Oxidative Effects of Animal-Originated Porphyrins and Riboflavin on Cholesterol Oxidation in an Aqueous Model System

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ABSTRACT: This study was executed to investigate effects of animal-originated porphyrins and riboflavin on cholesterol oxidation in aqueous model systems. Changes of headspace oxygen contents, cholesterol, and cholesterol oxide products (COP) in the model systems were measured by gas chromatography during storage under light. As concentration of protoporphyrin increased, contents of headspace oxygen decreased and COP increased. The same trend as that of protoporphyrin occurred with riboflavin in terms of contents of headspace oxygen, but production of COP was the highest at 5 ppm riboflavin. As concentrations of hemoglobin and myoglobin increased, headspace oxygen content and COP production were not changed significantly. Consequently, protoporphyrin could be the most active catalyst on the cholesterol oxidation in the aqueous system, but myoglobin and hemoglobin did not accelerate cholesterol oxidation.

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KEY WORDS: Cholesterol oxidation products, headspace oxygen, hemoglobin, myoglobin, protoporphyrin, riboflavin.

Cholesterol plays important roles in several biological functions in the body, including serving as a precursor for all of the steroid hormones, vitamin D, and bile acids as well as providing a balance in the fluidity of tissue membranes (1). However, numerous cholesterol oxidation products (COP) have been implicated in adverse human health effects such as angiotoxicity, arteriosclerosis, cytotoxicity, mutagenicity, carcinogenicity, and others (2–4).

The susceptibility of cholesterol to oxidation has been well documented, and over 60 products formed through auto-oxidation, photo-oxidation, and photosensitized oxidation have been reported (5). Therefore, the occurrence of COP in foods becomes an important issue in food science as well as the medical research area. Numerous investigators have studied the conditions for the formation of cholesterol oxides, i.e., (i) exposure to elevated temperatures in the presence of air for even a brief period (6) and (ii) prolonged storage in air at ambient temperatures (7), or (iii) storage under illumination (8). The auto-oxidation of cholesterol in foods has been reported for many years by numerous researchers, and mechanisms of formation of COP have been identified.

Food pigments are potential photosensitizers. Among the natural pigments, porphyrins of plant or animal origin, such as chlorophylls or myoglobins, are well-known singlet oxygen sensitizers. Red meat is one of the most important sources of dietary cholesterol as well as one of the major reservoirs of myoglobin. Myoglobin was reported to be an effective photosensitizer in generating singlet oxygen in methyl linoleate model system (9,10). However, Whang and Peng (11) reported that myoglobin and apomyoglobin did not act as photosensitizers under 1200 ft-candle light. The sensitizing effect of animal-originated porphyrins in photosensitized oxidation of methyl linoleate is still a controversial issue. Furthermore, information on the relationship between photosensitizers and formation of cholesterol oxides in red meat is not generally available at present. While riboflavin was reported to act as a photosensitizer of milk lipid in light-induced oxidation (12), oxidative effects of riboflavin on cholesterol oxidation under light have scarcely been investigated.

The objective of the present study was to examine the effects of animal-originated porphyrins such as protoporphyrin, myoglobin, hemoglobin, and riboflavin on cholesterol oxidation in the aqueous model system during storage at different concentrations.

MATERIALS AND METHODS

Materials. Cholesterol, 5α-cholestane, 5-cholesten-3β,7β-diol, 5-cholesten-3β,25-diol, 5-cholesten-3β-ol-7-one, 5α-cholestan-3β,5α,6β-triol, 3,5-cholestadien-7-one, 5α-cholestan-3-one, and 5-cholesten-3-one were purchased from Sigma Chemical (St. Louis, MO). 5-Cholesten-3β,7α-diol, 5,6α-epoxy-5α-cholestan-3β-ol, and cholestan-β,6β-epoxy-3β-ol were purchased from Steraloids, Inc. (Wilton, NH).

Cholesterol dispersion preparation. The aqueous model system used to examine cholesterol oxidation was prepared according to the modified method of Rankin and Pike (13). The aqueous dispersion consisted of 0.0025 M cholesterol prepared by dissolving cholesterol and sodium dodecyl sulfate in ethanol and then adding 0.01 M histidine buffer prepared with distilled, deionized water. Cholesterol dispersion (15 mL) was pipetted

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into 30-mL serum bottles (Supelco Inc., Bellefonte, PA). Hemoglobin, myoglobin, protoporphyrin, and riboflavin were added to the serum bottles and the pH was then adjusted to 5.5 by using 2 N HCl. The concentrations of hemoglobin and myoglobin were 0, 50, 100, 250, 500, and 1000 ppm, and protoporphyrin and riboflavin were 0, 5, 15, 30, and 60 ppm, respectively.

Storage. The sample bottles were sealed air-tight with rubber septa and aluminum caps. The bottles were placed in a Biochamber (Nippon Medical & Chemical Co., Tokyo, Japan) under 4,500 lux illumination at 25°C for 48 h.

Headspace oxygen contents. The contents of headspace oxygen were measured by gas chromatography (GC) (model 5890; Hewlett-Packard, Avondale, PA) equipped with stainless steel column (6 ft × 2 mm i.d.) and a thermal conductivity detector.

COP. Cholesterol oxides were measured according to the methods of Park and Addis (6) with slight modification. A 2.5-mL sample of cholesterol dispersion was removed from each 30-mL serum bottle and extracted three times with 10 mL ethyl acetate after adding 1 mL saturated NaCl solution and 5α-cholestane as an internal standard. Moisture in the collected extracts was removed with Na_2SO_4. Samples were concentrated by R110 Büchi Rotary Evaporator under nitrogen gas flow and then dissolved in 1 mL reagent grade pyridine. The samples were mixed well with 50 µL Sylon BTZ (*N*-trimethylsilylimidazol/*N,O*-bis-[trimethylsilyl]acetamide/trimethylchlorosilane, 3:2:3, by vol; Supelco) for derivatization of sterols into corresponding trimethylsilyl ether sterols in vials with Teflon-lined caps. Quantification of COP was performed using GC (PerkinElmer) with a flame-ionization detector. A 1-µL sample was injected onto a DB-1 capillary column (J&W Scientific Inc., Rancho Cordova, CA; 15 m × 0.25 mm i.d., 0.1 µm film thickness); integrator (PerkinElmer) quantified peaks.

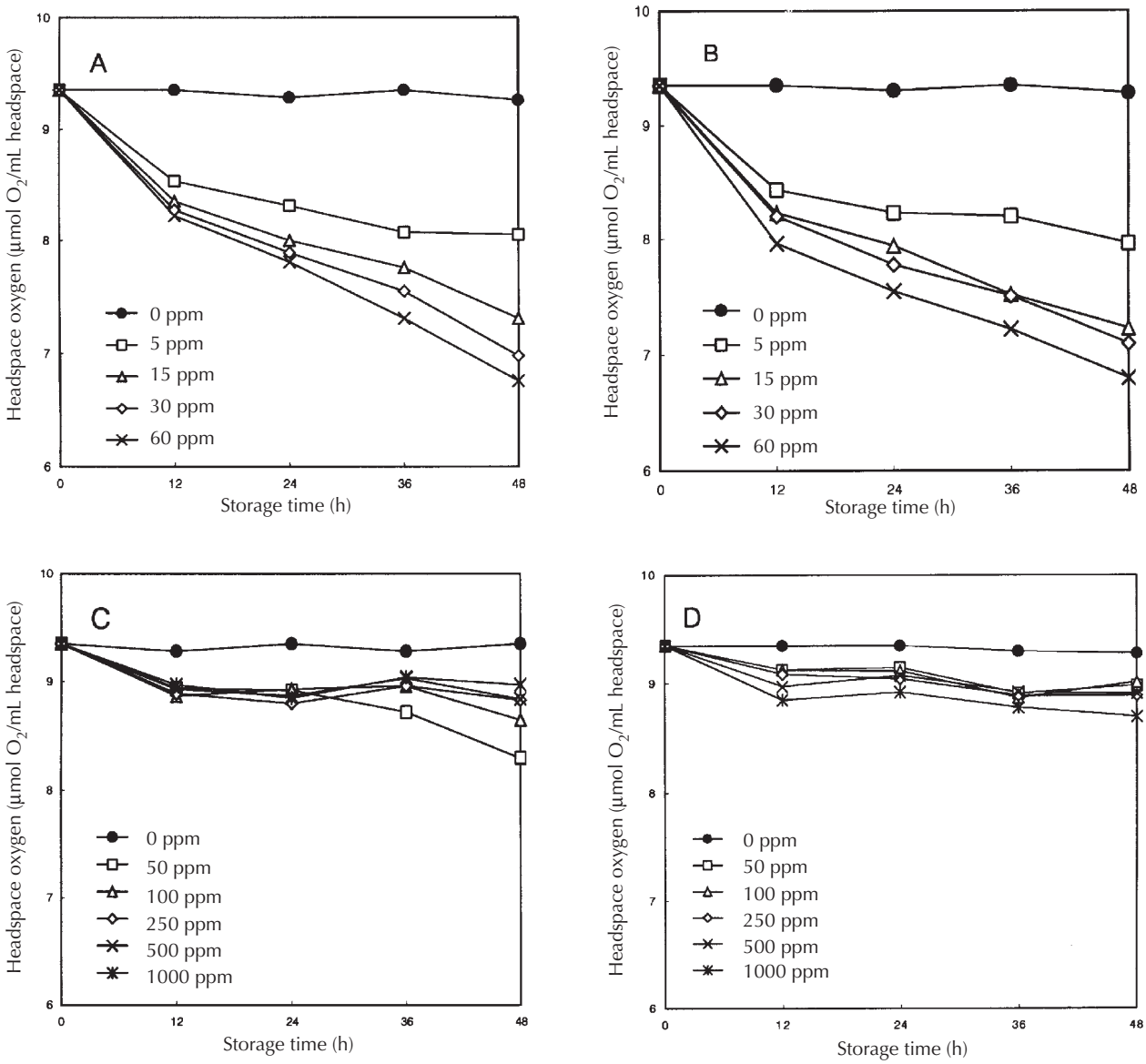

FIG. 1. Effects of animal-originated porphyrins and riboflavin on the headspace oxygen depletion in an aqueous cholesterol dispersion during storage under fluorescent light at 25°C. (A) Protoporphyrin, (B) riboflavin, (C) hemoglobin, and (D) myoglobin.

Flow rate of the nitrogen carrier gas was 2 mL/min, and the split ratio was 1:100. Initial column temperature was 180°C, which was increased to final 250°C at 3°C/min with final holding time of 5 min. Temperatures of injection port and detector were 280 and 300°C, respectively. Peak identification was based on retention time of standards.

RESULTS AND DISCUSSION

Headspace oxygen depletion. Figure 1 shows the changes of headspace oxygen contents measured by GC in the sample bottles containing different concentrations of animal-originated porphyrins during 48-h storage. The coefficient of variation for the headspace oxygen analysis by GC was 1.78%. There were no significant changes of headspace oxygen contents during storage in sample bottles that did not contain animal-originated porphyrins during 48-h storage. As the concentrations of protoporphyrin and riboflavin increased from 0 to 60 ppm (Fig. 1A,B), the depletion rate of headspace oxygen in the aqueous model system increased. After 48-h light storage, the contents of headspace oxygen in the serum bottles containing different concentrations of the protoporphyrin and riboflavin were markedly decreased.

The headspace oxygen contents in sample bottles containing different concentrations of myoglobin and hemoglobin were decreased gradually during 48-h storage. The concentration of the myoglobin and hemoglobin did not affect depletion rate of headspace oxygen contents. (Figs. 1C, 1D).

These results suggested that protoporphyrin and riboflavin accelerate oxidation of cholesterol, but myoglobin and hemoglobin do not. Denatured myoglobin, which released Fe ion, could be an effective catalyst of lipid oxidation (9,10,14,15). Whang and Peng (11) reported that protoporphyrin was the most effective sensitizer among myoglobin and its derivatives in their research on singlet oxygen oxidation measured by electron paramagnetic resonance (EPR) spectroscopy. Bradley and Min (16) reported that formation of singlet oxygen in milk containing riboflavin under illumination was confirmed by EPR technique.

COP. The effects of animal-originated porphyrins and riboflavin on the formation of COP in the aqueous model system

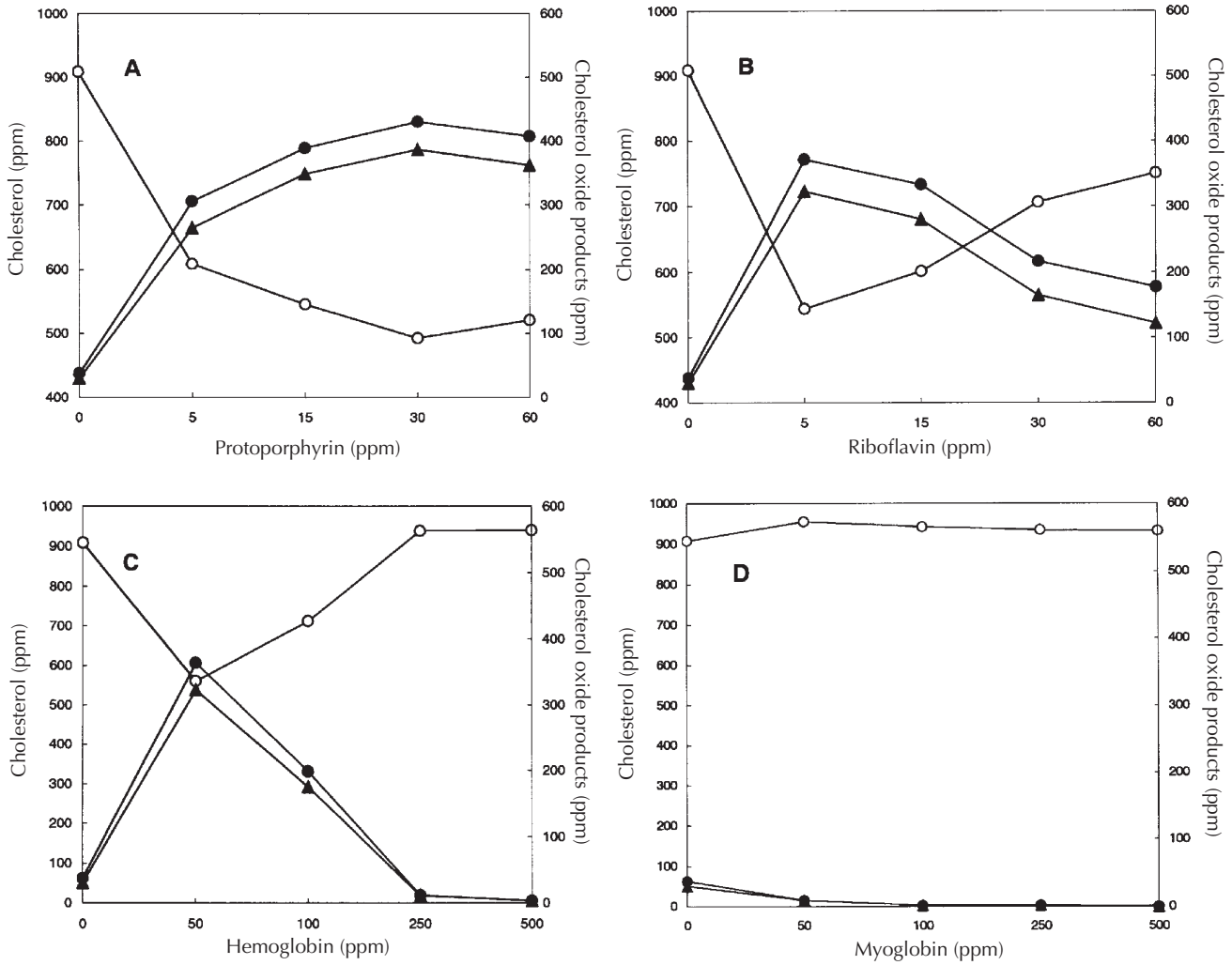

FIG. 2. Effects of animal-originated porphyrins and riboflavin on cholesterol oxidation in an aqueous cholesterol dispersion during storage under fluorescent light at 25°C for 48 h. Cholesterol (○), 7-cholesterol oxide products (▲) , and total cholesterol oxide products (●).

containing different concentrations during 48-h storage are shown in Figure 2. As the concentration of the protoporphyrin increased from 0 to 60 ppm, the amount of COP in the aqueous model system increased. However, as the concentration of the riboflavin increased from 0 to 60 ppm, the formation of COP decreased. The formation of COP in the aqueous model system was highest at 5 ppm riboflavin, followed by 15, 30, and 60 ppm (Fig. 2A,B). Trends of total COP formation in the aqueous model system were similar to those of the formation of 7-derivatives, which are 7α-hydroxy cholesterol, 7β-hydroxy cholesterol, and 7-ketocholesterol.

The concentrations of myoglobin did not significantly affect the formation of COP in the aqueous model system after 48-h light storage. The formation of COP by hemoglobin in the aqueous model system was highest at the 50 ppm hemoglobin concentration followed by 100 ppm, but there were no significant changes at 250, 500, and 1,000 ppm hemoglobin (Figs. 2C, 2D). Nakamura and Nishida (17) reported that oxidation of unsaturated fatty acid by high concentrations of hemoglobin is dependent upon the binding ability between hemoglobin and fatty acids.

Protoporphyrin and riboflavin accelerated formation of COP in the model system. As the concentration of the protoporphyrin increased from 0 to 60 ppm, the amount of COP in the aqueous model system increased gradually. However, as the concentration of the riboflavin increased from 5 to 60 ppm, the formation of COP decreased. The formation of COP was not affected significantly by the concentration of myoglobin and hemoglobin.

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