Antioxidant Properties of Deferoxamine

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Deferoxamine, a natural trihydroxamate, was tested for its antioxidant properties. A significant antioxidant effect was detected in linoleic and linolenic acid suspensions, as well as in linoleic acid and fish oil emulsions. Its antioxidant activity was compared to that of butylated hydroxyanisole (BHA) and quercetin. In both emulsions and suspensions, the antioxidant effect of deferoxamine was in the same concentration range of BHA and quercetin. The antioxidant effect of deferoxamine in emulsions was lower than that of BHA. The effect of 0.770 mM deferoxamine in suspensions was the same as that of 0.555 mM BHA. Deferoxamine antioxidant activity in these systems was independent of its capability to bind iron. Inhibition of β-carotene degradation in linoleic acid-Tween 40 emulsion was lower than that of BHA. Deferoxamine inhibited the activity of soy lipoxygenase. The inhibition of lipoxygenase was reversible, but its mechanism is still unknown.

KEY WORDS: Antioxidant; deferoxamine (desferrioxamine, desferal); soy lipoxygenase.

Deferoxamine (DFO), isolated from *Streptomyces pylosus*, belongs to the group of hydroxamic acids that form complexes with iron ions (1–3). Today it is recognized primarily as a chelator with particularly high affinity for trivalent iron ions ($K_b = 10^{31}$) (4). It is used therapeutically in chronic hyperferremia, acute iron poisoning and chronic aluminum overload (5).

Trihydroxamate–DFO has recently been shown to inhibit oxidation in various biological systems, such as the sickle erythrocyte membranes (6). Additionally, the antioxidant activity detected in brain homogenate, erythrocyte suspension or mouse liver has mostly been attributed to the iron-binding capacity of this reagent (7). Other investigators, however, have attributed its antioxidant activity to the donation of a proton or electron in mixtures of horseradish peroxidase with hydroperoxide (8,9) or to its interaction with superoxide radicals (10). Harteley *et al.* (11) have demonstrated that DFO acts as a chain-breaking radical scavenger, independently of its chelating capacity, by donating an electron or hydrogen atom from the hydroxamate center. DFO is poorly absorbed when administered orally so it needs to be injected when used medicinally (12).

DFO contains three units of hydroxamic acid, and because the latter is known to inhibit human lipoxygenase 5′, it is speculated that the inhibition stems from its interaction with a ferric ion at the active site (13). Because known derivatives of hydroxamic acid undergo rapid metabolism to form inactive carboxylic acid, attempts are being made to devise stable derivatives of this acid for oral administration in the treatment of asthma, psoriasis and rheumatoid arthritis (13,14).

In the present study, we explored the possibility of using DFO as an antioxidant in model systems, taking advantage of its diverse properties as antioxidant, chelator and possible inhibitor of plant lipoxygenase.

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MATERIALS AND METHODS

Methansulfonic DFO [Desferal (c)], was obtained from Ciba Co. (Basle, Switzerland). Linoleic and linolenic acids and quercetin were products of Fluka (AG Buchs, Switzerland). The fish oil was medicinal cod liver oil DAB7 (Henry Lamotte, Bremen, Germany). Butylated hydroxyanisole (BHA), β-carotene, Tween 40 and the soy lipoxygenase (Type 1-B, EC 1.13.11.12.) were products of Sigma Israel Chemical Co. (Holon, Israel). Tween 20 was purchased from Serva Biochemical (Westbury, NY).

Alcoholic suspensions. The antioxidant effect was assayed in the water/alcohol system of Osawa and Namiki (15). Antioxidant was added to a mixture containing 4 mL phosphate buffer (0.01 M) pH 7.0, 2 mL distilled water, 2 mL ethanol and 2 mL of a 2.5% solution of either linoleic or linolenic acid in ethanol. The mixture was then incubated in the dark at 42°C. Every 24 h, 50-μL samples were withdrawn into 3 mL of borate buffer, pH 8.5. The oxidation was measured by absorption of the conjugated dienes at 234 nm (ultraviolet-visible spectrophotometer M330; Camsped Ltd., Saloston, Cambridge, United Kingdom).

Emulsions. The antioxidant activity was assayed by the method of Cillard *et al.* (16) with minor modifications. Briefly, a solution of 0.5% Tween 20 and the tested antioxidant in phosphate buffer, pH 7.0, was mixed with an equal volume of 0.5% Tween 20 and 0.28% of the tested fatty acid or fish oil in phosphate buffer (pH 7.0) and the mixture placed in the dark at 22°C. The oxidation was determined on samples of 100 μL taken every 24 h into 2 mL of phosphate buffer (pH 7.0), by reading the absorption at 234 nm.

Emulsion with β-carotene and linoleic acid. Destruction of β-carotene was determined by the method of Marco (17) with minor modifications. The β-carotene (6 mg), 1 g linoleic acid and 2 mL of Tween 40 were dissolved in 25 mL chloroform. The chloroform was subsequently evaporated in a rotary evaporator, and its remaining traces were removed by nitrogen. The model emulsion was prepared by adding 25 mL of double-distilled water to the viscous, uniform lipid by stirring and violent shaking, and the volume was increased to 500 mL with double-distilled water. Two mL of the antioxidant solution were added to each test vial, together with 50 mL of the model emulsion, and it was incubated at 50°C (2 mL of 95% ethanol was added to the control). Zero time was the time of addition of the model emulsion to each vial. Destruction of β-carotene was determined after withdrawing a 2-mL sample into 7 mL of 95% ethanol every 20–30 min by reading the absorbance at 450 nm.

Iron analysis. Iron was determined by atomic absorption spectrometry (18) (aa/ae-Spectrophotometer-157; Instrumentation Laboratory Inc., Wilmington, MA). Samples were ashed by dry-ashing, with magnesium nitrate as ash aid (19).

Soy lipoxygenase. Lipoxygenase activity was measured by the method of Gibian and Galaway (20) with minor modifications. Briefly, to 3 mL of 0.2 M borate buffer (pH 9.0), were added 25 μL linoleic acid solution (2.5 mg/mL in absolute ethanol), 20 μL of DFO solution (in 0.2 M

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borate buffer, pH 9.0), and 25 μL of lipoxygenase solution (0.5 mg/mL in borate buffer). Enzyme activity was monitored by reading the difference in the absorbance at 234 nm. The time of substrate or enzyme addition was designated as zero time. The extent of inhibition was measured proportionally to the enzyme activity in the absence of inhibitor.

Reversibility of inhibition was assessed by incubation of lipoxygenase with DFO for 20 min in the absence of substrate, measuring its activity after dialysis and comparing it to that of nondialyzed lipoxygenase, which had been incubated for the same time. Dialysis was performed in dialysis tubing [Spectra/por 6 mm, molecular weight cut-off (MWCO) 12,000–14,000; Spectra Medical Industries, Inc., Los Angeles, CA] against 0.2 M borate buffer (pH 9.0), at 4°C, for 2 h.

Data and statistical analysis. Experiments in oxidation of emulsions and suspensions were performed in duplicate. Days to induction of oxidation were designated as days before absorption exceeded o.d. = 0.2 and are expressed as the means ± SD. Experiments in lipoxygenase inhibition were performed in triplicate. Lipoxygenase activity without DFO was designated as 100%, and results are expressed as mean percent ± SD. The difference between mean values was tested by Student's *t*-test for unpaired results. Significance limit was $P < 0.05$, unless stated otherwise. Linearity was evaluated by simple linear regression.

RESULTS

Oxidation inhibition in suspensions. The antioxidant activity of DFO was tested at various concentrations in comparison to that of BHA and quercetin (Fig. 1). The presence of DFO delayed the onset of linoleic acid oxidation by up to 30.5 ± 0.71 d at a concentration of 0.770 mM (500 ppm) and by 17 ± 1.41 d at a concentration of 0.154 mM (100 ppm). The number of days to start of oxidation in linoleic acid suspensions (Fig. 1A) was linear with DFO concentration ($r = 0.972$, $P < 0.03$). BHA [0.555 mM (100 ppm)] and DFO [0.770 mM (500 ppm)] showed the same relative antioxidant effect in both linoleic and linolenic acid suspensions. The inhibition of oxidation by 0.331 mM (100 ppm) quercetin was not significantly higher then that of 0.154 mM (100 ppm) DFO in both suspensions.

Oxidation inhibition in emulsions. Protection afforded by various concentrations of DFO was compared to that of BHA (Fig. 2). In linoleic acid emulsion (Fig. 2B), all tested concentrations of DFO delayed the start of

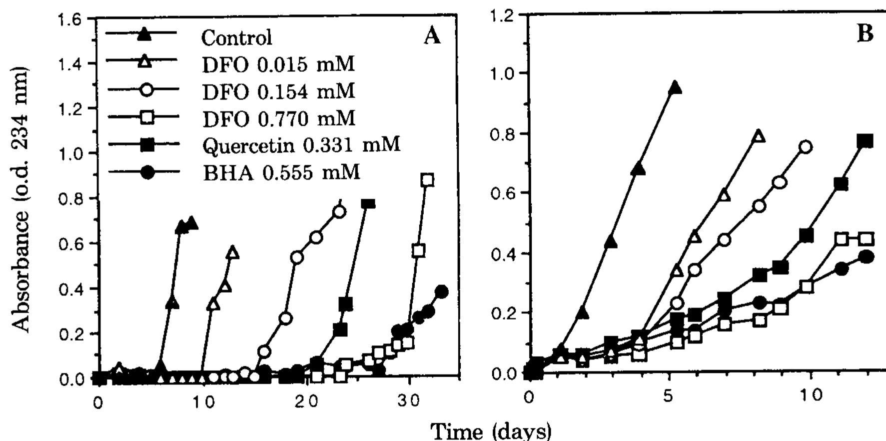

FIG. 1. Conjugated dienes of (A) linoleic acid and (B) linolenic acid suspensions in the presence of deferoxamine (DFO), butylated hydroxyanisole (BHA) and quercetin.

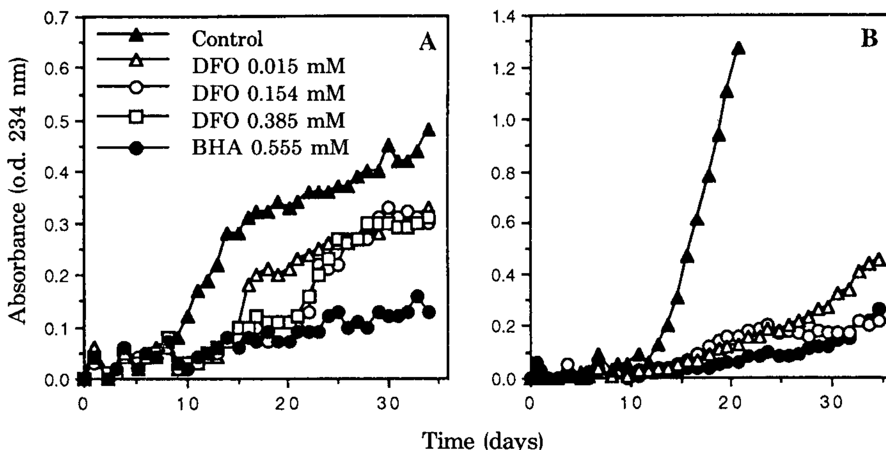

FIG. 2. Conjugated dienes of (A) fish oil and (B) linoleic acid emulsions with various concentrations of DFO and BHA. Abbreviations as in Figure 1.

TABLE 1

Typical Fatty Acid Profile of the Fish Oil[a]

Fatty acid	Content (%)	Fatty acid	Content (%)
C12:0	0.03	C18:3	2.48
C14:0	3.69	C18:4	0.17
C14:1	0.09	C20:0	0.20
C15:0	0.29	C20:1	11.69
C16:0	10.38	C20:4	0.79
C16:1	7.04	C20:5	8.81
C16:2	0.43	C21:5	0.47
C16:4	0.39	C22:1	8.37
C17:0	0.13	C22:4	0.30
C18:0	2.33	C22:5	1.29
C18:1	23.26	C22:6	10.94
C18:2	1.80		

[a]Fatty acid profile was obtained from Henry Lamotte (Bremen, Germany).

oxidation by at least 28 ± 2 d ($P < 0.03$). Antioxidant effect of DFO in fish oil emulsion (iodine value, 167; vitamin A, 1.437 IU/g; fatty acid profile in Table 1) was identical for 0.154 mM (100 ppm) and 0.385 mM (250 ppm) (Fig. 2A). Both delayed oxidation for 23 d, and the control was oxidized after 12 ± 0.71 d and BHA inhibited the start of oxidation by over 34 d. At all concentrations of DFO, the extent of fish oil oxidation was lower than that of the control ($P < 0.01$).

The protection against oxidation afforded by DFO to β-carotene in emulsion was compared to that of BHA (Fig. 3). The results showed that both DFO and BHA inhibited the loss of β-carotene compared to the control ($P < 0.05$). The effect of 15 μM DFO was not significantly different from that of 1.7 μM BHA.

Iron analysis. No iron was detected (detection limit 1 μM) in the alcoholic suspensions and in the linoleic acid/fish oil emulsions.

Lipoxygenase inhibition. Activity of the enzyme was tested in the presence of two concentrations of DFO (Table 2). Increasing DFO concentration showed better inhibition effect. Lipoxygenase that was dialyzed after incubation with DFO regained its activity, whereas enzyme that was incubated with DFO for the same time without dialysis did not show any recovery of its activity.

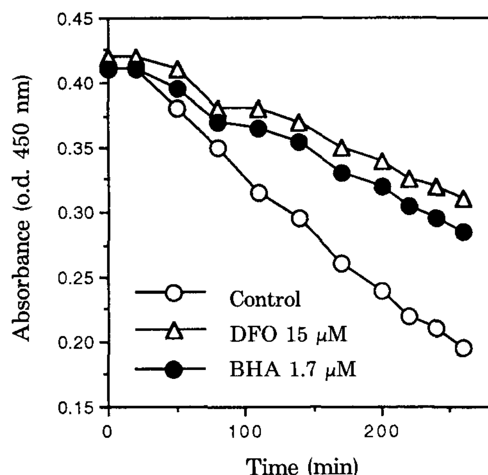

FIG. 3. Absorbance of β-carotene emulsions with linoleic acid in the presence of DFO (15 μM) or BHA (1.7 μM).

TABLE 2

Lipoxygenase Activity in the Presence of Deferoxamine (DFO) as Compared to Its Activity in the Absence of DFO, with or without Dialysis (compared to the enzyme's activity in the absence of DFO)

Time (h)	Activity (% ± SD), DFO concentration 73.6 (μM) Nondialyzed	73.6 (μM) Dialyzed	147.2 (μM) Nondialyzed	147.2 (μM) Dialyzed
0	90.4 ± 0.9[a]	90.4 ± 0.9[a]	72.1 ± 4.4[a]	72.1 ± 4.4[a]
2	87.5 ± 4.4[a]	99.7 ± 1.2	70.1 ± 3.2[a]	96.4 ± 7.1

[a]Significant inhibition ($P < 0.01$).

DISCUSSION

In assessing DFO protection against oxidation of fatty acids and fish oil, it was found that DFO possessed antioxidant activity even at low concentration, such as 15 μM, and this effect increased proportionally with increasing DFO concentrations in linoleic acid (Fig. 1A). These findings are in agreement with earlier observations that showed DFO inhibitory potential on lipid oxidation (6,21). The fact that the inhibition was observed in systems devoid of iron supports earlier findings that DFO has antioxidant properties that are independent of its capability to bind iron as a peroxyl-radical scavenger in linoleic acid suspension (22). Explanations for the possible mechanism of DFO antioxidant activity are: (i) DFO acts as an electron donor (23), and (ii) it is capable of reacting with the hydroxyl radical (24) or the superoxide anion (25). The present study also showed that the antioxidant activity of DFO is effective at concentrations (100 ppm) typical for antioxidants including BHA and quercetin (Fig. 1).

DFO also displayed antioxidant activity in emulsions (Fig. 2). The delay in onset of the oxidation process was greater in the model emulsion of linoleic acid than in fish oil emulsion (Fig. 2). More specifically, oxidation inhibition in the fish oil emulsion was lower, but the extent of oxidation was also lower in emulsions containing DFO. The cause of the lower extent of oxidation in the fish oil control in comparison to the linoleic acid control (Fig. 2) may reside in the presence of vitamin A that can act as an antioxidant in the fish oil (26). The concentration of DFO needed for β-carotene protection in emulsion was higher than that of BHA (Fig. 3). The reason for this difference may be explained by the high affinity of BHA for the lipid phase, whereas DFO is soluble in the aqueous phase, so its effective concentration in the lipid is lower.

Activity of soy lipoxygenase was inhibited by various concentrations of DFO (Table 2). At DFO concentration of 73.6 μM, the enzymatic activity declined to 90.4 ± 0.9%, and at 147.2 μM DFO, to 72.1 ± 4.4%. This result agrees with previous findings that showed lipoxygenase 5′ inhibition by hydroxamic acids (13,27,28). The ability of these substances to inhibit this enzyme has been attributed to interaction with an iron ion present at the active site of the enzyme (13), although other sources support a competitive inhibition mechanism (28). Our

findings (Table 2) suggest that the inhibition of lipoxygenase by DFO is a reversible process because lipoxygenase that was dialyzed after incubation with DFO regained its activity. This is possibly a consequence of a spatial disruption or molecule folding because DFO is known to bind onto iron during this process (3). The manner whereby DFO inhibits the lipoxygenase is not yet known.

The results obtained in the present study illustrate the ability of deferoxamine to inhibit oxidation. The study also shows that these properties of DFO are effective at acceptable concentration ranges for commercial antioxidants. DFO inhibition of soy lipoxygenase indicates its potential to inhibit other plant lipoxygenases. Inhibition of oxidation in suspensions and emulsions necessitates our assessment of DFO activity in additional systems with industrial application.

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