INHIBITION OF OXYMYOGLOBIN OXIDATION BY VITAMIN E

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INTRODUCTION

The consumer's initial decision to buy fresh meat is influenced by the surface appearance of the meat (Cornforth, 1994). In red meats, consumers associate a bright red colour with freshness and discriminate against meat which has turned brown in colour. Underlying the undesirable colour change from bright red to brown is the oxidation of the muscle pigment oxymyoglobin to metmyoglobin. The rate and extent of metmyoglobin formation is influenced by factors intrinsic to the muscle itself (muscle type, pH, oxidation-reduction reactions) and by environmental factors (temperature, oxygen pressure, packaging) (Renerre, 1990).

It has been established that, in certain feeding regimes, supplementation of beef animal diets with the natural antioxidant vitamin E (α-tocopherol) can enhance the colour stability of bovine muscle post-mortem (Faustman et al., 1989; Vega et al., 1996). The mechanism by which vitamin E, a lipid soluble antioxidant, inhibits the oxidation of myoglobin, a water soluble protein, remains to be clarified. In the present study, the effect of muscle α-tocopherol level on lipid and oxymyoglobin oxidation was investigated in bovine muscle systems.

MATERIALS AND METHODS

Vitamin E levels. *M. longissimus dorsi* samples were obtained from Romagnola x Friesian and Piedmontese x Friesian steers fed either a control diet containing silage and concentrates or a control diet supplemented with vitamin E (2000 I.U./day) for 40 days before slaughter. The α-tocopherol content of *M. longissimus dorsi* samples (3 control, 3 supplemented) was determined by HPLC.

Lipid and oxymyoglobin oxidation in a microsome-enriched muscle fraction. A microsome-enriched muscle fraction (MF) was prepared by first subjecting a *M. longissimus dorsi* homogenate to centrifugations at 17,600g for 30 min as described by Apgar and Hultin (1982). Half of the supernatant obtained was centrifuged at 104,000g for 60 min to yield a microsome pellet and a soluble fraction (SF) containing oxymyoglobin and other water-soluble proteins. The SF was retained and the microsomal pellet obtained was resuspended in the other half of the supernatant obtained at 17,600g to give a microsome-enriched fraction (MF). Lipid oxidation was initiated in incubations of MF and SF by the addition of 45μM $FeCl_3$/ascorbate (1:1) (O'Grady et al., 1995). Lipid oxidation (2-thiobarbituric acid reactive substances, TBARS) and oxymyoglobin oxidation (Krzywicki, 1982) were assessed over a 24h storage period.

RESULTS AND DISCUSSION

M. longissimus dorsi samples from control and vitamin E-supplemented animals were grouped on the basis of α-tocopherol content into low vitamin E (<2.0 μg α-tocopherol/g muscle) and high vitamin E (>2.5 μg α-tocopherol/g muscle) samples. The mean muscle α-tocopherol levels for the low vitamin E group was 1.6 ± 0.4 μg/g and 2.8 ± 0.4 μg/g for the high vitamin E group.

In the model system stimulation of lipid oxidation with $FeCl_3$/ascorbate led to an increase in both lipid and oxymyoglobin oxidation (Figure 1A). Oxymyoglobin oxidation did not occur when the microsomal lipids were omitted from the incubations containing the prooxidant (Table 1). The data show that oxymyoglobin oxidation can be catalysed by oxidising lipids. A possible mechanism for catalysis of oxymyoglobin oxidation is through interaction of free radical intermediates of lipid oxidation with oxymyoglobin (Sevanian and Hochstein, 1985).

In the model system containing bovine microsomal lipids and oxymyoglobin isolated from the high vitamin E group, both lipid and myoglobin oxidation were inhibited (Figure 1B). Thus, a likely mechanism for the inhibition of oxymyoglobin oxidation in red meats containing elevated vitamin E levels is through the inhibition of the formation of free radical lipid intermediates of lipid oxidation.

It appears from the data obtained in this experiment that a threshold level of lipid oxidation needs to be attained for myoglobin oxidation to occur. Thus, while considerable lipid oxidation occurred in the soluble fraction (SF) obtained after removal of the microsomes, oxymyoglobin was largely unoxidised in these samples (Table 1). To effect oxidation of oxymyoglobin, enrichment of the extract with microsomal lipid was necessary suggesting that a critical ratio of oxidisable lipid to oxymyoglobin needs to be present.

The model system used in this study differs from that used in previous studies (Lin and Hultin, 1977; Anton et al., 1991; O'Grady et al., 1995): (i) the pH used (pH 5.5) is typical of that in post-mortem muscle, (ii) the temperature used (4°C) is typical of that used for meat storage, (iii) lipids and myoglobin native to the muscle are used, (iv) water soluble components of muscle which can affect susceptibility to oxidation, e.g. antioxidant enzymes, are present in the system, (v) the prooxidant used to initiate lipid oxidation does not cause oxidation of oxymyoglobin in the absence of lipids. However, it remains a model system and future studies should focus on examining oxidative changes at the exposed muscle surface.

CONCLUSION

The data presented support the contention that oxidising lipids could cause oxidation of oxymyoglobin in fresh fed meats and that the mechanism of enhancement of colour stability by vitamin E is via its role as lipid antioxidant. The work was undertaken with model systems in which lipid oxidation was stimulated by the addition of a prooxidant system capable of degrading preformed lipid hydroperoxides. Further studies of oxidation with muscle lipids and oxymyoglobin *in situ* are necessary to verify these results.

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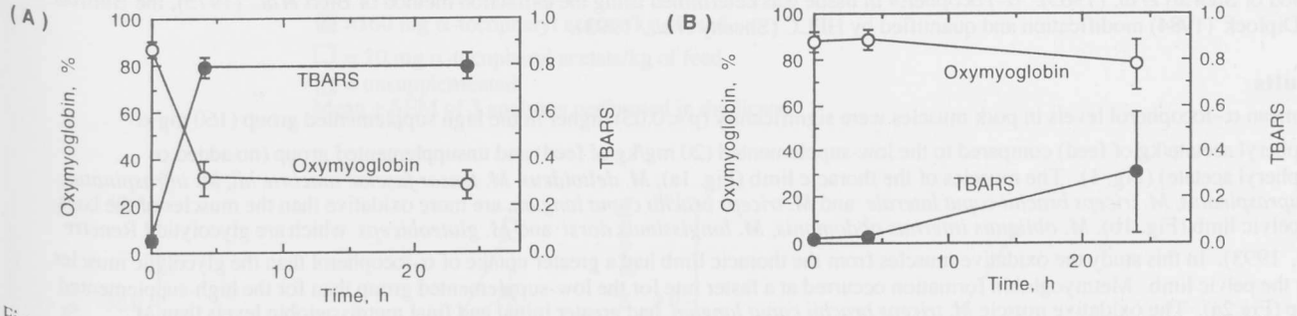

Figure 1. Lipid and myoglobin oxidation in a microsome-enriched muscle fraction from (A) low vitamin E and (B) high vitamin E *M. longissimus dorsi*.

Table 1. Lipid and oxymyoglobin oxidation in microsome-enriched muscle extracts from *M. longissimus dorsi* containing high and low vitamin E levels.

Incubation		Storage time @ 4°C			
		0 h		4 h	
		TBARS[1]	OMb, %[2]	TBARS	OMb, %
Low E muscle:	MF[3] + Fe/ascorbate	0.056 ± 0.026[a]	87.3 ± 3.5[a]	0.793 ± 0.043[a]	31.8 ± 7.7[a]
	MF	0.028 ± 0.009[b]	88.0 ± 3.0[a]	0.028 ± 0.006[c]	87.8 ± 1.3[b]
	SF[4] + Fe/ascorbate	0.059 ± 0.031[a]	90.7 ± 1.9[a]	0.485 ± 0.059[b]	84.2 ± 2.5[b]
	SF	0.027 ± 0.006[b]	91.7 ± 1.4[a]	0.025 ± 0.009[c]	91.0 ± 1.3[b]
High E muscle:	MF + Fe/ascorbate	0.022 ± 0.007[b]	87.8 ± 4.6[a]	0.023 ± 0.007[c]	88.7 ± 4.5[b]
	MF	0.011 ± 0.008[b]	87.7 ± 4.5[a]	0.010 ± 0.005[c]	87.5 ± 4.8[b]
	SF + Fe/ascorbate	0.014 ± 0.003[b]	91.0 ± 4.4[a]	0.022 ± 0.002[c]	90.0 ± 3.5[b]
	SF	0.011 ± 0.005[b]	90.7 ± 4.5[a]	0.013 ± 0.007[c]	90.5 ± 4.8[b]

[1] nmoles malondialdehyde/mg protein. [2]Oxymyoglobin, % of total. [3]Microsome-enriched muscle fraction. [4]Soluble fraction. [a,b,c,d] Mean values in the same column bearing different superscripts are significantly different, $P<0.05$.