Microsomal Lipid Peroxidation in Relation with Oxidation of Bovine Myoglobin

M. ANTON, P. GATELLIER AND M. RENERRE.

Meat Research Station, INRA, 63122 Ceyrat, France.

SUMMARY

The aim of this experiment was to appreciate with two bovine muscles different from the viewpoint of colour stability (LD / PM), the relationships between microsomal lipid peroxidation and myoglobin oxidation. It was shown that enzymic microsomal lipid peroxidation (TBA Test) in presence of cofactors, generated oxygen radicals which increased myoglobin oxidation (DO 580 nm) at the same extent for the two muscles. Fluorescence decay, used as a test of tryptophan loss, was more prononced with microsomes extracted from PM than from LD. The inhibitory effect of SOD and Vit.E on myoglobin oxidation and lipid peroxidation were confirmed and participation of superoxide anion underlined. For nonenzymic catalysis, it was observed that metmyoglobin activated by hydrogen peroxide enhanced lipid peroxidation at the same extent for the two muscles.

INTRODUCTION

The autoxidation of unsaturated lipids in meats results in significant deterioration in quality characteristics such as colour, flavor, texture, nutritive value and safety. Phospholipids are the primary contributors to lipid oxidation and warmed-over flavors (WOF) development in raw and cooked meats. Furthermore the ability of hematin and heme proteins, such as myoglobin, to promote lipid peroxidation has been demonstrated. Significant amounts of superoxide radical has been proved as generated by different organelles as microsomes and mitochondria in muscles tissues or by autoxidation of molecules such as oxymyoglobin (MbO2) (Gotoh & Shikama,1976) and this radical can cause or initiate lipid peroxidation in meat. It has been reported that enzymic microsomal lipid peroxidation might be involved in the initiation process (Rhee et al; 1986). These enzymic systems catalyse the oxidation of microsomal fractions in the presence of cofactors such as NADPH, ADP and Fe^{++}/Fe^{+++}(Hultin, 1980). Furthermore lipid peroxidation in raw meat has also been regarded as a non-enzymic reaction and Kanner et Harel (1985) reported that the interaction of H2O2, generated by microsomes and mitochondria with metmyoglobin (MetMb) gave activated-MetMb capable of initiating lipid oxidation. The major objective of this study was to provide information about the relationships between microsomal lipid oxidation and MetMb formation, from two different beef muscles, which largely regule the colour stability of raw meat.

MATERIAL AND METHODS

Two muscles different from the viewpoint of colour stability and oxidative capacity (Renerre, 1984) : Psoas major (PM) and Longissimus dorsi (LD) were taken 1h post-mortem on Charolais cull cows and put in liquid nitrogen. Extraction of oxymyoglobin (MbO2) in cold 10 mM Tris HCl buffer (pH 8.4) and its precipitation with ammonium sulfate between 70 and 100% saturation was realized. Oxymyoglobin solution (MbO2) was passed through a mono-Q (Pharmacia) column which was equilibrated with 10mM Tris HCl buffer (pH 8.4) and eluted with a linear gradient (0 to 100 mM) of NaCl in the same buffer. MbO2 purity was analysed by SDS- polyacrylamide electrophoresis and isoelectrofocusing (unpublished results).

Isolation of the microsomal fraction was done by a procedure described by Apgar & Hultin (1982) after homogenization of meat extract in 5mM histidine buffer, 0, 6M KCl (pH 6.8) and centrifugation at 600g, 17000g and 100000g for 60 min. Protein determination was conducted by the Biuret procedure. Lipid peroxidation was measured using the thiobarbituric (TBA) assay described by Bueg & Aust (1978) with the results reported as nM malonaldéhyde (MDA) per mg protein.

The extent of enzymic lipid peroxidation in the presence of MbO2 was appreciated with a reaction mixture containing 0.72 mg microsomal protein/ml, 0.16 mM FeCl3, 0.83 mM ADP, 0.4 mM NADPH and 0.66 mg/ml MbO2 in 10 mM Tris HCl buffer (pH 8.4) (Lin & Hultin; 1977). Measurement was effectued for a period of 64 min. at 37°C. The inhibitory effect on MbO2 oxidation and lipid peroxidation of different radical scavengers and antioxidants were tested as: superoxide dismutase (SOD) (0.3 U/ml), mannitol (10mM), dimethylsulfoxide (DMSO) (240 mM), BHT (1mM) and vitamine E (8 mM). Tryptophan loss in the mixture was appreciated by fluorescence measurement (280 nm excitation / 340 nm emission) in the presence of MbO2 (0.02 mg/ml) and microsomal fraction (0.02 mg/ml).

Non-enzymic catalysis of lipid peroxidation with the MetMb-H2O2 complex was studied at 37°C in the presence of MetMb (0.5 mg/ml), H2O2 (30 µM) and microsomal fraction (1mg/ml) (Kanner et Harel; 1985). Lipid peroxidation was evaluated following the determination of TBA- reactive substances (MDA) and by fluorescence emission at 420 nm (340 nm excitation) (Kagan; 1988).

RESULTS AND DISCUSSION.

After chromatography on Mono-Q column, a single band corresponding to a molecular weight of about 17000 was detected by staining with silver nitrate and identified by spectrophotometry as MbO2. Analytical IEF, by staining with Coomassie blue, revealed 3 bands corresponding to oxy- (pI= 6.8 and pI=7.0) and met-myoglobin (pI=7.3). These results are according to our previous results obtained after purification of the haeminic pigment in different chromatographic conditions (Renerre et al; in press).

Interactions between microsomes and oxymyoglobin.

The results of these experiments (Table 1 and Table 2) indicated the presence in microsomal fractions from beef muscles of enzymic systems NADPH dependent which catalyse the oxidation of microsomal lipids in vitro in the presence of co-factors as previously shown in different species (Lin & Hultin; 1977; Rhee & Ziprin; 1987). Moreover it was shown that these enzymic lipid peroxidation systems isolated from the two studied (LD and PM) muscles were capable of oxidize MbO2 in vitro. After addition of the microsomal fraction to MbO2 solution, the oxidation % of the pigment in 40 min. was multiplied by about 1.5 and by 3.0 after addition of microsomes+cofactors. With a pH in our reaction medium equal to 7.0, we were not in the most favorable conditions to enhance lipid peroxidation (Rhee et al; 1984). Furthermore, it must be noted (table 1) that there was no difference in MbO2 autoxidation % between LD and PM muscles.

In our reaction medium, MbO2 autoxidation was linked to the microsomal lipid peroxidation as indicated by the TBA test (Table 2). The maximum was reached after 40 min. with production of about 10 nM MDA / mg microsomal protein. With different conditions, Rhee et al (1984) found a similar effect of incubation time on lipid peroxidation in beef muscle microsomes. The autoxidation process was followed by a plot of log % MbO2 vs time and it was observed two different rates (Figure not represented). For Stewart (1990), this fact could be explained by a competition between MetMb which acts as a peroxidase, decreasing in situ concentrations of H2O2, and MbO2 which also is oxidized by the peroxide. Moreover, it must not be excluded that in our experimental conditions, MetMb reductase localized in microsomes and in more or less intact mitochondria (Echevarne et al; 1990), and identified as a NADH-cytochrome b5 reductase, can interfere in MbO2 autoxidation rate.

The use of different radical scavengers and antioxidants allowed us to observe that SOD lowered MbO2 autoxidation rate and, with a less extent, TBA reactive substances concentration indicating that interactions between MbO2 and microsomal lipid fraction were of radical nature and that superoxide anion was well generated in our reaction medium (Tables 1 and 2). It is well admitted that isolated microsomal fractions have been shown to produce H2O2 rapidly in vitro in the presence of NADPH via dismutation of superoxide anion. Inversely, it was observed that addition of mannitol and DMSO did not lead to a decrease in radicals production from the reaction medium suggesting that hydroxyl radicals were not produced (or in a too low quantity to be detected). In all these experiments, no muscle effect was noted. These results could indicate that in our reaction medium, iron could not be released from MbO2 and that, consequently, we were not in good conditions leading to hydroxyl radical generation by the Fenton reaction (Halliwell & Gutteridge, 1989) .

The use of BHT and Vit.E had no effect towards MbO2 autoxidation but leaded to an important decrease in TBA reactive substances formation (Table 2); no difference was noted between LD and PM muscles. The major action of these antioxidants is to react with peroxy and alkoxy radicals donating labile hydrogen to them and so terminating the chain reaction of peroxidation (Halliwell & Gutteridge, 1989). Being hydrophobic, Vit. E is a fat-soluble molecule which may also protect against peroxidation by modifying membrane structure.

Tryptophan loss in the reaction medium containing microsomal fraction+ cofactors and MbO2 was appreciated by fluorescence measurement at 340 nm (280 nm excitation) and after 6 h, it was important to note that the fluorescence loss was more prononced with the PM muscle than with the LD muscle (Figures 1and 2). As addition of SOD inhibited the fluorescence loss (figures 1and 2), it could be concluded that oxygen radicals (and particularly superoxide anion) were formed. In this case, compared to previous results (tables 1and 2), the inhibition of oxygen radicals formation was fast complete during all the length (6h) of the reaction. For Davies et al (1987), the loss of tryptophan fluorescence is the result of the protein to superoxide anion and hydroxyl radical exposure. The observed differences between muscles in

fluorescence loss indicated that PM muscle produced more oxygen radicals than LD muscle and this could be due, for one part, to variations in the fatty acid composition of the membranes. Protein characteristics cannot be implicated in these changes because it was shown that MbO2 extracted and purified from LD and PM muscles presented identical autoxidation rates (Renerre et al; in press).

Nonenzymic catalysis of lipid peroxidation with the MetMb-H2O2 complex was studied as described by Kanner & Harel (1985) and no difference was noted between LD and PM muscles either by the TBA test (figure 3) or by the fluorescence emission at 420 nm (Figure 4).The lipid oxidizing activity may be due to MetMb activated by H2O2 (Rhee , 1988). A ferryl specie, called the porphyrin cation radical, could be formed during the reaction where heme protein acts as peroxidase. By using this test, Freybler et al (1989) demonstrated that the peroxidative reactions in meat systems are well initiated in the membranes. For Kanner et al. (1986), the cytotoxic activity of H2O2 with MetMb is due in particular to its capacity to be reduced to the hydroxyl radical.

CONCLUSIONS

These results have shown that enzymic systems, present in microsomes, and H2O2 activated-MetMb are responsible for initiation of lipid peroxidation in raw meat; lipid peroxidation is linked up with MetMb formation and free oxygen radicals are formed during these oxidation processes as, in all likelihood, superoxide anion and hydroxyl radicals. Differences in superoxide anion formation have been detected between LD and PM muscles but more investigations are needed to clearly define the relationships between the microsomal lipid peroxidation and meat colour stability.

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Fig1: Tryptophan destruction by oxygen radicals (m. PM)

Fig 2: Tryptophan destruction by oxygen radicals (m. LD)

Fig 3: TBA test for non-enzymic catalysis

Fig 4: Fluorescence emission for non-enzymic catalysis

TABLE 1: MbO2 oxidation % in presence of microsomal peroxidation system cofactors, radical scavengers and antioxidants after 40 min.(n=7)

treatment \ muscle	LD	PM
MbO2	8.8±1.1	8.1±0.9
MbO2+micro	13.6±2.0	14.0±3.9
MbO2+micro.+cof	26.4±4.2	24.6±4.6
id. + SOD	16.4±4.0	12.3±1.6
id. + mannitol	23.0±3.8	18.6±2.4
id. + DMSO	24.6±4.0	25.8±2.2
id. + Vit. E	20.2±2.0	20.6±2.6
id. + BHT	20.0±2.6	21.2±1.7

TABLE 2 : Lipid peroxidizing activity (nM MDA/mg.P.) in presence of MbO2, cofactors, radical scavengers and antioxidants after 40 min.(n=7)

treatment \ muscle	LD	PM
MbO2	1.03±0.38	0.96±0.38
MbO2+micro.	5.06±0.64	5.44±0.83
MbO2+micro.+cof.	10.83±0.83	10.26±0.77
id + SOD	8.30±0.70	8.97±0.70
id + mannitol	9.10±0.96	9.29±0.83
id + DMSO	10.64±0.77	9.93±0.77
id. + Vit.E	1.47±0.40	1.73±0.39
id. + BHT	2.18±0.70	1.99±0.57