SALT EFFECTS ON MYOGLOBIN DERIVATIVES OF THE SARCOPLASMIC EXTRACT FROM PRE- AND POST-RIGOR BEEF WITH AND WITHOUT MITOCHONDRIA AND MICROSOMES

A. ASGHAR, E. TORRES, J.I. GRAY AND A.M. PEARSON
Dept. Food Sci. & Human Nutrition, Michigan State University, East Lansing, MI 48824 USA.

INTRODUCTION

Color in raw meat is dependent on the relative proportions of the different myoglobin derivatives, i.e., oxymyoglobin (MbO_2), metmyoglobin (MetMb, ferrimyoglobin) and reduced myoglobin (Mb, ferromyoglobin). MetMb unlike Mb is a poor electron donor at the sixth ligand (George & Stratman, 1954; Rifkind, 1973), and is responsible for the brown to black appearance of oxidized meat. Salt is known to promote oxidation in post-rigor meat (Chang & Watts, 1950; Ellis et al., 1968; Greene et al., 1971; Greene & Price, 1975), but Torres et al. (1988) have recently reported that adding salt to pre-rigor ground beef stabilizes the red color by maintaining a high proportion of MbO_2. Pre-rigor grinding of raw meat while the pH is still high has also been reported to minimize lipid oxidation (Owen & Lawrie, 1975; Whang et al., 1986).

Bearing the above facts in mind, the present study was designed to further explore the role of salt and pH in meat pigment oxidation. Thus, the effects of salt level, pH, and rigor state (pre-versus post-rigor) upon the activity of the mitochondrial and microsomal fractions of muscle were related to the relative proportions of Mb, MbO_2 and MetMb over 96 hr. at 4^oC.

MATERIALS AND METHODS

Pre-rigor bottom round muscle was obtained from beef carcasses within 45 min. of slaughter from the abattoir of the Meat Laboratory at Michigan State University. The post-rigor samples were excised from the contralateral muscle of the same carcasses at 24 hr. post-mortem as described by Torres et al. (1988).

Preparation of Sarcoplasmic Extract

Both the pre- and post-rigor meat samples were minced by twice passing through a meat grinder. A 150g minced sample was homogenized in a cold (4^oC) 0.25M glycerol solution containing 5μM sodium iodoacetate to arrest glycolysis. The sarcoplasmic extract was obtained by centrifugation at 1000g for 10 min. at 0^oC and then was divided into two portions. One portion was adjusted to pH 7.4 and the other to 5.4 by adding NaOH or lactic acid, respectively. The extracts were kept cool in crushed ice. Each extract was further divided into 3 parts, with the first part containing both the mitochondrial and microsomal fractions. From the second part, the mitochondria were eliminated by centrifugation and from the third part both the mitochondria and microsomes were removed by sequential centrifugation using the method of Schenkman & Cinti (1978). Each extract was further divided into 4 parts and NaCl was added to make the final concentration 0.0, 0.5, 2.0 and 4.0% salt. All samples were stored at 4^oC, and analyzed for myoglobin derivatives after 0, 24, 48 and 96 hr.

Determination of Myoglobin Derivatives

Relative percentages of MbO_2 and MetMb were determined by the method of Broumand et al. (1958). A 5 ml sample was centrifuged at 600g for 5 min. and subjected to spectrophotometric readings at 475, 507, 573 and 597 nm with the percentages of MbO_2 and MetMb being computed

using the method of Broumand et al. (1958).

Statistical Analysis

The data were analyzed statistically using a factorial and complete block design as outlined by Steel & Torrie (1980) in order to partition the influence of the variables (rigor state, pH values, subcellular organelles, salt concentrations) and their interactions. The storage periods were treated as blocks for analysis of variance. The significance between mean differences was determined by using either least significant difference (LSD) or by Duncan's multiple range test as explained by Duncan (1955).

RESULTS AND DISCUSSION

Data in Table 1 demonstrate that the sarcoplasmic extract from post-rigor meat contained significantly more MbO_2 and less MetMb than that from pre-rigor meat ($P < 0.05$). This is surprising in view of the fact that Torres et al. (1988) found a higher proportion of MbO_2 in pre-rigor salted than in post-rigor salted meat. The low percentage of MbO_2 in the pre-rigor sarcoplasmic extract may be associated with the sustained demand for oxygen by the mitochondria, since the electron transport system of mitochondria in the sarcoplasmic extract from pre-rigor meat is likely to be potentially active (Andrews et al., 1952; Saleh & Watts, 1968).

Influence of pH

The sarcoplasmic extract adjusted to pH 5.4 contained a significantly higher percentage of MbO_2 ($P < 0.05$), and a lower percentage of Mb than the sample adjusted to pH 7.4 (Table 1). This agrees with Cornforth & Egbert (1985) who observed high "a" values (redness) for minced beef at low pH. In the present study, however, the proportion of MetMb was not significantly influenced ($P > 0.05$) by pH. These observations are at variance with the general concept that oxidation of Mb is directly related to pH in the range of 5 to 7 (Brown & Mebine, 1969).

The decrease in the affinity of oxygen for haem with decreasing pH values is called the "Bohr effect" (Perutz, 1970), which is based on an allosteric interaction of haem-iron (active center) and the proton-binding site (allosteric center). Depending on the degree of dissociation of the proton-binding site, the protein moiety of haem acquires one of two conformations (T- or R-state), which differ in their oxygen affinity (Antonini & Brunori, 1971). It is proposed that a high pH may enhance the stability of the salt bridge between the protonated imidazole of histidine and carboxylate of the aspartic acid residue, and hence stabilizes the deoxy- relative to the oxy-conformation of haem. A high concentration of protons may weaken the bond between oxygen and haem iron, thereby facilitating dissociation of oxygen from myoglobin (Antonini & Brunori, 1971). Moreover, the rate of haem-globin dissociation at low pH is reported to be higher than at physiological pH (Gotoh & Shikama, 1974).

All these observations may hold true for a pure myoglobin system. However, the interactions of different organelles in the sarcoplasmic extract may have an important bearing on the MbO_2 concentration, and thus its characteristics may deviate from that in pure solution. For instance, it has been reported that the respiratory activity of mitochondria in the sarcoplasmic extract increases upon raising the pH from 5.1 to 7.1 (Lawrie, 1953; Stewart et al., 1965). If this is so, then the sarcoplasmic extract at

high pH is likely to be depleted of oxygen. This may lead to a decrease in MbO_2 levels and an increase in the percentage of deoxymyoglobin (Mb). The greater proportion of Mb in the sarcoplasmic extract at pH 7.4 in comparison to pH 5.4 (Table 1) substantiates these views. However, the pH effect accounted for only about 3% of the variation in the MbO_2 level of the sarcoplasmic extract.

Influence of Mitochondria and Microsomes

It is apparent from the data in Table 1 that the sarcoplasmic extract maintained a significantly higher percentage of MbO_2 in the absence of both mitochondria and microsomes. This is understandable in view of the fact that cytochrome c oxidase, which is the terminal enzyme of the mitochondrial respiratory chain, requires oxygen to reduce 4 electrons from the chain to ultimately form 2 molecules of water (Brunori et al., 1975), thereby minimizing oxygen radical formation. Thus, the presence of mitochondria would be expected to deplete the oxygen level in the sarcoplasmic extract and would lead to a reduction in the MbO_2 concentration. The data for the subcellular organelles (mitochondria and microsomes) suggests that they collectively account for only 7% of the variation in the percentage of MbO_2 in the sarcoplasmic extract. As the samples were stored at low temperature (4°C) in the present study, the activity of the subcellular organelles is likely to be smaller than would occur at ambient temperature.

Effect of Salt Concentration

The data shown in Table 1 demonstrate that salt concentration had a significant effect ($P < 0.05$) on MbO_2 percentage, which decreased with increasing concentrations of NaCl in the sarcoplasmic extract. Salt concentration accounted for about 11% of the variation in the percentage of MbO_2. The percentage of MetMb increased with increasing salt levels, but the Mb percentage remained relatively constant. This agrees with the findings of Wallace et al. (1982) and Caughey & Kamanishi (1983) which indicated that certain anions (Cl^-, CN^-, N_3^-) increase the rate of oxidation in MbO_2. Possibly, these anions bind to haem-iron and change its redox potential to favor oxidation of Fe^{2+} to Fe^{3+}, or they may preferentially stabilize the Fe^{3+} relative to the Fe^{2+} state of haem-iron. It is emphasized that to a large extent the oxidation rate of haem-iron depends on the concentration and nucleophilic characteristics of the anions. Thus, chloride, being the least nucleophilic ion, would be required in a high concentration to affect the reaction. On the other hand, a high concentration of NaCl (5%) would inhibit the MetMb-reducing activity of the meat system, thus favoring formation of MetMb (Stewart et al., 1965).

These inferences are contrary to that reported in a recent paper by Torres et al. (1988), which showed that the addition of salt helped to maintain the desirable red color in pre-rigor minced beef, whereas, the unsalted sample rapidly lost its characteristic red color. It should be pointed out, however, that the discrepancy in the behavior of MbO_2 with the results of Torres et al. (1988) could be due to differences between an intact meat system and an isolated system of meat organelles. The results of the present study suggest that salt per se does promote oxidation of MbO_2 at low pH. However, in pre-rigor minced meat as studied by Torres et al. (1988) the beneficial role of salt in maintaining the desired color may possibly be related to its ability in suppressing the growth of aerobic

psychrophilic bacteria (Robach & Costilow, 1961), mainly fluorescent pseudomonads (Silliker et al., 1958; Lin, 1973), which cause depletion of oxygen and lead to color deterioration (Kraft & Ayres, 1952; Butler et al., 1953).

The present study indicated that the sarcoplasmic extract from post-rigor meat contained more MbO_2 and less MetMb than that from pre-rigor meat. The low pH of the sarcoplasmic extract from the post-rigor samples seems to account for this difference since the sarcoplasmic extract from post-rigor meat adjusted to pH 7.4 had a lower percentage of MbO_2 than the sarcoplasmic extract maintained at pH 5.4. The presence of subcellular organelles (mitochondria and microsomes) in the sarcoplasmic extract at high pH significantly influenced the proportion of MbO_2 and MetMb but at a low pH their presence had little affect. Possibly the mitochondrial and microsomal enzymes that effect myoglobin derivatives become active at high pH, but at low pH their activity is inhibited. With increasing salt concentrations at low pH the level of MetMb in the sarcoplasmic extract increased and that of MbO_2 decreased proportionately. This may partly be due to the catalytic action of chloride ion in promoting oxidation of MbO_2 to MetMb, and partly due to inhibiting the MetMb-reducing activity of the sarcoplasmic extract. In general, the percentage of MbO_2 in the sarcoplasmic extract was highly dependent on the interaction of pH, salt concentration and subcellular organelles of the muscle.

CONCLUSIONS

Results indicated that the sarcoplasmic extract (SPE) from both pre- and post-rigor meat adjusted to pH 5.4 contained more MbO_2 than that maintained at pH 7.4. The presence and absence of mitochondria and microsomes in the SPE at pH 5.4 had little effect on percentage of MbO_2. At pH 7.4, however, the proportion of MbO_2 decreased in the SPE containing microsomes. The amount of MbO_2 decreased and MetMb increased with increasing salt concentration and with length of storage in the SPE from both pre- and post-rigor meat. It is suggested that depression of microbial growth may be a mechanism by which salt stabilizes the color in pre-rigor meat rather than by enzymic effects of subcellular organelles.

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Table 1. Mean values showing effects of rigor, pH, subcellular organelles and salt concentration on percent of myoglobin derivatives in the sarcoplasmic extract from beef muscle.

Variables	Mean Percent of Myoglobin Forms		
	MbO_2	MetMb	Mb
Rigor state (N=120)			
Pre-rigor	62.6	26.0	11.4
Post-rigor	64.0	28.1	7.9
S.E.	0.54	0.36	0.20
pH values (N=120)			
pH 5.4	65.0	27.1[a]	7.9
pH 7.4	60.6	27.0[a]	12.4
S.E.	0.54	0.36	0.20
Subcellular organelles (N=80)			
Mito. + Micro. present	58.3	30.1	11.6
Only micro. present	63.0	28.4	8.6[a]
Both absent	68.6	22.7	8.7[a]
S.E.	0.67	0.44	0.25
Salt concentrations (N=60)			
0.0%	71.1	21.9	7.0
0.5%	63.4	26.1	10.5
2.0%	59.3	28.8	11.9
4.0%	57.6	31.4	11.0
S.E.	0.77	0.50	0.29
Storage periods (N=48)			
0 hours	66.4[a]	23.3	10.3[b]
24 hours	65.7[a]	27.0[b]	10.3[b]
48 hours	62.8[b]	26.0[b]	8.0
72 hours	62.4[b]	28.3	9.3
96 hours	59.5	30.6	10.2[b]
S.E.	0.86	0.56	0.32

Mean values with the same letters within a column are not significantly different (P>0.05) from each other.