of mass 17112 appeared in addition to the native porcine Mb (Fig. 1). This corresponded to a mono-adduct of HNE with porcine Mb and was 158 Daltons greater than the mass of porcine Mb, a mass shift equivalent to the molecular weight of HNE. In bovine samples, a mono-adduct peak (17097) was prominent after 24 h incubation and a di-adduct peak (17254) apparent at 24 h, was prominent by 48 h incubation. The unadducted Mb, mono-adduct and di-adduct were separated by a mass shift of 157 Daltons and these results indicated that HNE adducts were formed by Michael addition (Fig. 2). If the adduct had resulted from Schiff base formation, a molecular mass addition of 140 Da would have been expected as one mole of water is lost per mole of HNE: Mb in the reaction. After 48 h incubation with HNE, porcine Mb formed only mono-adducts and the abundance of mono-adduct was considerably less than unadducted Mb (Fig. 1). Whereas in bovine Mb, mono- and di-adducts were present after 48 h incubation with HNE, the intensity of the mono-adduct peak was similar to that of unadducted Mb indicating its similar relative abundance (Fig. 2). These results are similar to those observed by Phillips et al. (2001) who identified the formation of up to three covalently bound HNE molecules per bovine Mb molecule following incubation at pH 7.4 and 37°C.

Conclusions

The results of the present study suggested that bovine Mb is more susceptible to nucleophilic attack by HNE and subsequent adduction than porcine Mb at typical meat storage conditions. This could explain the relatively greater apparent stability of pork color in the presence of lipid oxidation when compared to beef. Work is underway to further characterize the specific sites of adduction on porcine myoglobin.

Figures

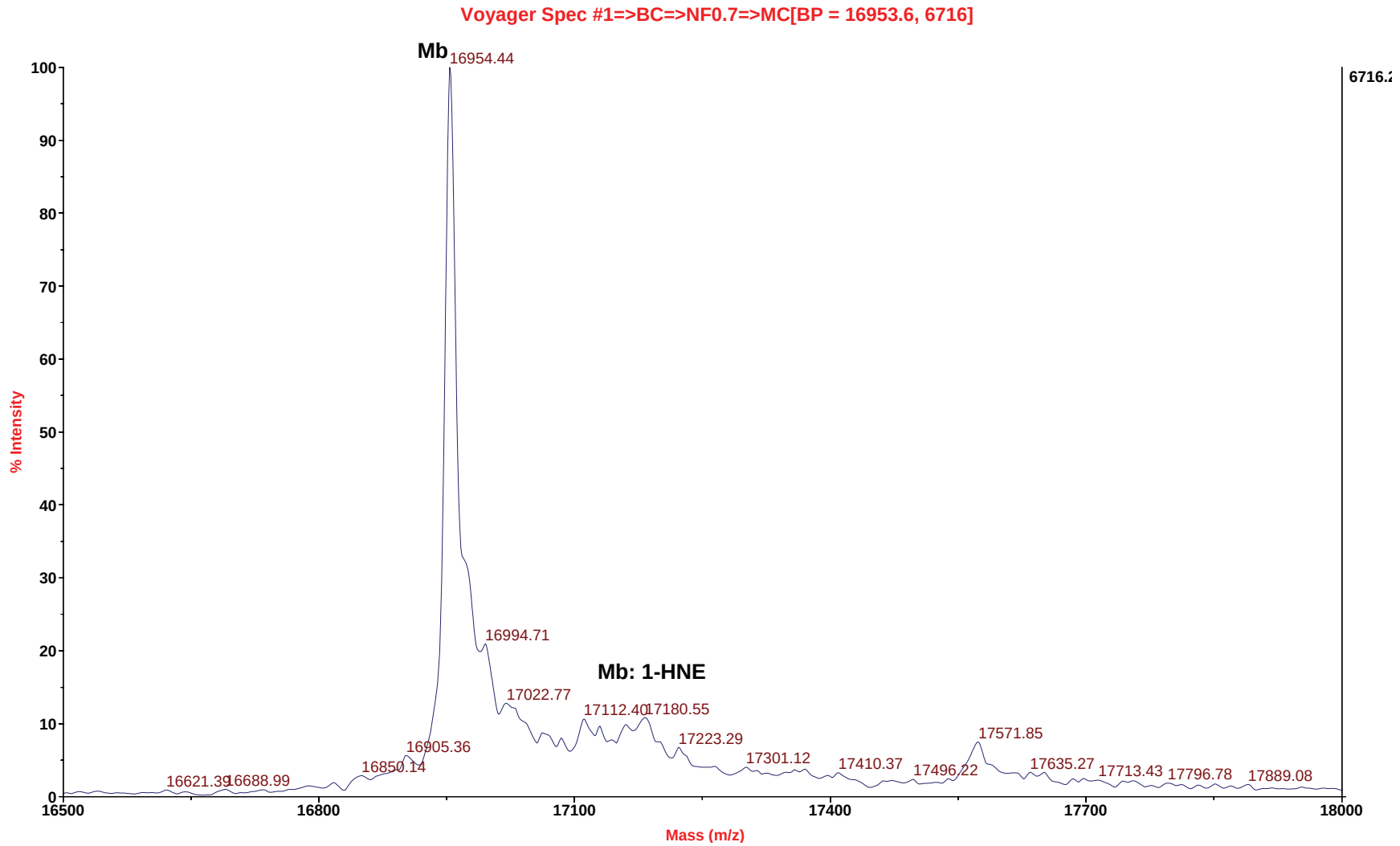

Fig. 1. MALDI-TOF MS spectra of porcine oxymyoglobin (0.075 mM) following reaction with HNE (0.5 mM) at pH 5.6 and 4°C for 48 h.

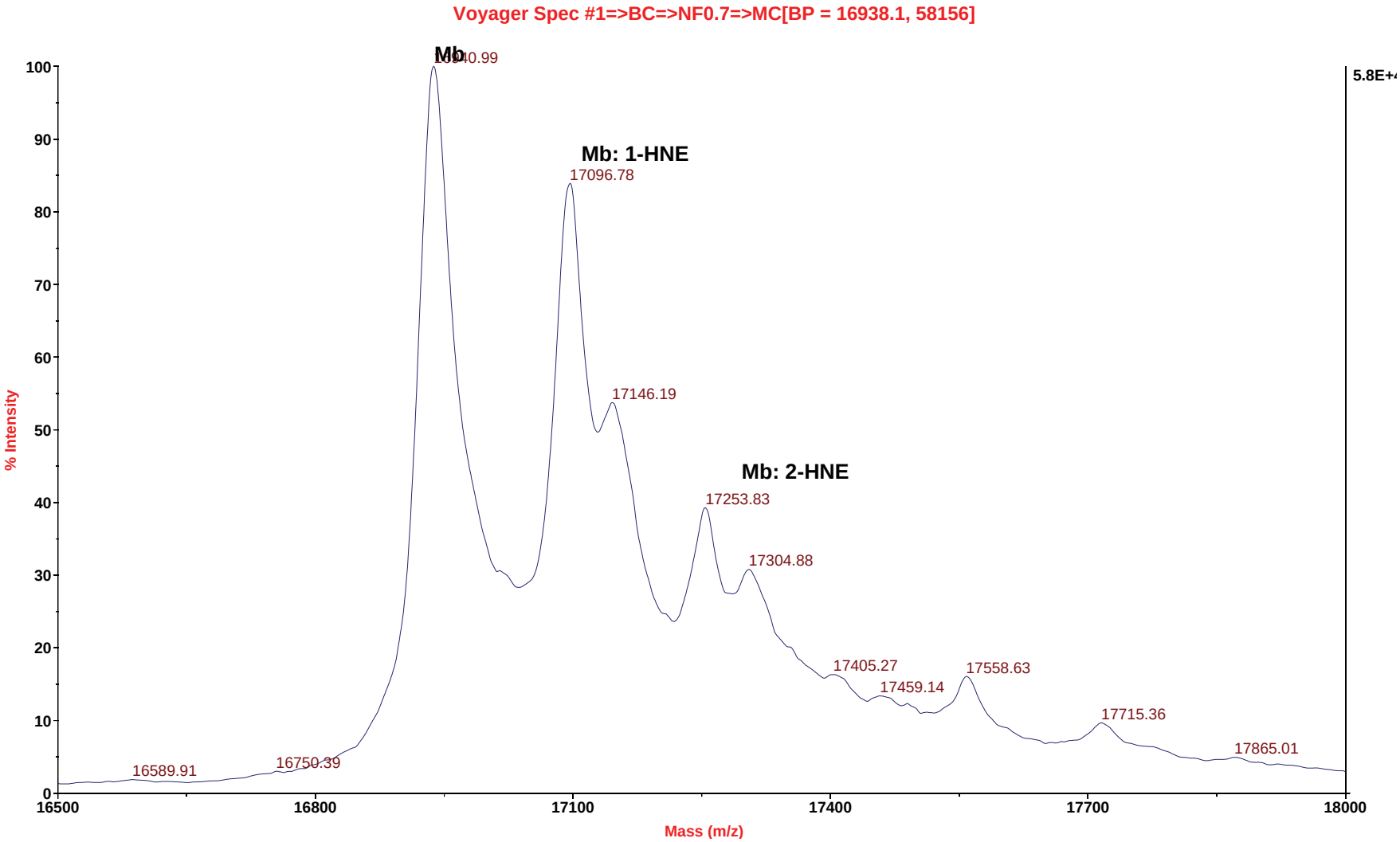

Fig. 2. MALDI-TOF MS spectra of bovine oxymyoglobin (0.15 mM) following reaction with HNE (1.0 mM) at pH 5.6 and 4°C for 48 h.

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