Ability of Surfactant Hydrophobic Tail Group Size To Alter Lipid Oxidation in Oil-in-Water Emulsions

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Oxidation of oil-in-water emulsion droplets is influenced by the properties of the interfacial membrane surrounding the lipid core. Previous work has shown that an important factor in the oxidation of oil-in-water emulsions is surfactant properties that impact interations between water-soluble prooxidants and lipids in the emulsion droplet. The purpose of this research was to study the impact of surfactant hydrophobic tail group size on lipid oxidation in oil-in-water emulsions stabilized by polyoxyethylene 10 lauryl ether (Brij-lauryl) or polyoxyethylene 10 stearyl ether (Brij-stearyl). The ability of iron to decompose cumene peroxide was similar in hexadecane emulsions stabilized by Brij-stearyl and Brij-lauryl. Oxidation of methyl linoleate in hexadecane emulsions containing cumene peroxide was greater in droplets stabilized by Brij-lauryl than in those stabilized by Brij-stearyl at pH 3 with no differences observed at pH 7.0. Oxidation of salmon oil was greater in emulsions stabilized by Brij-lauryl than in those stabilized by Brij-stearyl as determined by both lipid peroxides and headspace propanal. These results suggest that surfactant hydrophobic tail group size may play a minor role in lipid oxidation in oil-in-water emulsions.

Keywords: *Lipid oxidation; emulsions; surfactants; prooxidants; iron*

INTRODUCTION

Lipids are commonly found in foods as oil-in-water emulsions that are stabilized by surfactants. Such foods can be susceptible to lipid oxidation especially if they contain high amounts of polyunsaturated fatty acids. The oxidation of lipids in oil-in-water emulsions is influenced by the properties of the droplet interfacial membrane, since this membrane impacts interactions between continuous phase prooxidants, such as iron, with fatty acids and lipid hydroperoxides in the emulsion droplet core.

A number of studies have recently been conducted to investigate the influence of emulsion droplet interfacial characteristics on lipid oxidation. The charge of the emulsion droplet interfacial membrane is an important determinant of the oxidative stability of oil-in-water emulsions. Lipid peroxides are more rapidly broken down by Fe^{2+} in anionic emulsion droplets prepared with sodium dodecyl sulfate (SDS) than in emulsions stabilized with nonionic (polyoxyethylene 10 lauryl ether, Brij) or cationic (dodecyltrimethylammonium bromide, DTAB) surfactants (Mei et al., 1998a; Mancuso et al., 1999, 2000). While Fe^{2+} can promote decomposition of lipid peroxides in anionic, cationic, and nonionic emulsion droplets, Fe^{3+} was only observed to promote cumene hydroperoxide decomposition in anionic emulsion droplets (Mancuso et al., 2000). Oxidation of emulsified corn and salmon oil behaves very similar to the stability of lipid peroxides, with oxidation rates being greatest in emulsion droplets stabilized with anionic SDS compared to cationic DTAB and nonionic Brij or Tween 20 (Mei et al., 1998a; Mancuso et al., 1999). This increased reactivity of iron in emulsions stabilized by anionic surfactants is believed to be due to the electrostatic attraction between the positively charged metal and the negatively charged emulsion droplet membrane.

Jacobsen et al. (1999) found that the physical location and chemical state of iron are also important determinants in the oxidative stability of the lipid emulsion in mayonnaise. In their study, mayonnaise containing egg yolk was found to oxidize faster in the presence of ascorbic acid. This was attributed to the ascorbate-dependent release of iron from proteins such as phosvitin that were located at the emulsion droplet interface since the presence of ascorbate increased aqueous-phase iron concentrations. The authors postulated that ascorbate's ability to both reduce iron (ferrous iron is a stronger prooxidant than ferric iron; Dunford, 1987) and release iron from phosvitin (phosvitin is able to inhibit iron-promoted lipid oxidation; Lu and Baker, 1987) increased the ability of iron to decompose existing lipid peroxides and thus promote lipid oxidation.

Lipid oxidation reactions are also influenced by the thickness of the emulsion droplet interfacial membrane as recently observed in a study where oil-in-water emulsions were prepared with polyoxyethylene 10 stearyl ether (Brij 76) or polyoxyethylene 100 stearyl ether (Brij 700), which are structurally identical surfactants except for the length of their hydrophilic headgroups, with Brij 700 containing 10 times more polyoxyethylene groups than Brij 76 (Silvestre et al., 2000). Fe^{2+}-promoted decomposition of cumene hydroperoxide was lower in Brij 700- than Brij 76-stabilized hexadecane emulsions. Fe^{2+}-promoted α-tocopherol oxidation rates were similar in hexadecane emulsions regardless of surfactant type. Brij 700 decreased production of hexa-

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nal from methyl linoleate and the formation of lipid peroxides and propanal from salmon oil compared to by Brij 76. These results indicate that the emulsion droplet interfacial thickness may also be an important determinant in the oxidative stability of food oil-in-water emulsions.

Surfactant properties can potentially be important in lipid oxidation through their ability to alter the properties of the emulsion droplet interfacial membrane. The objective of this research was to use two different surfactants which varied in hydrophobic tail group size to evaluate whether this factor also influenced the oxidative stability of oil-in-water emulsions since hydrophobic tail group size could influence surfactant packing properties that would alter interactions between droplet lipids and continuous phase prooxidants. To produce emulsions with differences in hydrophobic tail group size, polyoxyethylene 10 lauryl ether (Brij-lauryl) or polyoxyethylene 10 stearyl ether (Brij-stearyl) was used as surfactant. These are structurally identical with Brij-stearyl and Brij-lauryl containing 18 and 12 carbon atoms in their hydrophobic tail groups, respectively.

MATERIALS

Salmon oil was isolated by centrifuging minced salmon muscle (Mei et al., 1998b). Hexadecane, cumene hydroperoxide, Brij-lauryl, Brij-stearyl, ferrous sulfate, imidizole, sodium acetate, and methyl linoleate were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade or purer.

METHODS

Preparation of Emulsions. Emulsions were prepared by mixing either hexadecane or salmon oil with aqueous solutions of Brij-lauryl or Brij-stearyl (0.017 M) in acetate−imidizole buffer solution (5 mM each) to give a final emulsion of 5% lipid. Cumene hydroperoxide (1.1 mmol/L of emulsion) or cumene hydroperoxide (5.7 mmol/L of emulsion) plus methyl linoleate (1 mmol/L of emulsion) was added to hexadecane prior to sonication. Solutions were sonicated using a Braun-Sonic 2000 U ultrasonic generator (Braun Biotech, Allentown, PA) equipped with a 5T standard probe at a power setting of +150 and 0.3 s repeating cycle. Emulsions were sonicated for different times (hexadecane, Brij-lauryl 150 s, Brij-stearyl 110 s; salmon oil, Brij-lauryl 240 s, Brij-stearyl 190 s) to obtain emulsion droplets with average diameters ranging from 0.38 to 0.42 μm with an overall mean diameter of 0.40 + 0.03 μm. Particle size distributions were measured using a laser scattering particle size distribution analyzer (LA-900 Horiba Instruments, Irvine, CA) (Weiss et al., 1996). Particle size distributions were measured periodically and did not change over the course of the experiments, indicating that coalescence or Oswald ripening did not occur. The sonication conditions used did not alter initial concentrations of peroxides, hexanal, or propanal. All emulsions were adjusted to pH 3.0 or 7.0 by the addition of NaOH or HCl after sonication. Hexadecane emulsions were oxidized at 55 °C in the presence or absence of ferrous sulfate (500 μM for cumene hydroperoxide and 50 μM for cumene hydroperoxide plus methyl linoleate). Salmon oil emulsions were oxidized at 32 °C in the dark.

Determination of Lipid Peroxide Concentrations. Lipid peroxide concentrations in the emulsions were extracted by adding 0.3 mL of the emulsion to 1.5 mL of isooctane−2-propanone (3:2; v/v), followed by vortexing three times for 10 s each. After centrifugation for 2 min at 2000*g*, 0.2 mL of the clear upper layer was collected, and peroxides were quantitated using a modified method of Shanta and Decker (1994). The sample extract (0.2 mL) was mixed with 2.8 mL of methanol/1-butanol (2:1; v/v) and 30 μL of thiocyanate/Fe^{2+} solution and then vortexed. The thiocyanate/Fe^{2+} solution was made by mixing one part 3.94 M thiocyanate solution with one part 0.072 M Fe^{2+} solution (obtained from the supernatant of a mixture of 1 part 0.144 M $FeSO_4$ and 1 part 0.132 M $BaCl_2$ in 0.4 M HCl). After 20 min of incubation at room temperature, absorbance was measured at 510 nm. Lipid peroxide concentrations were determined using a cumene hydroperoxide standard curve.

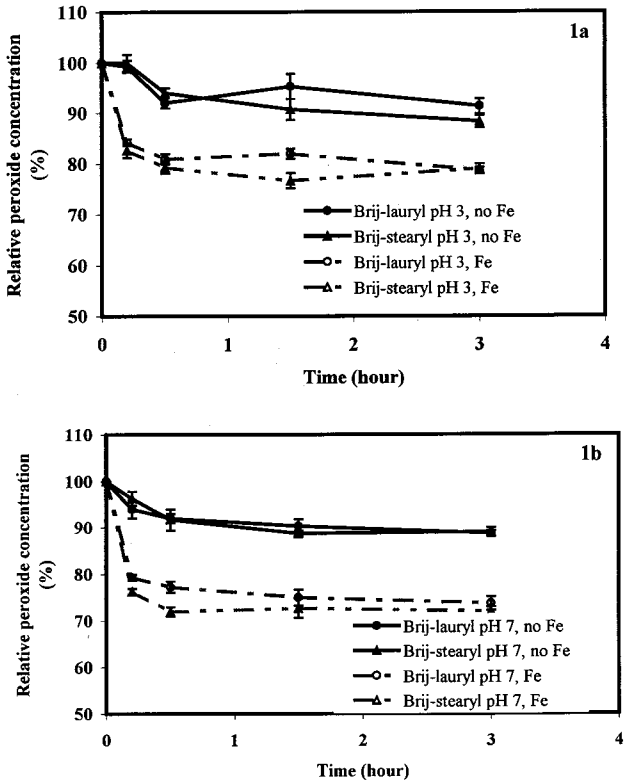

Figure 1. Decomposition of cumene hydroperoxide (initial concentration 1.1 mM) in hexadecane emulsion stabilized by Brij-lauryl or Brij-stearyl at pH 3.0 (a) and 7.0 (b) and 55 °C in the presence and absence of ferrous sulfate (500 μM). Data points represent means ($n = 3$) ± standard deviations.

Determination of Hexanal Concentrations in Hexadecane Emulsions. Hexanal concentrations in the hexadecane emulsions containing methyl linoleate (1.0 mL) that were sealed in 10 mL headspace vials with poly(tetrafluoroethylene)/butyl rubber septas were determined using a Hewlett-Packard (HP) 5890A gas chromatograph (Avondale, PA) with a HP 19395A headspace sampler and coupled to a HP 3392A integrator. The headspace conditions were as follows: sample temperature, 55 °C; sample loop and transfer line temperature, 110 °C; pressurization, 10 s; venting, 10 s; injection, 1.5 min. The aldehydes were separated isothermally at 65 °C on a HP methyl silicone (DB-1) fused silica capillary column (50 m, 0.31 mm i.d., 1.03 μm film thickness). The splitless injector temperature was 180 °C, and the eluted compounds were detected with a flame ionization detector at 250 °C. Concentrations were determined from the peak areas using a standard curve made from authentic hexanal in hexadecane emulsions containing Brij-lauryl or Brij-stearyl (Silvestre et al., 2000).

Determination of Propanal Concentrations in Salmon Oil Emulsions. Propanal concentrations in the salmon oil emulsions were determined using the same conditions as described for headspace hexanal with the exception of sample temperature, 40 °C; equilibration time, 5 min; oven temperature, 70 °C; and detector temperature, 200 °C (Silvestre et al., 2000). Concentrations were determined from the peak areas using a standard curve made from authentic propanal since it was determined that the emulsions did not influence the amount of propanal partitioning into the headspace.

Statistical Analysis. Assays were carried out using triplicate samples. Statistical analysis was performed using

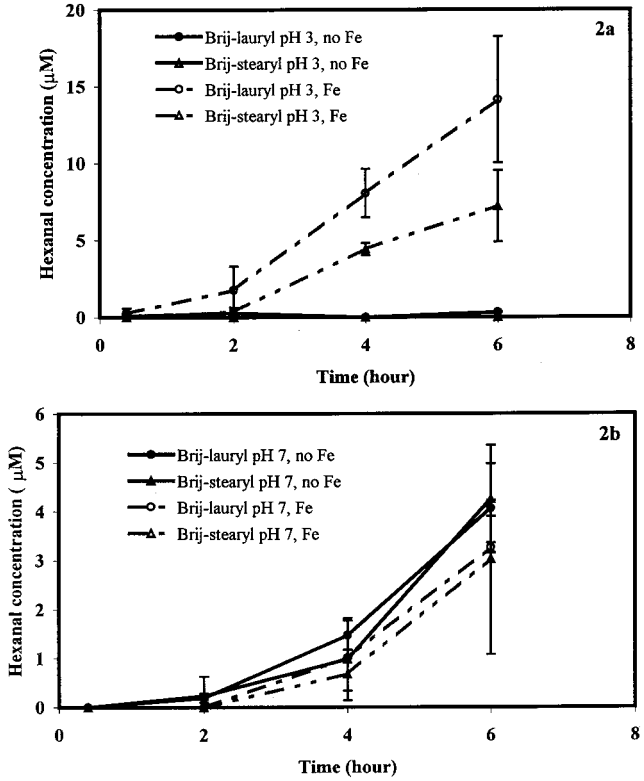

Figure 2. Formation of hexanal in hexadecane and methyl linoleate (1 mM) emulsion stabilized by Brij-lauryl or Brij-stearyl at pH 3.0 (a) and 7.0 (b) and 55 °C in the presence and absence of ferrous sulfate (50 μM). Emulsions contained 5.7 mM cumene hydroperoxide. Data points represent means (n = 3) ± standard deviations.

analysis of variance. Mean separations were achieved using the Student t test (Snedecor and Cochran, 1989).

RESULTS

The ability of hydrophobic tail group length to impact Fe^{2+}-promoted breakdown of cumene hydroperoxide was studied in emulsions prepared with hexadecane (a nonoxidizable lipid that does not form additional peroxides in the presence of free radicals). Cumene hydroperoxide was used as a model of a lipid peroxide. Fatty acid hydroperoxides were not used due to their potential to undergo further oxidation reactions, thus creating more hydroperoxide. Cumene hydroperoxide concentrations in Brij-stearyl- and Brij-lauryl-stabilized emulsions decreased during storage in the absence of added Fe^{2+} at both pH 3.0 and pH 7.0 (Figure 1), with no significant differences occurring between different surfactants. Addition of Fe^{2+} significantly ($p \leq 0.05$) increased cumene hydroperoxide decomposition compared to no added Fe^{2+} controls in both the Brij-stearyl- and Brij-lauryl-stabilized emulsions (Figure 1). Fe^{2+}-promoted decomposition of cumene hydroperoxide occurred rapidly, with the majority of peroxide breakdown occurring within the first 0.2 h, after which only small changes in peroxide concentrations were observed. This pattern of rapid peroxide decomposition by Fe^{2+} has also been observed in emulsions stabilized by other surfactants (Brij 76, Brij 700, Tween 20, and DTAB) and is likely due to the rapid depletion of Fe^{2+} with the resulting Fe^{3+}, being unable to significantly promote additional cumene hydroperoxide decomposition (Silvestre et al., 2000; Macuso et al., 2000). Decomposition of cumene peroxide by Fe^{2+} was similar in emulsions

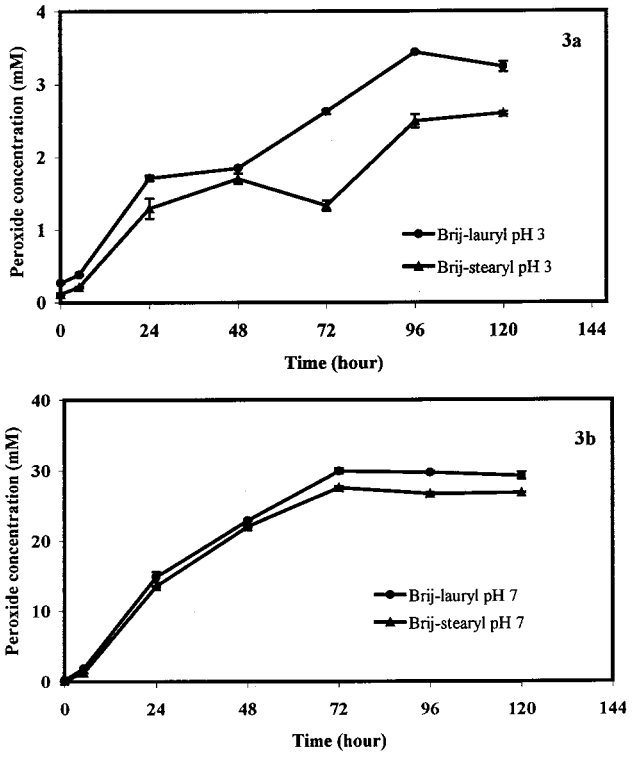

Figure 3. Formation of lipid peroxide in salmon oil emulsion stabilized by Brij-lauryl or Brij-stearyl at pH 3.0 (a) and 7.0 (b) and 32 °C in the dark. Data points represent means (n = 3) ± standard deviations.

droplets stabilized by the two different surfactants, with significant differences ($p \leq 0.05$) only being observed at 1.5 h at pH 3.0 (Brij-stearyl > Brij-lauryl; Figure 1a) and at 0.2 and 0.5 h at pH 7.0 (Brij-stearyl > Brij-lauryl; Figure 1b). Although some significant differences were observed between the surfactants, these differences were small, ranging from 3% to 5.3%.

The impact of hydrophobic tail group length on the ability of free radicals arising from the decomposition of cumene peroxide to promote oxidation of fatty acids was determined in hexadecane emulsions containing cumene hydroperoxide and methyl linoleate at both pH 3.0 and pH 7.0 (Figure 2). Headspace hexanal, a common breakdown product of the oxidation of linoleic acid, was used to monitor methyl linoleate oxidation. For both surfactants, hexanal concentrations did not increase in the absence of cumene hydroperoxide. At pH 3.0, Fe^{2+}-promoted hexanal formation was significantly ($p \leq 0.05$) greater (1.1−5.0-fold) at 4 and 6 h of incubation in the Brij-lauryl-stabilized than in the Brij-stearyl-stabilized hexadecane emulsions. Hexanal formations in the Brij-lauryl- and -stearyl-stabilized emulsions were similar at pH 7.0.

The impact of surfactant hydrophobic tail group length on lipid oxidation in salmon oil emulsions stabilized with Brij-stearyl or Brij-lauryl at pH 7.0 and 3.0 in the absence of added iron was determined by monitoring the formation of lipid peroxides and headspace propanal (a common lipid oxidation product of n − 3 fatty acids). Formation of lipid peroxides was significantly ($p \leq 0.05$) greater in the Brij-lauryl-stabilized than in the Brij-stearyl-stabilized emulsions (differences ranged from 1.04-fold to 2.2-fold) at both pH 3.0 and pH 7.0 (Figure 3). Propanal formation was greater in the Brij-lauryl-stabilized than in the Brij-stearyl-stabilized salmon oil emulsions at all times

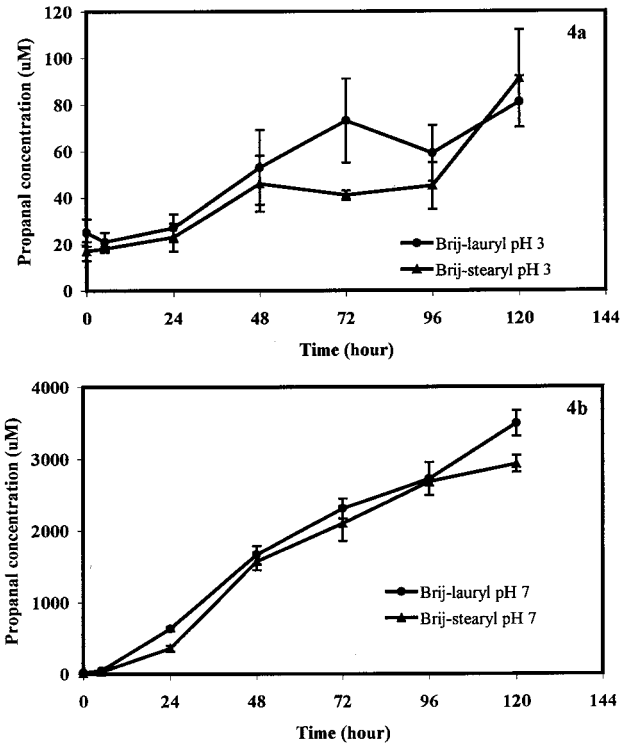

Figure 4. Formation of headspace propanal in salmon oil emulsion stabilized by Brij-lauryl or Brij-stearyl at pH 3.0 (a) and 7.0 (b) and 32 °C in the dark. Data points represent means ($n = 3$) ± standard deviations.

analyzed (Figure 4); however, this difference was only significant at 72 h (1.8-fold greater propanal) of incubation at pH 3.0 and at 5 (1.7-fold greater propanol), 24 (1.8-fold greater propanol), and 120 (1.2-fold greater propanal) h of incubation at pH 7.0.

Little to no difference in cumene hydroperoxide stability in the presence of Fe^{2+} was observed as a function of surfactant hydrophobic tail group size. Much larger differences were observed between surfactants during the oxidation of methyl linoleate and salmon oil, with oxidation rates decreasing with increasing hydrophobic tail group size. Lipid oxidation reactions have also been evaluated in emulsions prepared with surfactants that had varying hydrophillic headgroup sizes (Brij 76 and Brij 700; Silvestre et al., 2000). The impact of hydrophobic tail groups versus hydrophilic headgroups in these two studies is difficult to compare since the hydrophobic tail groups differed in only 6 carbons while the hydrophilic headgroups differed by 180 carbons. However, similar trends were observed in both studies, with oxidative reactions decreasing as the size of the surfactant increased.

The greater polarity of cumene hydroperoxide compared to hexadecane would drive the peroxide to accumulate at the surface of the emulsion droplet. Since both Brij-stearyl and Brij-lauryl have identical hydrophilic headgroups, it is not surprising that no major differences were observed in the ability of Fe^{2+} to decompose cumene hydroperoxide. Upon the decomposition of lipid peroxides at the emulsion droplet interface, the resulting free radicals would have to migrate into the lipid core of the emulsion droplet to promote lipid oxidation. The ability of increasing hydrophobic tail group length to decrease the oxidation of methyl linoleate and salmon oil suggests that the longer tail group size decreases the ability of the free radicals originating from cumene hydroperoxide to reach the polyunsaturated fatty acids. This could be due to the thicker barrier provided by the larger hydrophobic tail groups that would make it more difficult for the free radicals to reach the fatty acids in the lipid core or could be due to tighter packing of the surfactant tail groups since intermolecular interactions (e.g., van der Waals interactions) between the tail groups would be expected to increase with increasing carbon number. Tight packing of the hydrophobic tail groups could again make transfer of free radicals into the lipid core more difficult and could also decrease the ability of unsaturated fatty acids to partition into the interfacial region of the emulsion droplet membrane.

This research further supports the notion that the characteristics of the interfacial membrane of emulsion droplets is an important factor in lipid oxidation reactions. While it may not be practical to substantially increase the oxidative stability of food emulsions by increasing surfactant hydrophobic tail group length alone, this may be an additional factor to consider when a multifaceted system is designed to inhibit lipid oxidation.

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