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Interactions between Mitochondrial Lipid Oxidation and Oxymyoglobin Oxidation and the Effects of Vitamin E

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Off-flavor and discoloration of meat products result from lipid oxidation and myoglobin (Mb) oxidation, respectively, and these two processes appear to be interrelated. The objective of this study was to investigate their potential interaction in mitochondria and the effects of mitochondrial α-tocopherol concentrations on lipid oxidation and metmyoglobin (MetMb) formation in vitro. The addition of ascorbic acid and ferric chloride (AA-Fe^{3+}) increased ovine and bovine mitochondrial lipid oxidation when compared with their controls ($p < 0.05$); MetMb formation also increased with increased lipid oxidation relative to controls ($p < 0.05$). Reactions containing Mb and mitochondria with greater α-tocopherol concentrations demonstrated less lipid oxidation and MetMb formation than mitochondria with lower α-tocopherol concentrations. Greater mitochondrial α-tocopherol concentration was also correlated with increased mitochondrial oxygen consumption in vitro and with a more pronounced effect at pH 7.2 than at pH 5.6. Relative to controls, succinate addition to bovine mitochondria resulted in increased concentrations of ubiquinol 10 and α-tocopherol and decreased lipid and Mb oxidation ($p < 0.05$). Mitochondrial lipid oxidation was closely related to MetMb formation; both processes were inhibited by α-tocopherol in a concentration-dependent manner.

KEYWORDS: Mitochondria; myoglobin; lipid oxidation; α-tocopherol; succinate

INTRODUCTION

Color and flavor are important parameters used to judge meat quality (*1*). The discoloration of meat from red to brown during storage results from the oxidation of ferrous myoglobin (Mb) to ferric metmyoglobin (MetMb); rancidity in meat primarily involves oxidation of unsaturated fatty acids of phospholipids that are an integral part of mitochondrial and microsomal membranes (*2*, *3*). Mitochondria are membrane-bound organelles and the primary function of which is the synthesis of ATP in living cells, while microsomes are a heterogeneous set of vesicles 20−200 nm in diameter formed from the endoplasmic reticulum when cells are disrupted. Mb oxidation and lipid oxidation appear interrelated in meat (*4*−*6*), and support for this interaction has been demonstrated using liposome and microsome model systems (*7*−*12*). However, Arnold et al. (*4*) concluded that Mb and lipid oxidation may be regulated by different mechanisms because lipid oxidation and Mb oxidation responded to vitamin E supplementation differently in *longissimus lumborum* and *gluteus medius* muscles. Vitamin E only slightly decreased MetMb formation but markedly inhibited lipid oxidation in these muscles (*4*). Mitochondria represent a relevant membrane system for studying lipid:Mb interactions in meat; the quantity of the mitochondrial fraction in beef muscle is twice that of the microsomal fraction (*4*). More than 50% of fatty acids in mitochondria are unsaturated and thus susceptible to lipid oxidation (*3*, *13*).

Dietary supplementation of cattle, sheep, and pigs with α-tocopherol acetate leads to increased α-tocopherol concentrations in muscles and improved lipid or color stability in meat subsequently obtained from these animals (*4*−*6*, *14*, *15*). α-Tocopherol levels in mitochondrial fractions from muscles of supplemented animals were also elevated in a dose-dependent manner (*4*, *15*). This was not surprising, given the importance of mitochondria in energy metabolism in living cells and the associated generation of reactive oxygen species from mitochondrial electron transport; the latter has been implicated in damage to mitochondria, cytosolic components, and DNA (*16*). Like α-tocopherol, coenzyme Q (ubiquinone) is also present in mitochondria to provide protection from oxidative damage (*17*, *18*). Coenzyme Q acts as an electron carrier in the mitochondrial respiratory chain by transferring electrons to complex III and was reported to inhibit lipid oxidation directly in its reduced form (ubiquinol) in freshly isolated submitochondrial particles (*17*). Furthermore, a mechanism for α-tocopherol regeneration by ubiquinol has been reported in freshly isolated mitochondria with succinate as substrate (*13*, *16*, *19*, *20*). The latter was

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proposed to be responsible for an observed inhibitory effect on NADPH-dependent lipid oxidation in intact mitochondria (*21*).

Despite loss of morphological integrity postmortem, mitochondria retain the ability to consume oxygen and affect Mb redox stability in meat (*22*). If membrane changes that occur postmortem are related to lipid oxidation, then α-tocopherol would be expected to protect mitochondrial membrane integrity and enzyme activity (*18*, *23*, *24*) and this would impact Mb stability. Given that mitochondria are one of the major reservoirs of α-tocopherol in meat and that this antioxidant nutrient delays lipid and Mb oxidation (*4*), it appeared logical to ascertain the degree of oxidative interaction between Mb and lipid in systems containing mitochondria.

The objective of this study was to investigate the interaction between lipid oxidation in mitochondria and Mb oxidation and the effect of α-tocopherol on these two processes and on postmortem mitochondrial oxygen consumption.

MATERIALS AND METHODS

Materials and Chemicals. Bovine or ovine hearts were obtained locally within 0.5 h of exsanguination, placed on ice, transported to the laboratory, and stored at 0 °C for 96 h. Equine heart Mb, sodium hydrosulfite, magnesium chloride ($MgCl_2$), bovine serum albumin (BSA, essentially fatty acid free), sucrose, tris[hydroxymethyl]aminomethane hydrochloride (Tris-HCl), ethylenediaminetetraacetic acid (EDTA), potassium phosphate monobasic (KH_2PO_4), ethylene glycolbis-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES), maleic acid, adenosine 5′-diphosphate (ADP), ubiquinone 10, α-tocopherol, α-tocopherol acetate, sodium perchlorate, perchloric acid, myxothiazol, and Nagarse protease (10.5 unit/mg) were obtained from Sigma Chemical Co. (St. Louis, MO); sodium succinate, ethanol, methanol, and hexane were purchased from Fisher Scientific (Fair Lawn, NJ). Sodium perchlorate, α-tocopherol, α-tocopherol acetate, ubiquinone 10, and solvents were HPLC grade; all other chemicals were reagent grade. The electron-transfer inhibitor myxothiazol was dissolved in ethanol prior to incorporation into reactions.

Equine OxyMb Preparation. OxyMb was prepared according to the method of Brown and Mebine (*25*) as follows. Commercial MetMb (Sigma Chemical Co., St. Louis, MO) was dissolved in 50 mM Tris-HCl buffer (pH 7.4), reduced chemically by mixing with sodium hydrosulfite (0.1 mg/mg MetMb), and oxygenated by bubbling air through the solution. Residual sodium hydrosulfite was removed by dialysis against 50 mM Tris-HCl buffer.

Mitochondria Isolation. Mitochondria were isolated from bovine or ovine cardiac muscle according to the method of Smith (*26*) with minor modification. Briefly, 100 g of ground cardiac muscle was washed with 250 mM sucrose twice and suspended into 200 mL of mitochondrial isolation buffer (250 mM sucrose, 10 mM Tris-HCl, 1 mM EGTA, pH 7.4). The suspension was stirred slowly and hydrolyzed with Nagarse protease (protease/tissue = 0.5 mg/g) for 20 min; the pH was maintained between 7.0 and 7.4. After proteolytic digestion, the suspension was diluted to 1 L with mitochondrial isolation buffer and subjected to two homogenization processes. The first of these was accomplished in a Kontes Duall grinder (Vineland, NJ) with three passes and was followed by treatment with a Wheaton Potter−Elvehjem grinder (Millville, NJ) with one pass; pestles of these grinders were driven by a heavy-duty drill at 1400 rpm. The homogenate was centrifuged (1200*g*) for 20 min with a Sorvall RC-5B centrifuge (Newtown, CT), and the resulting supernatant was then centrifuged (26 000*g*) for 15 min. The pellet was washed twice and suspended in mitochondrial suspension buffer (250 mM sucrose, 10 mM Tris-HCl, pH 7.4) and was ready for use. All steps were performed at 0−4 °C. Mitochondrial protein content was determined by a Bicinchoninic Acid Protein Assay Kit from Sigma Chemical Co. (St. Louis, MO).

Oxygen Consumption Measurement. Mitochondrial oxygen uptake was measured with a Clark oxygen electrode using a polarizing voltage of 0.60 V, which was clamped to the bottom of an 8 mL incubation chamber. Reaction components were added to the chamber and the temperature maintained at 25 °C by a water jacket and Lauda RE120 circulating water bath (Westbury, NY). The chamber was stirred with a 10-mm Teflon-covered bar at 600 rpm. Additions were made with Hamilton syringes through a 1-mm port. The electrode was attached to a Rank Brothers digital model 20 oxygen controller (Cambridge, England), and connected to a personal computer with data logger. Oxygen consumption was recorded over time at pH 7.2 (250 mM sucrose, 5 mM KH_2PO_4, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.1% BSA, 20 mM HEPES) and pH 5.6 (same as pH 7.2 except maleic acid replaced HEPES). Calculations for state III and IV oxygen consumption rate (OCR) were based on the method of Estabrook (*27*).

Acquisition of Mitochondria with Different Concentrations of α-Tocopherol. Sheep cardiac muscles were obtained from control animals or from animals supplemented with α-tocopherol acetate, in an attempt to obtain mitochondria containing different concentrations of α-tocoperol. Eight Dorset/Shropshire sheep were divided into four groups and fed a diet containing different levels of α-tocopherol acetate (Blue Seal Feeds, Inc., Londonderry, NH) for 7 weeks. The diet contained 15.0% crude protein, 2.5% crude fat, 7.0% crude fiber, 0.6−1.0% calcium, 0.4% phosphorus, 0.75−1.25% salt, 0.40 ppm selenium, and 4000 IU/lb vitamin A. Animals were given free access to water. All animals were slaughtered at a local commercial facility, and mitochondria were isolated from cardiac muscle at 96 h postmortem as noted above.

Measurement of α-Tocopherol, Ubiquinone 10, and Ubiquinol 10. Lipid extracts were prepared from mitochondria using a modification of the procedure described by Lang (*28*). A mitochondrial suspension containing 5 or 10 mg of mitochondrial protein was mixed with 20 μL of internal standard (α-tocopherol acetate) and 2 mL of ethanol. The mixture was vortexed for 30 s, 2 mL of hexane was added, and the tightly screwed test tube was vortexed vigorously for 2 min. The sample was then centrifuged for 3 min at 1000*g* to separate the phases and the hexane phase was transferred to a second tube. Triplicate hexane extractions were applied to each sample, and the hexane phases were pooled and dried under nitrogen. The residue was redissolved in ethanol and analyzed by HPLC. An HP1100 HPLC system (Agilent Technologies, Waldbronn, Germany) with a Zorbax ODS column (C18, 4.6 × 150 mm, 70 A) was used for separation and α-tocopherol was detected by fluorescence with an excitation wavelength of 295 nm and emission wavelength of 320 nm. The α-tocopherol acetate internal standard was detected at 286 nm, ubiquinone 10 at 275 nm, and ubiquinol 10 at 290 nm with a diode array detector. Two mobile phases, methanol/water (98:2, v/v) (solvent A) and ethanol/methanol (90:10, v/v) (solvent B), were used with a flow rate of 1.5 mL/min, and both contained 0.7% $NaClO_4$ and 0.1% $HClO_4$ (70%). Solvent A was used for separation of α-tocopherol and solvent B for separation of ubiquinone 10 and ubiquinol 10 (*28*). The gradient procedure was as follows: (1) 100% solvent A for 11 min, (2) increasing solvent B from 0% to 100% between 11 and 12 min, (3) maintaining 100% solvent B for 8 min between 12 and 20 min, (4) decreasing solvent B from 100 to 0% between 20 and 22 min, (5) maintaining 100% solvent A for at least 2 min prior to the next injection. Standard ubiquinol 10 was reduced from ubiquinone 10 according to the procedure of Lang (*28*). Quantification was based on peak heights, using external standardization.

Interactions between Mitochondrial Lipid and OxyMb Oxidation. Mitochondria (1 mg of mitochondrial protein/mL) isolated from bovine or ovine cardiac muscles were combined with OxyMb (20 μM) or ascorbic acid (100 μM) + ferric chloride (50 μM) and incubated at pH 5.6 and 25 °C. OxyMb and mitochondria were used as controls for lipid and Mb oxidation, respectively, and incubation time for bovine mitochondria:OxyMb systems was extended to 150 min because OxyMb in these systems oxidized slower than that in ovine mitochondria:OxyMb systems. Samples were withdrawn for determination of lipid oxidation and MetMb formation every 30 min. For the experiment of vitamin E supplementation, mitochondria were isolated from ovine cardiac muscles, and α-tocopherol concentrations of isolated mitochondria were determined using HPLC, and then mitochondria were combined with OxyMb as described above to investigate the effect of α-tocopherol on lipid and OxyMb oxidation. The effects of α-tocopherol concentrations on mitochondrial OCR were determined at pH 5.6 and

7.2 and 25 °C using isolated ovine mitochondria. The effects of succinate addition on OxyMb and lipid oxidation were investigated with isolated bovine mitochondria at pH 5.6 and 25 °C. Isolated bovine mitochondria (1 mg of mitochondrial protein/mL) were combined with 20 μM OxyMb, 10 μM myxothiazol, 100 μM ascorbic acid, and 50 μM ferric chloride. Treatments also contained 8 mM succinate. Samples were withdrawn for determination lipid and OxyMb oxidation every hour and for HPLC determination of α-tocopherol, ubiquinone 10, and ubiquinol 10 at 0, 1, and 5 h.

Lipid Oxidation. Lipid oxidation in samples was determined by measuring thiobarbituric reactive substances according to the method of Schmedes and Holmer (*29*) with minor modification. Each sample of 0.5 mL was mixed with an equal volume of 11% trichloroacetic acid solution and then centrifuged for 3 min (15 000*g*) with an Eppendorf 5415D centrifuge (Westbury, NY). The resulting supernatant (0.5 mL) was combined with 0.5 mL of 20 mM thiobarbituric acid and incubated at 25 °C for 20 h. The absorbance at 532 nm was measured with a Shimadzu UV 2101-PC (Kyoto, Japan) spectrophotometer.

MetMb Determination. One-milliliter samples were withdrawn at specific incubation times and centrifuged for 3 min (15 000*g*) with an Eppendorf 5415D centrifuge (Westbury, NY). The resulting supernatants were scanned from 650 to 500 nm with a UV2101-PC Shimadzu (Kyoto, Japan) spectrophotometer. MetMb concentrations were calculated according to Tang et al. (*30*).

Statistical Analysis. Results are expressed as mean values (±SE) of three independent trials. Data were analyzed using the GLM procedure of SAS, and differences among means were detected at the 5% level using LSD with appropriate correction for multiple comparisons (*31*). Correlation and regression analyses were performed using SigmaPlot (*32*). The optimal equation was determined as the equation that maximized the coefficient of determination and minimized the root-mean-square error of the model (*32*).

RESULTS AND DISCUSSION

Mitochondrial Lipid Oxidation and OxyMb Oxidation. The interactions between mitochondrial and equine OxyMb are presented in **Figures 1** and **2**. Relative to mitochondria or Mb controls, greater lipid oxidation and MetMb formation were observed upon the combination of ovine mitochondria with OxyMb (**Figure 1**; $p < 0.05$), a result in agreement with observations in liposomes (*7*, *12*). The addition of ascorbic acid−ferric chloride (AA−Fe^{3+}) to ovine mitochondria or Mb alone accelerated lipid oxidation in mitochondria samples (**Figure 1A**; $p < 0.05$), but had no effect on MetMb formation when compared with their respective controls (**Figure 1B**; $p > 0.05$). However, AA−Fe^{3+} addition to ovine mitochondria/Mb systems increased both lipid oxidation and MetMb formation (**Figure 1**; $p < 0.05$). These results suggested that lipid oxidation and Mb oxidation were interrelated. Similar results were observed with bovine mitochondria with two differences noted (**Figure 2**): (1) adding bovine mitochondria to Mb had no effect on MetMb formation relative to Mb controls, and (2) AA-Fe^{3+} addition to Mb delayed MetMb formation relative to Mb controls (**Figure 2B**; $p < 0.05$). A clear basis for these observations is not readily explained. These differences may result from the different sources of mitochondria (ovine vs bovine) that were used. Different responses of membrane lipid and Mb to an AA−Fe^{3+} system were also reported by Gorelik et al. (*9*). They noted that AA−Fe^{3+} addition to microsomes or Mb increased microsomal lipid oxidation but decreased bovine MetMb formation, and they attributed the inhibitory effect on Mb oxidation to the reducing action of ascorbic acid (*9*). We used 100 μM ascorbic acid in our experiment and the prooxidant effect on lipid oxidation was consistent with previous reports (*9*), but this concentration was antioxidant to proteins such as Mb. The relationship between ovine mitochondrial lipid oxidation and MetMb formation was fitted into a sigmoid curve with a coefficient of determination (r^2) of 0.79 (**Figure 3**).

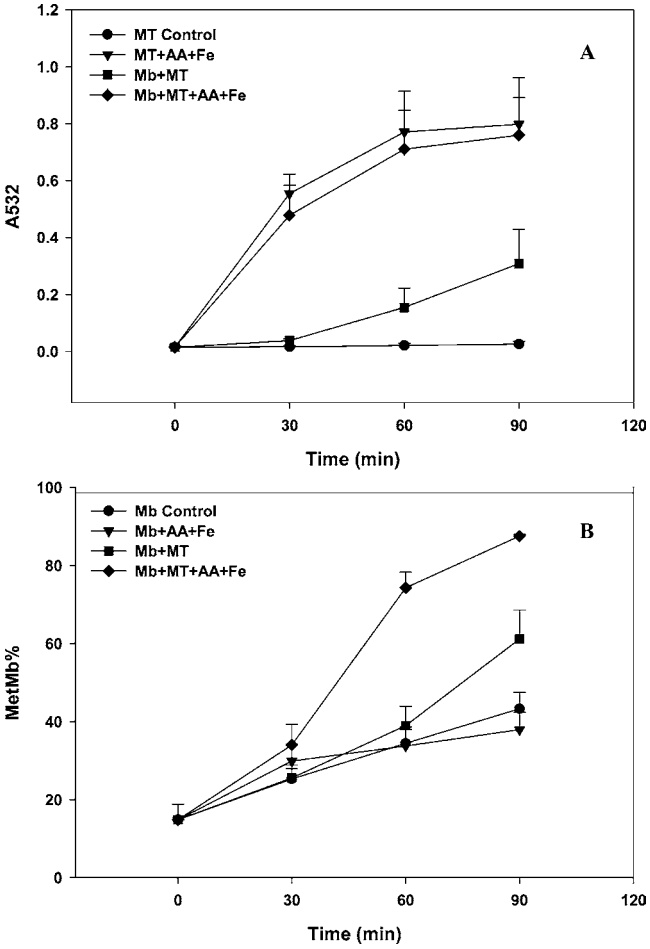

Figure 1. Lipid oxidation (A_{532}) (A) and MetMb formation (%) (B) in an ovine mitochondria–equine OxyMb system during incubation at pH 5.6 and 25 °C. Mitochondria (MT), 1 mg of MT protein/mL; Mb, 20 μM; ascorbic acid (AA), 100 μM; ferric chloride (Fe), 50 μM. Standard error bars are indicated ($n = 3$).

Effects of α-Tocopherol on Lipid Oxidation and Mb Stability. Mitochondria that contained greater α-tocopherol concentrations demonstrated less lipid oxidation than those containing lower α-tocopherol concentrations (**Figure 4A**); this was accompanied by concomitantly less MetMb formation during 30 min incubation at 25 °C (**Figure 4B**). These results further suggested that lipid oxidation and Mb oxidation were interrelated. The relationship between lipid oxidation and α-tocopherol concentration was fitted into an inverse first-order curve with $r^2 = 0.94$, while the relationship between MetMb formation and α-tocopherol concentration was fitted into a single-exponential decay curve with $r^2 = 0.93$. It appeared that a critical α-tocopherol concentration existed for achieving an inhibitory effect in that concentrations greater than 0.2 nmol of α-tocopherol/mg of mitochondria protein provided minimal benefit for preventing lipid oxidation and Mb oxidation in vitro. In related observations with beef muscles, Faustman et al. (*33*) and Arnold et al. (*4*) reported that approximately 3.0 μg of α-tocopherol/g of muscle was a threshold concentration necessary for maximum inhibition of lipid oxidation and MetMb formation in beef *longissimus lumborum* muscle.

Previous researchers reported that succinate addition to fresh mitochondria appeared to regenerate α-tocopherol and inhibit lipid oxidation (*20*, *21*) and we investigated this reaction in 96-h

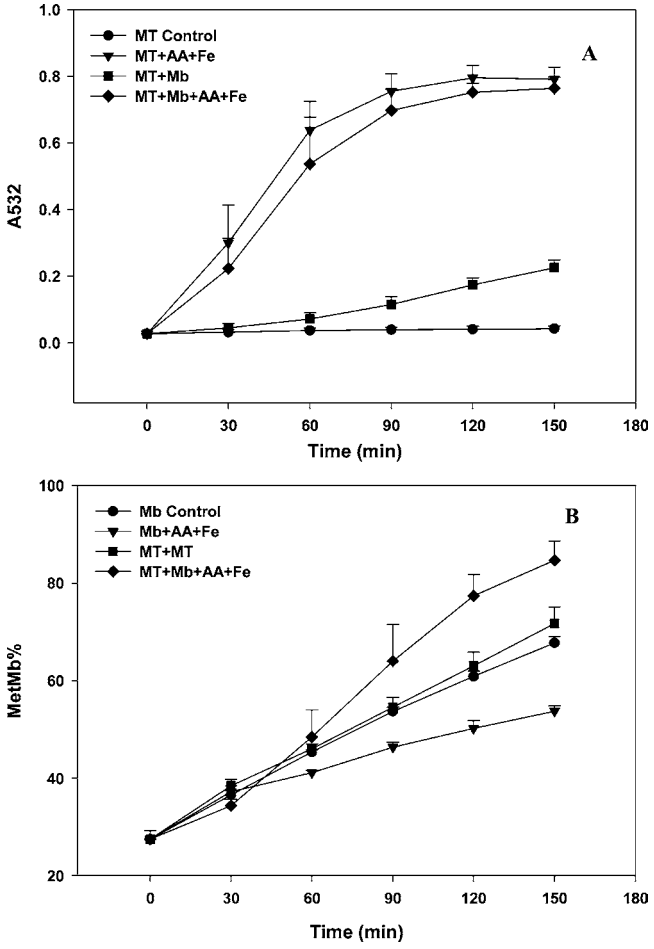

Figure 2. Lipid oxidation (A_{532}) (A) and MetMb formation (%) (B) in a bovine mitochondria–equine OxyMb system during incubation at pH 5.6 and 25 °C. mitochondria (MT), 1 mg of MT protein/mL; Mb, 20 μM; ascorbic acid (AA), 100 μM; ferric chloride (Fe), 50 μM. Standard error bars are indicated ($n = 3$).

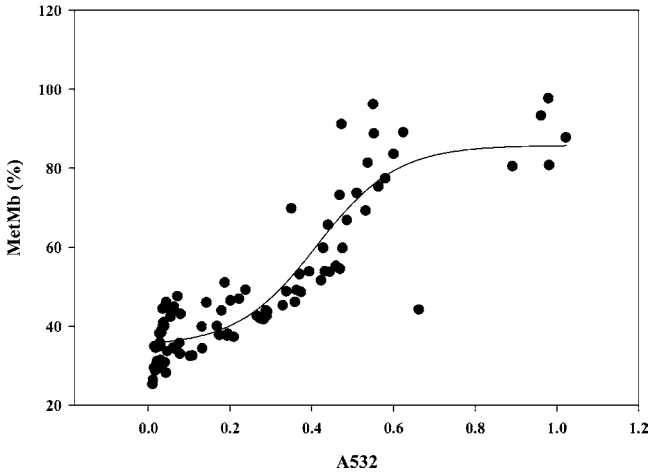

Figure 3. Correlation between ovine mitochondrial lipid oxidation (A_{532}) and equine MetMb formation (MetMb%). Mitochondrial lipid oxidation was initiated by addition of ascorbic acid (100 μM) and ferric chloride (50 μM) at pH 5.6 and 25 °C.

postmortem mitochondria. To eliminate any effect of mitochondrial oxygen consumption on Mb redox stability (*34*), myxothiazol was added to inhibit electron transfer from complex III to IV; its addition to Mb alone did not affect OxyMb stability (results not shown). α-Tocopherol was detected upon succinate addition, and 40−70% of CoQ 10 was present in a reduced form during 5 h incubation at 25 °C (**Table 1**); however, α-tocopherol was undetectable, and less than 30% CoQ 10 was found in a reduced form in nonsuccinate controls (**Table 1**, *p*

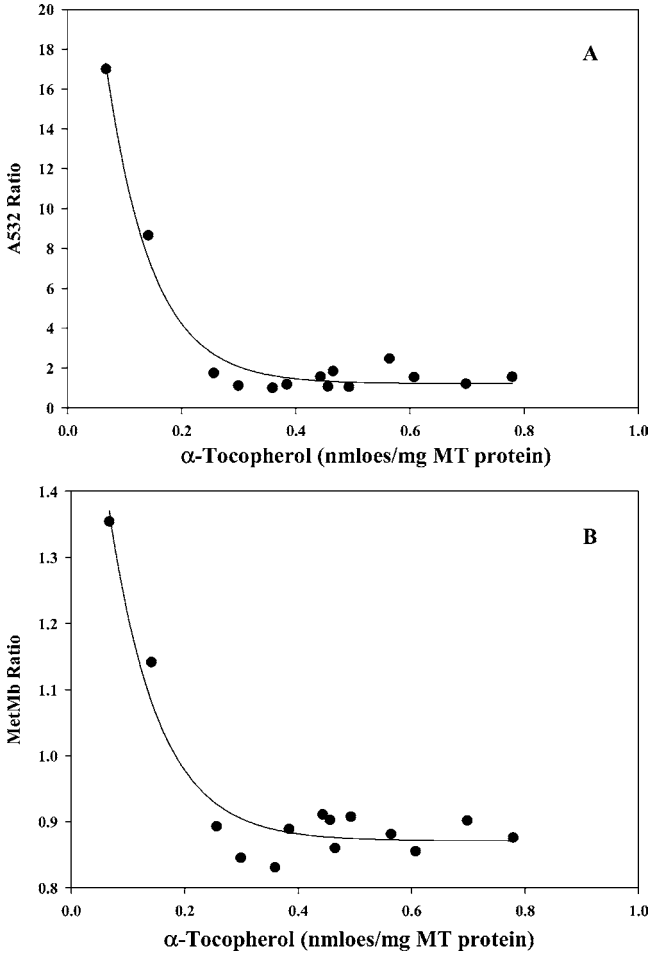

Figure 4. Relationship between ovine mitochondrial (MT) α-tocopherol concentration (nmol/mg of MT protein) and lipid oxidation (A) or MetMb formation (B). The ovine mitochondria–equine OxyMb system was incubated at pH 5.6 and 25 °C for 30 min; lipid oxidation was initiated by addition of ascorbic acid (AA, 100 μM) and ferric chloride (Fe, 50 μM). MT, 1 mg of MT protein/mL; Mb, 20 μM. The A_{532} ratio is the absorbance ratio at 532 nm between treatments (MT + Mb + AA + Fe^{3+}) and their respective controls (MT + Mb); the MetMb ratio is the %MetMb ratio between treatments (MT + Mb + AA + Fe^{3+}) and their respective controls (MT + Mb).

Table 1. Effects of Succinate Addition on Bovine Mitochondrial (MT) α-Tocopherol, Ubiquinone 10 (OCoQ10), and Ubiquinol 10 (RCoQ10) Concentrations (nmol/mg of MT protein)[a,c]

treatment	time (h)	α-tocopherol	OCoQ10	RCoQ10
Cont	0	ND[b]	1.48 ± 0.09a	0.50 ± 0.25a
	1	ND	0.25 ± 0.02c,d	0.08 ± 0.04d
	5	ND	0.13 ± 0.05d	0.23 ± 0.12d
Succ	0	0.24 ± 0.02a	1.30 ± 0.26a,b	1.70 ± 0.20b,c
	1	0.24 ± 0.04a	1.03 ± 0.21a,b,c	1.61 ± 0.09a,b
	5	0.24 ± 0.03a	0.70 ± 0.41b,c,d	2.17 ± 0.53a

[a] Each reaction system contained 120 mM KCl, 5 mM NaH_2PO_4, and 30 mM maleic acid with a final pH 5.6. Succinate (Succ) treatments contained 1 mg of MT protein/mL, 20 μM equine OxyMb, 10 μM myxothiazol, 8 mM succinate, 100 μM ascorbic acid, and 50 μM ferric chloride. Controls (Cont) did not contain succinate. [b] ND means not detected. [c] Values in the same column with different letters were different ($p < 0.05$).

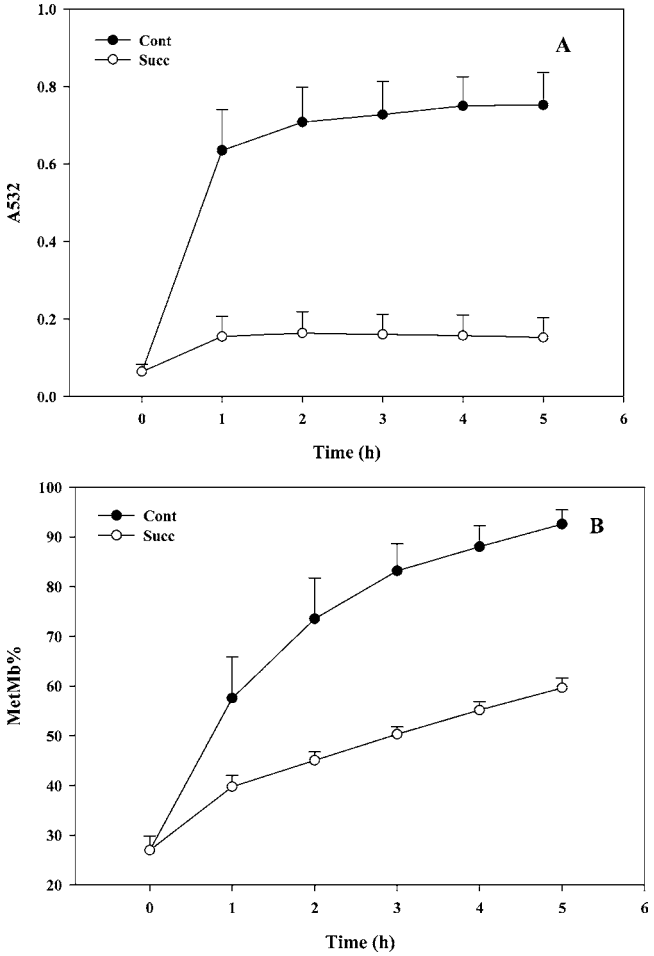

Figure 5. The effect of succinate oxidation on bovine mitochondrial (MT) lipid oxidation (A) and MetMb formation (B) during incubation at pH 5.6 and 25 °C. Each reaction system contained 120 mM KCl, 5 mM NaH_2PO_4, and 30 mM maleic acid with a final pH 5.6. The succinate treatment contained 1 mg of MT protein/mL, 20 μM equine OxyMb, 10 μM myxothiazol, 8 mM succinate, 100 μM ascorbic acid, and 50 μM ferric chloride. Controls did not contain succinate. Standard error bars are indicated ($n = 3$).

< 0.05). Furthermore, CoQ 10 was degraded in controls during the 5 h incubation period. Mitochondrial lipid was oxidized readily and MetMb formation was greater in controls when compared with succinate-treated samples (**Figure 5**, $p < 0.05$). Meszaros et al. (*21*) reported that succinate or β-OH-butyrate addition decreased NADPH-dependent production of malondialdehyde in intact mitochondria; an inhibitory effect of succinate was abolished by addition of malonate, a complex II inhibitor, but not affected by the complex I inhibitor rotenone or the complex III inhibitor antimycin A. They concluded that the reduction of ubiquinone by respiratory substrate was responsible for the observed inhibition of lipid oxidation. Our results demonstrated that both α-tocopherol and CoQ 10 concentrations were increased by succinate oxidation and likely protected mitochondria from lipid oxidation (**Table 1**, **Figure 5A**) as previously suggested by Noack et al. (*16*). Therefore, the presence of substrates such as succinate, appears to affect Mb stability both by favoring MetMb formation via enhanced mitochondrial oxygen consumption (*22*) and decreasing MetMb formation by inhibition of mitochondrial lipid oxidation.

Effects of α-Tocopherol on Mitochondrial Oxygen Consumption. No transition from state IV to state III respiration was observed upon the addition of ADP to mitochondria isolated 96 h postmortem and this is due to the disruption of mitochondrial structure under storage at 0 °C (*22*, *34*). Therefore, only state IV OCR is reported as a function of α-tocopherol concentrations. For mitochondria that contained greater α-tocopherol concentrations, OCR increased in a concentration-dependent manner at pH 7.2. The regression equation for this relationship was $y = 72.0x + 55.8$, where y was OCR, and x the α-tocopherol concentration; the r^2 value was 0.73 (**Figure 6**). The relationship between α-tocopherol concentration and oxygen consumption rate at pH 5.6 was weaker (**Figure 6**; $y = 41.8x + 15.1$, $r^2 = 0.42$), and it is possible that mitochondrial oxygen consumption was inhibited at pH 5.6 (*22*). It was not surprising that mitochondria with greater α-tocopherol concentration had greater OCR because α-tocopherol would be expected to protect mitochondrial lipids and proteins from oxidative stress, thereby maintaining greater enzymatic activity and oxygen consumption (*18*, *23*, *35*). Our present results demonstrated that increasing α-tocopherol concentration had the potential to affect Mb stability by both inhibition of mitochondrial lipid oxidation and increasing mitochondrial oxygen consumption; the former increased Mb stability, while the latter decreased Mb stability. Faustman et al. reported that α-tocopherol supplementation of cattle increased lipid and color stability in postmortem muscle (*5*, *33*). Taken together, those results suggested that the primary function of α-tocopherol was to protect meat flavor and color by inhibition of lipid and Mb oxidation; its effect on Mb oxidation by enhancing oxygen consumption would have been expected to be negligible, probably due to the lack of an appropriate substrate (i.e., succinate or NADH).

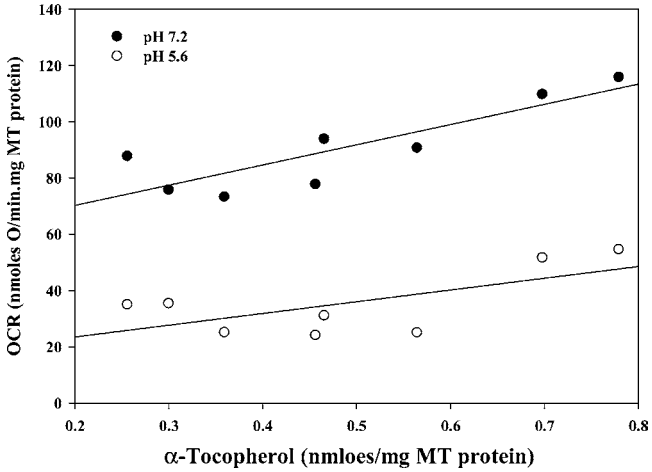

Figure 6. The relationship between mitochondrial (MT) α-tocopherol concentration (nmol/mg of MT protein) and state IV oxygen consumption rate (OCR, nmol of O/min mg of MT protein) at 25 °C and pH 7.2 or 5.6. The reaction systems contained mitochondria (0.38 mg of MT protein/mL), rotenone (0.02 mM), and succinate (8 mM).

Postmortem mitochondrial lipid oxidation and Mb oxidation were interrelated in vitro, and both processes were inhibited by greater α-tocopherol concentrations. Mitochondrial OCR was also increased with elevated α-tocopherol concentrations in vitro.

ABBREVIATIONS USED

MT, mitochondria; Mb, myoglobin; OxyMb, oxymyoglobin; MetMb, metmyoglobin; OCR, oxygen consumption rate; Tris-

HCl, tris[hydroxymethyl]aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; BSA, bovine albumin; EGTA, ethylene glycolbis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]; ADP, adenosine 5′-diphosphate; HPLC, high performance liquid chromatography.

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