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PROOXIDATIVE ACTIVITY OF DIFFERENT MYOGLOBIN SPECIES IN LINOLEIC ACID EMULSIONS

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Keywords: Metmyoglobin, ferryl myoglobin, perferryl myoglobin, lipid peroxidation

Introduction

An unsolved problem regarding lipids peroxidation in muscle foods is the source of the primary catalyts. Free iron, metmyoglobin and activated myoglobin have all been proposed as important prooxidants in meat. H_2O_2 activated myoglobin has received increasing attention as it generates a short-life protein radical, perferryl myoglobin $^{\bullet}MbFe^{IV}=O$ that subsequently is reduced to ferryl myoglobin $MbFe^{IV}=O$ in a fast reaction. Model systems, with the necessary simplifications, like the linoleic acid emulsion used in the present study, provide a possibility for a direct comparison of the prooxidative activity of metmyoglobin, perferryl myoglobin and ferryl myoglobin, with the purpose to get a more detailed understanding of the mechanisms responsible for initiation of lipid peroxidation in meat.

Materials & Methods

Chemicals. Equine metmyoglobin (Type III), linoleic acid, chelating resin (Chelex-100) and Tween 20, were obtained from Sigma. Analytical grade chemicals and double deionized water were used throughout. Buffers were passed through a chelating resin columm to remove any metal ions.

Preparation of linoleic acid emulsion. Linoleic acid emulsion was prepared by mixing Tween 20 (0.012 g) and linoleic acid (21 mM) with phosphate buffer (5.0 mM; pH 6.5). The pH was addjusted to approximately 9.0 in order to provide the highest stabillity of the emulsion. Emulsions were prepared fresh every day.

Preparations of MMb and $MbFe^{IV}=O$. MMb was dissolved in 5.0 mM phosphate buffer, centrifugated, passed through a chelating resin columm and dilluted to 0.2 mM or 0.4 mM using ϵ_{525}:7700 $l \cdot mol^{-1} \cdot cm^{-1}$. MMb was stored at 5 °C in the dark. $MbFe^{IV}=O$ was prepared by mixing equal volume of 0.4 mM MMb with 1.2 mM H_2O_2 and allowed to react for 10 min to ensure complete conversion of MMb to $MbFe^{IV}=O$ before use.

Reactions of linoleic acid with different myoglobin species.
Initiation of oxidation of linoleic acid by MMb was studied after mixing 2.4 ml prethermostatted (25°C) O_2-saturated phosphate buffer (0.15 M; pH 5.50) with 50 μl linoleic acid emulsion and 50 μl (0.2 mM) MMb. $MbFe^{IV}=O$ initiated reactions were studied using the same procedure, substituting MMb by $MbFe^{IV}=O$. Experiments with $^{\bullet}MbFe^{IV}=O$ were carried out by adding 25 μl (0.4 mM) MMb into 2.475 ml prethermostatted (25°C) O_2-saturated phosphate buffer (0.15 M; pH 5.5) containing 0.42 mM linoleic acid emulsion and 0.012 mM H_2O_2. All samples were prepared directly in a 1 cm quartz cuvette placed in a thermostated cell compartment (25°C) of an HP8452 UV-VIS diode array spectrophotometer. Spectra ($190 < \lambda < 700$ nm) were recorded immediatly at time intervals of 1 min for 10 min.

Determination of conjugated dienes.
Conjugated dienes were determined by extraction of 1 ml reaction mixture with 4 ml of cold diethylether. The ether phase was collected and evaporated under N_2. The residue was dissolved in 1 ml ethanol and the absorption spectrum was recorded ($190 < \lambda < 700$ nm). The relative amount of conjugated dienes was obtained by calculating the second derivative at 234 nm: Procedure 1 (F. P. Corongiu et al., 1986). Cojugated dienes were also determined from the absorption spectra using the second derivative at 244 nm of the absorption spectra: Procedure 2 .

Results & Discussion.

The results presented in Figure 1 show that formation of conjugated dienes (cd) was induced by MMb and $MbFe^{IV}=O$, while $^{\bullet}MbFeIV=O$ did not induce any detectable formation of cd. This qualitative pattern in formation of cd was independent of the methods applied. The observed rate of formation of cd was approximately equal for MMb and $MbFe^{IV}=O$.

Lipid oxidation is known to influence the stability of heme protein and the effect of lipid peroxidation on heme protein degradation is presented in Figure 2. The observed relative change in absorption at 410 nm (Soret band) is expected to provide an indication of the integrity of the myoglobin derivates. The absorbance at 410 nm for MMb and H_2O_2 activated MMb (Fig. 2) decreased during incubation with the initial change being most dramatic for the perferryl species.

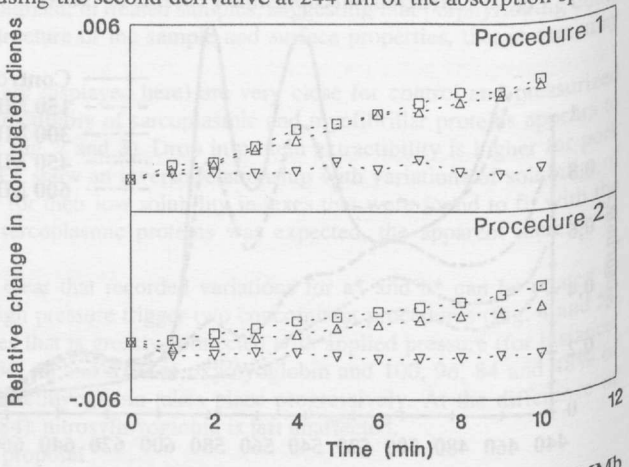

Figure 1. Relative change in conjugated dienes during MMb (--□--),$MbFe^{IV}=O$ (--△--) and $^{\bullet}MbFe^{IV}=O$ (--▽--) initiated peroxidation of linoleic acid using Procedure 1 and Procedure 2 . Each point is a mean of three determinations.

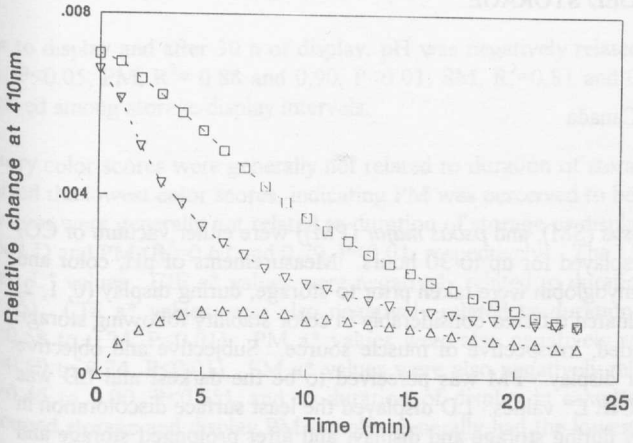

Figure 2. Relative change in the Soret band (410 nm), using the secondderivative spectra, during heme protein initiated peroxidation of linoleic acid. Each point is a mean of three determinations. Symbol as Fig. 1.

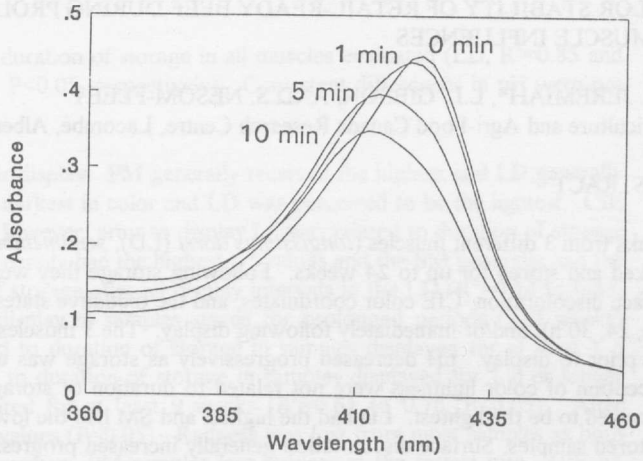

Figure 3. Blue-shift in the Soret region during $MbFe^{IV}=O$ induced oxidation of linoleic acid 0, 1, 5 and 10 min after addition of $MbFe^{IV}=O$.

The decrease at 410 nm for MMb equal heme protein degradation, while the change at 410 nm for H_2O_2 activated MMb has to be attributed to a combination of heme protein degradation and a steady state production of the hypervalent iron state which has an extinction coefficient (88300 $l \cdot mol^{-1} \cdot cm^{-1}$), which is lower than that of MMb (178000 $l \cdot mol^{-1} \cdot cm^{-1}$). The latter might explaine why the initial decrease of H_2O_2 activated MMb is faster than that of MMb at 410 nm even though only negligible amount of $^{\bullet}MbFe^{IV}=O$ was formed during the reaction as deduced from the obtained spectra (data not shown).

Beside the initial lower absorbance of $MbFe^{IV}=O$ due to its lower extinction coefficient at 410 nm (Fig. 2) the absorbance increased initially and then leveled out at 410 nm during the reaction in contrast to the experiments with MMb and H_2O_2 activated MMb. This can be explained by the results in Figure 3, which show a shift in the Soret band from 420 nm towards 410 nm, typical for the reduction of ferrylmyoglobin to metmyoglobin. However, Fig. 2 also show that the amount of heme protein equal the same level independent of the initiating species during prolonged incubation, which indicate that the degree of heme protein degradation during the oxidation is independent of the initiating species in contrast to the formation of cd as mentioned above.

The fact that perferryl myoglobin initiated peroxidation of linoleic acid does not result in any detectable formation of cd in a system where the linoleic acid 9-hydroperoxide has to be expected is in agreement with earlier results by Galaris et al. (1990), who in return observed another unidentified lipid peroxidation product as the major product. Somehow surprisingly the rate of lipid peroxidation as evaluated from relative formation of cd was found to be independent of whether the oxidation was initiated by MMb or $MbFe^{IV}=O$. The latter compound is reduced to MMb simultaneous with a degradation of the heme protein typical for MMb induced linoleic acid peroxidation (Mikkelsen et al., 1992). This indicate that the hemeprotein-catalyzed lipid hydroperoxide-dependent lipid peroxidation mechanism proposed by Tappel (1955) is dominant for both MMb and $MbFe^{IV}=O$ under the present conditions. Further investigations focusing on the mechanisms involving $^{\bullet}MbFe^{IV}=O$ as initiating species in the present system is in progress.

References.

Corongiu F.P., Poli G., Dianzani M.U., Cheeseman K.H. & Slater T.F. 1986. *Chem.-Biol. Interac.* **59**:147-155

Galaris D., Sevanian A., Cadenas E. & Hochstein P. 1990. *Arch. Biochem. Biophys.* **281(2)**:163-169.

Mikkelsen A., Sosniecki L. & Skibsted L.H. 1992. *Z. Lebensm. Unters. Forsch.* **195**:228-234.

Tappel A.L. 1955. *J. Biol. Chem.* **217**:721-733.