Antioxidant Properties of Desferrioxamine E, a New Hydroxamate Antioxidant

E. Shimoni[a], M. Ampel[a], H. Zähner[b], and I. Neeman[a,*]

[a]Department of Food Engineering and Biotechnology, Technion-Israel Institute of Technology, Haifa 32000, Israel, and [b]University of Tübingen, D-7400 Tübingen, Federal Republic of Germany

ABSTRACT: Antioxidant properties of desferrioxamine E, a cyclic trihydroxamate produced by microorganisms, were tested and compared to those of desferrioxamine B and butylated hydroxyanisole (BHA). Desferrioxamine E exhibited significant antioxidant effects in linoleic acid emulsions as well as in emulsions of linoleic acid and β-carotene. In concentrations of 100 ppm, the effect of both desferrioxamines in linoleic acid emulsion was equal to that of BHA. The antioxidant activity of the desferrioxamines in emulsions of β-carotene and linoleic acid was significantly higher than that of BHA. In addition, the initial rate of β-carotene destruction was significantly lower when desferrioxamines were added to the emulsion.
JAOCS 75, 1453–1455 (1998).

KEY WORDS: Antioxidant, desferrioxamine, hydroxamate.

Desferrioxamine E, a cyclic trihydroxamate, belongs to a group of microbial siderophores which form complexes with iron ions (1). Stoll *et al.* (2) described it first as an antibiotic. Desferrioxamines were primarily isolated from actinomycetes, such as *Streptomyces pilosus* (3,4), but they were also described in the genus *Arthrobacter, Chromobacterium* (5), *Pseudomonas* (6), and *Erwinia herbicola* (7). These trihydroxamates are recognized primarily as chelators with a particularly high affinity for trivalent iron ions ($K_b = 10^{31}$) (8).

A well-studied analog of desferrioxamine E is the noncyclic desferrioxamine B. Several studies have demonstrated the ability of desferrioxamine B to inhibit oxidation in various biological systems (9–11). The antioxidant activity of desferrioxamine B was first attributed to its iron binding capacity (10). Later, however, it was shown that desferrioxamine B acts as a chain-breaking radical scavenger by donating an electron or hydrogen atom from the hydroxamate center (11). This capacity could be attributed to the hydroxamate centers that produce a resonance-stabilized center following the donation of a hydrogen atom (12).

While the biological activities of desferrioxamine B have been extensively investigated, little is known about the properties of the related desferrioxamine E (Fig. 1). Due to the antioxidant capacity of the hydroxamate moieties of desferrioxamines, they may prove useful as primary antioxidants. In a previous study, desferrioxamine B exhibited significant antioxidant activity in model emulsions and suspensions of fatty acids and oils (13). As a result of the structural difference between these two desferrioxamines, desferrioxamine B is freely soluble in water, while desferrioxamine E possesses poor solubility in water and cold methanol (14). Therefore, the authors examined the effect of these structural differences on the antioxidant potential of desferrioxamine E.

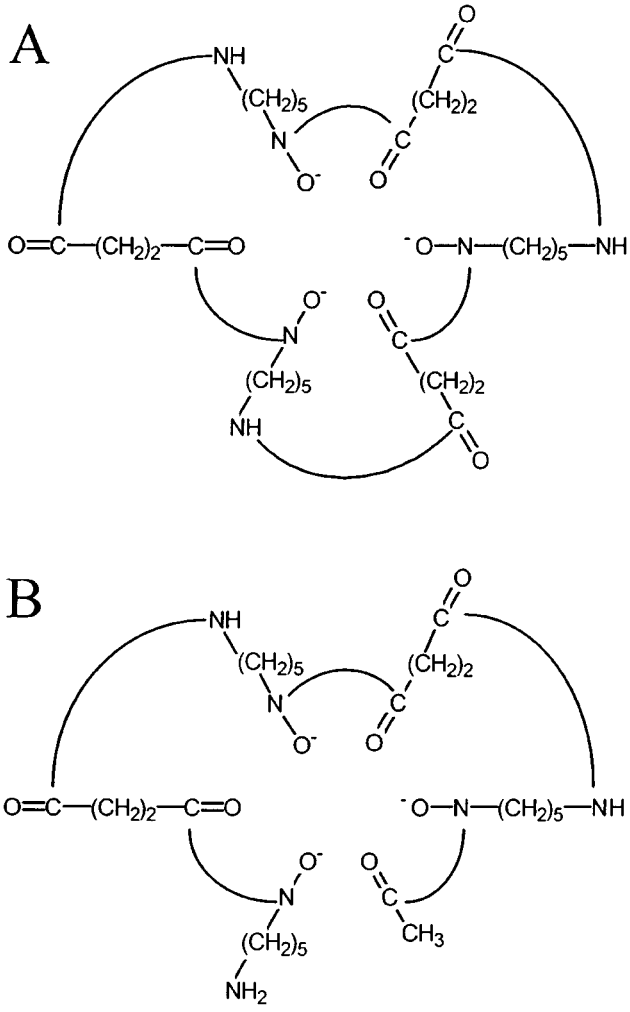

FIG. 1. Structure of desferrioxamine E (A) and B (B).

*To whom correspondence should be addressed.
E-mail: eshimoni@techunix.technion.ac.il.

EXPERIMENTAL PROCEDURES

Desferrioxamine E was provided by Prof. H. Zähner (University of Tübingen, Tübingen, Germany). Desferrioxamine B [Desferal (c)] was obtained from Ciba Co. (Basel, Switzerland). Linoleic acid was a product of Fluka AG (Buchs, Switzerland). Butylated hydroxyanisole (BHA), β-carotene, Tween 22, and Tween 40 were purchased from Sigma Israel Chemical Co. (Holon, Israel).

Antioxidant activity in linoleic acid emulsion. The antioxidant activity was assayed by the method of Cillard *et al.* (15) with minor modifications. Briefly, a solution of 0.5% Tween 20 (Serva Biochemicals, Westbury, NY) and the tested antioxidant in pH 7.0 phosphate buffer was mixed with an equal volume of 0.5% Tween 20 and 0.28% linoleic acid (Fluka) in phosphate buffer (pH 7.0), and the mixture was placed in the dark at 37°C. Oxidation was determined on 100-μL samples taken into 2 mL of phosphate buffer (pH 7.0) by reading the absorption of conjugated dienes at 234 nm (ultraviolet-visible spectrophotometer M330, Camsped Ltd., Saloston, Cambridge, United Kingdom).

Antioxidant activity in emulsion of β-carotene and linoleic acid. Destruction of β-carotene was determined by the method of Hammerschmidt and Pratt (16) with some modifications. The β-carotene (0.1 mg), 20 mg linoleic acid, and 200 mg Tween 40 (Sigma) were dissolved in 20 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 50 mL double-distilled water to the viscous, uniform lipid by stirring and violent shaking. Five milliliters of the emulsion was added to test tubes that contained 2 mL of solution [1:1 distilled water/dimethyl sulfoxide (DMSO)] of the tested antioxidant, and the tubes were immediately incubated at 50°C (2 mL ethanol was added to the control). Zero time was the time of addition of the model emulsion to each test tube. Destruction of β-carotene was monitored by reading the absorption at 470 nm. The antioxidant activity was measured by the residual absorption after 250 min of incubation and the rate of β-carotene loss.

Data and statistical analysis. Oxidation experiments were performed in duplicate and are expressed as means. Days to oxidation induction were designated as days before absorption exceeded o.d. = 0.2. 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 AND DISCUSSION

Emulsion of linoleic acid. The antioxidant effect of desferrioxamine E was tested in various concentrations and compared to that of desferrioxamine B and BHA (Fig. 2). The presence of desferrioxamine E delayed the onset of linoleic acid oxidation by up to 24 d at a concentration of 0.17 mM (100 ppm) and by 17 d at a concentration of 0.085 mM (50 ppm). Desferrioxamine E inhibition of linoleic acid oxidation was not significantly higher than that of desferrioxamine B. The antioxidant effects

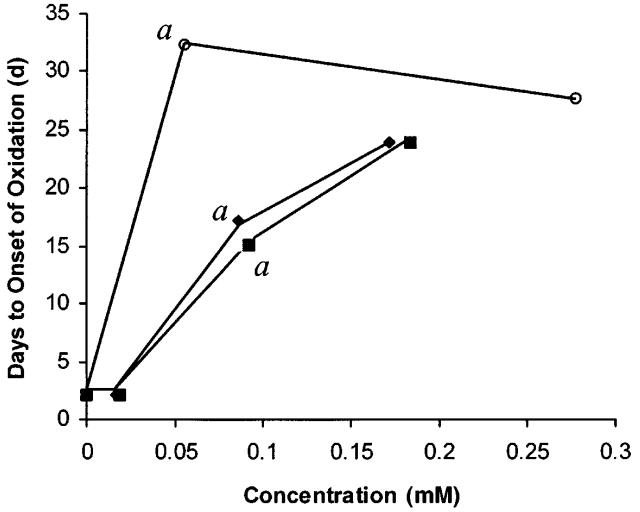

FIG. 2. Days before onset of oxidation of linoleic acid emulsions with no antioxidant (–), and in the presence of various concentrations of desferrioxamine E (◆), desferrioxamine B (■), and butylated hydroxyanisole (BHA) (○). [a]Significant difference between desferrioxamines and BHA.

of BHA were significantly higher than that of both desferrioxamines. However, the antioxidant effect of both desferrioxamine E and B was not significantly different to that of BHA only at concentrations of over 100 ppm (0.278 mM BHA, 0.171 mM desferrioxamine E, 0.184 mM desferrioxamine B). One possible explanation is that due to its poor solubility in water, BHA accumulates in the hydrophobic sites of the emulsion. Thus, BHA is concentrated close to the linoleic acid micelles, and its effective concentration is higher.

Emulsion of β-carotene and linoleic acid. Protection against oxidation afforded by various concentrations of des-

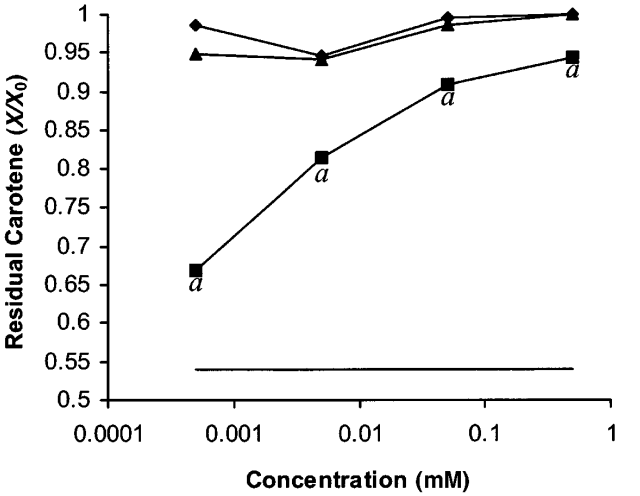

FIG. 3. Absorbance of ß-carotene emulsions with linoleic acid after 250 min of incubation with no antioxidant (–), and in the presence of various concentrations of desferrioxamine E (◆), desferrioxamine B (▲), and BHA (■). [a]Significantly different from both desferrioxamines. In X/X_0, X is absorbance at 250 min, and X_0 at time zero. See Figure 2 for other abbreviation.

TABLE 1
Initial Rate of β-Carotene Destruction in Emulsion of β-Carotene and Linoleic Acid in the Presence of Desferrioxamine E, Desferrioxamine B, and BHA

	Rate of β-carotene destruction (%/min)		
		Desferrioxamine	
Concentration (μM)	BHA	E	B
0	0.22	0.22	0.22
0.5	0.16	0.004[a]	0.03[a]
5	0.08	0.002[a]	0.03[a]
50	0.04	<0.001[a]	<0.001[a]
500	0.02	<0.001[a]	<0.001[a]

[a]Significantly lower than butylated hydroxyanisole (BHA).

ferrioxamine E to β-carotene in emulsion with linoleic acid was compared to that of desferrioxamine B and BHA (Fig. 3). The antioxidant effect of desferrioxamine E was significantly higher than that of BHA at concentrations of 0.5, 5, 50, and 500 μM ($P < 0.001$). However, it was not significantly different than that of desferrioxamine B. The loss of β-carotene (after 250 min) at antioxidant concentration of 0.5 μM was 1.4, 5.1 B, and 34.1% desferrioxamine E, desferrioxamine B, and BHA, respectively.

The rate of β-carotene destruction was linear at the first stages of oxidation. Thus, the antioxidant activity of all tested chemicals was also compared based on the rate of β-carotene destruction (Table 1). At all concentrations, the rate of β-carotene destruction was lower in the presence of desferrioxamines E and B than in the presence of BHA. At concentrations of 0.5 μM, both desferrioxamines inhibited β-carotene degradation, while BHA had only a moderate effect. However, a previous study (17) showed that the antioxidant activity of desferrioxamine B was lower than that of BHA in β-carotene emulsion. The controversy of results is explained by the addition of DMSO to the emulsion in the present study, thus achieving a better distribution of the desferrioxamines in the lipid fraction.

The results obtained in the present study demonstrate the ability of desferrioxamine E to inhibit oxidation of linoleic acid and β-carotene in a concentration range typical for commercial antioxidants. Many studies clearly indicate that the hydroxamate moiety is most effective in preventing oxidation (18,19). Thus, desferrioxamine E joins the expanding group of hydroxamate antioxidants. Because desferrioxamine E is a microbial secondary metabolite that can be produced in high yields (20), it is suggested that its activity should be assessed in food and biological model systems.

REFERENCES

1. Emery, T., Hydroxamic Acids of Natural Origin, *Adv. Enzymol. Relat. Areas Mol. Biol. 35*:135–185 (1971).
2. Stoll, A., A. Brack, and J. Renz, Nocardamin, Ein Neues Antibioticum aus Einer *Nocardia, Art. Schweiz. Z. Pathol. Bacteriol. 14*:225–233 (1951).
3. Bickel, H., R. Bosshardt, E. Gaumann, P. Reusser, E. Vischer, W. Voser, A. Wettstein, and H. Zähner, Über die Isolierung und Characterisierung der Ferrioxamine A-F, neuer wusstoffe der sideramin-gruppe, *Helv. Chim. Acta 43*:2118–2128 (1960).
4. Bickel, H., G.E. Hall, W. Keller-Schierlein, V. Prelog, E. Vischer, and A. Wettstein, Über die Constitution von Ferrioxamin B, *Ibid. 43*:2129–2139 (1960).
5. Müller, A., and H. Zähner, Stoffwechselprodukte aus Mikroorganismen 65. Ferrioxamine aus Eubacteriales, *Arch. Mikrobiol. 62*:257–263 (1968).
6. Meyer, J.M., and M.A. Abdallah, The Siderophores of Non Fluorescent Pseudomonads: Production of Nocardamine by *Pseudomonas stutzeri. J. Gen. Microbiol. 118*:125–129 (1980).
7. Berner, I., S. Conetschny-Rapp, G. Jung, and G. Winkelmann, Characterization of Desferrioxamine E as the Principal Siderophore of *Erwinia herbicola (Enterobacter agglomerance), Biol. Metals 1*:51–56 (1988).
8. Keberle, H., The Biochemistry of Desferrioxamine and Its Relation to Iron Metabolism, *Ann. NY Acad. Sci. 119*:758–764 (1964).
9. Rice-Evans, C., S.C. Omorphos, and E. Baysal, Sickle Cells Membranes and Oxidative Damage, *Biochem. J. 237*:265–269 (1986).
10. Videla, L.A., M.I. Villena, C. Salgado, P. Cenales, and E.A. Lissi, Antioxidant Capacity of Desferrioxamine in Biological Systems, *Biochem. Int. 15*:205–214 (1987).
11. Harteley, A., M. Davies, and C. Rice-Evans, Desferrioxamine as a Lipid Chain-Breaking Antioxidant in Sickle Erythrocyte Membrane, *FEBS Lett. 264*:145–148 (1990).
12. Perkins, M.J., Acyl Nitroxides, *Rev. Chem. Intermed. 7*:133–141 (1986).
13. Shimoni, E., R. Armon, and I. Neeman, Antioxidant Properties of Deferoxamine, *J. Am. Oil. Chem. Soc. 71*:641–644 (1994).
14. Keller-Schierlein, W., V. Perlog, and H. Zähner, Siderochrome. (Naturliche Eisen(III)-trihydroxamat-Komplexe), *Fortchr. Chem. Org. Naturst. 22*:279–322 (1964).
15. Cillard, J., P. Cillard, M. Cormier, and L. Girre, α-Tocopherol Prooxidant Effect in Aqueous Media: Increased Oxidation Rate of Linoleic Acid, *J. Am. Oil. Chem. Soc. 57*:252–255 (1980).
16. Hammerschmidt, P.A., and D.E. Pratt, Phenolic Antioxidants of Dried Soybeans, *J. Food Sci. 43*:556–559 (1978).
17. Hinojosa, O., T.J. Jacks, T.A. Calamary, and D.P. Tibodeaux, Electron Spin Resonance Study of the Relationship of UV Irradiation to Nitroxide Radical Formation in Desferrioxamine (desferal) and Related Hydroxamate and Hydroxylamine Derivatives, *Photochem. Photobiol. 49*:1–5 (1989).
18. Denicola, A., J.M. Souza, R.M. Gatti, R. Augusto, and R. Radi, Desferrioxamine Inhibition of the Hydroxyl Radical-Like Reactivity of Peroxynitrile: Role of the Hydroxamic Groups, *Free Radical Biol. Med. 19*:11–19 (1995).
19. Kozlov, A.V., F. Yu. Meshchanov, I.G. Nikolaeva, G.N. Kol'tsova, and Yu. A. Vladimirov, Comparison of Antioxidant Activity of Polyhydroxamic Acid, Desferal and Other Iron Chelators, *Biofizika 38*:708–713 (1993).
20. Meiwes, J., H.P. Fiedler, H. Zähner, S. Konetschny-Rapp, and G. Jung, Production of Desferrioxamine E and New Analogues by Directed Fermentation and Feeding Fermentation, *Appl. Microbiol. Biotechnol. 32*:505–510 (1990).

[Received March 20, 1998; accepted June 19, 1998]