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EFFECTS OF α-TOCOPHEROL AND ASCORBYL PALMITATE ON OXYMYOGLOBIN AND LIPID OXIDATION *IN VITRO*

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BACKGROUND - Lipid oxidation and oxymyoglobin oxidation are deteriorative processes affecting consumer acceptability of meat. Antioxidants have been used to slow these processes and α-tocopherol has been especially effective as a fat-soluble antioxidant. In addition, water-soluble ascorbic acid has proven effective as an antioxidant towards both lipid and oxymyoglobin in a concentration-dependent manner *in vitro* (Yin et al. 1993). Ascorbyl palmitate, a fat-soluble ester of ascorbic acid, is an effective antioxidant in oil-based food systems (Hawrysh,1992). Its efficacy in aqueous based systems, such as muscle foods, has not been adequately addressed.

OBJECTIVES - The objective of this study was to investigate the antioxidant potential of ascorbyl palmitate at different concentrations, and also relative to α-tocopherol in an oxymyoglobin liposome system.

METHODS -

OXYMYOGLOBIN LIPOSOMES - Egg phosphatidylcholine (30mg), cholesterol (12mg), and dicetyl phosphate (3mg) were dissolved in 5 mL methylene chloride:methanol (2:1, v/v) in a round bottom flask. Solvent was removed by rotary evaporation to form a dry lipid film. An oxymyoglobin (OxyMb) solution was produced by chemical reduction of metmyoglobin (MetMb) with sodium hydrosulfite (.1mg/mg Mb) in sodium citrate (50mM, pH 5.6). Air was then bubbled through to facilitate oxygenation and the solution passed over a mixed-bed ion exchange column to remove excess sodium hydrosulfite. The resulting OxyMb solution was adjusted to a final concentration of 2.5 mg/ml. To each prepared lipid film, 10 mL OxyMb and 25 glass beads were added. Each of the flasks was then shaken at 4°C for 30 min. to permit formation of multilamellar vesicles. Solutions were transferred to test tubes and incubated at 37°C.

OXYMYOGLOBIN OXIDATION - MetMb formation was determined according to Krzywicki (1982) at 0, 0.5, 1, 1.5, 2, and 2.5 hr. incubation (37°C). A sodium citrate liposome preparation without oxymyoglobin was used as a blank.

LIPID OXIDATION - The Thiobarbituric acid procedure of Yin et al. (1993) was used to assess lipid oxidation at 0, 0.5, 1, and 2.5 hours incubation at 37°C and reported as thiobarbituric acid reactive substances (TBARS).

ANTIOXIDANT ADDITION - L-Ascorbic Acid 6-Palmitate was dissolved in methylene chloride:methanol (2:1,v/v). Aliquots of this solution were introduced to the lipid film to obtain final concentrations of 0, 2, 10, 100, 500 and 1000uM ascorbyl palmitate in the total 10 mL reaction assay. α-Tocopherol was delivered in solvent and incorporated into the lipid bilayer membrane at 0, 10, and 100uM according to the procedure of Lang et al.(1992).

STATISTICAL ANALYSIS - Data were analyzed by analysis of variance (ANOVA) and computed by using the SAS General Linear Model. Duplicate assays were prepared for each treatment on a single day and repeated on several different days.

RESULTS - Since there was no significant day effect ($P>.1$), data shown are for duplicate assays from one representative day.

The effect of ascorbyl palmitate on lipid oxidation was concentration-dependent (Fig.1). At 2.5 hr., 2uM ascorbyl palmitate demonstrated a strong antioxidant effect toward lipid oxidation (61% decrease of TBARS relative to controls $p<0.05$). At the same time point, greater concentrations of ascorbyl palmitate, from 10 to 1000uM, decreased TBARS by approximately 95.5% relative to controls ($p<0.05$). Ascorbyl palmitate's effect on OxyMb oxidation is presented in Fig.2. At 10 and 100uM, ascorbyl palmitate demonstrated antioxidant activity. At 2uM, ascorbyl palmitate showed no effect, and at 500 and 1000uM, it demonstrated an initial prooxidant effect that plateaued after 1 hr. incubation. This is similar to the effect of ascorbate on OxyMb oxidation in liposomes as presented by Yin et al. (1993). The similarity of this effect to that of ascorbate would suggest that ascorbate and ascorbyl palmitate are acting as antioxidants in a similar manner. However, Yin et al. (1993) reported an effect for ascorbate opposite to that of ascorbyl palmitate for lipid oxidation. Ascorbyl palmitate is fat-soluble and incorporates into the lipid bilayer. The actual orientation of the molecule within the membrane is unknown, but it is expected that the hydrophilic reducing end of the molecule would protrude from the lipid bilayer. The location of ascorbyl palmitate is likely different from that of ascorbate and may be responsible for the observed differences.

The effects of ascorbyl palmitate were also compared to those of α-tocopherol at 0, 10, and 100uM concentrations. α-Tocopherol and ascorbyl palmitate appeared to have equal ability to delay lipid oxidation (Fig.3). At 10uM, α-tocopherol was more efficient in protecting OxyMb from oxidation than ascorbyl palmitate. At 100uM, ascorbyl palmitate had no antioxidant activity, and α-tocopherol was a strong antioxidant, decreasing OxyMb oxidation by 33%, relative to controls (Fig.4).

CONCLUSION - Ascorbyl palmitate was similar to α-tocopherol in effectively delaying lipid oxidation. At low concentrations, ascorbyl palmitate was slightly antioxidant or had no effect toward OxyMb, but as its concentration was increased it became strongly prooxidative.

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FIGURE LEGENDS -

Fig. 1- Effect of ascorbyl palmitate on lipid oxidation (TBARS) in phosphatidylcholine Mb-liposomes during incubation at 37°C, pH 5.6.

Fig. 2- Effect of ascorbyl palmitate on metmyoglobin formation in phosphatidylcholine Mb-liposomes during incubation at 37°C, pH 5.6.

Fig. 3- Effect of ascorbyl palmitate or α-tocopherol on lipid oxidation (TBARS) in phosphatidylcholine Mb-liposomes at 1 hr. incubation at 37°C, pH 5.6.

Fig. 4-Effect of ascorbyl palmitate or α-tocopherol on metmyoglobin formation in phosphatidylcholine liposomes at 1 hr. incubation at 37°C, pH 5.6.