THE FENTON REACTION BETWEEN FERROUS-DIETHYLENETRIAMINEPENTAACETIC ACID AND HYDROGEN PEROXIDE

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1. Introduction

The iron chelating agent, diethylenetriaminepentaacetic acid (DTPA or DETAPAC), has gained popularity in recent years because it inhibits the formation of hydroxyl radicals ('OH) in systems where both superoxide (O_2^-) and hydrogen peroxide are generated. Thus, DTPA gives an appearance of blocking the 'iron-catalyzed Haber-Weiss reaction':

$$H_2O_2 + O_2^- \xrightarrow{Fe} {}^{\cdot}OH + OH^- + O_2 \qquad (1)$$

Eq. (1) does not proceed to any appreciable extent in 'pure' aqueous solution in the absence of iron contaminants ($k < 1\ M^{-1}\ s^{-1}$) [1,2]. However, the reaction is catalyzed by iron salts or iron chelates (e.g., iron–EDTA). The following mechanism has been proposed [3,4]:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\cdot}OH + OH^- \qquad (2)$$

$$Fe^{3+} + O_2^- \longrightarrow Fe^{2+} + O_2 \qquad (3)$$

Eq. (2) is a classical Fenton reaction, giving rise to 'OH. Eq. (3) represents a route for recycling of the iron so that it can participate in a Fenton reaction. Some investigators refer to this mechanism as 'superoxide-driven Fenton chemistry' [5].

If DTPA inhibits the iron-catalyzed Haber-Weiss mechanism, it could do so either by preventing the Fenton reaction [eq. (2)] or by preventing the reduction of Fe^{3+} [eq. (3)]. Many investigators have assumed that DTPA blocks the Fenton reaction. This error has persisted into some very recent publications.

Some investigators [6,7] have observed that Fe^{2+}–DTPA will react with hydrogen peroxide to generate ethylene gas from 2-keto-4-thiomethylbutyrate. This reaction and an analogous one with methional are believed to be mediated by the formation of hydroxyl radicals. However, to be certain that a Fenton-type reaction [eq. (2)] has indeed mediated the reaction, it is necessary to show disappearance of Fe^{2+} and appearance of Fe^{3+}. Moreover, the formation of 'OH should be confirmed by showing the formation of an appropriate product with a more traditional 'OH scavenger. This brief communication fulfills these needs.

2. Experimental

Ion exchange water was subsequently distilled in an all-glass still (Corning) and then passed through a Milli Q system (Millipore Corp.) immediately before use. The sources of chemicals were: ferrous ammonium sulfate · 6 H_2O (ACS Grade, Fisher); diethylenetriaminepentaacetic acid (DTPA, Sigma); 2-keto-4-thiomethylbutyric acid; sodium salt (KTBA, Sigma); and hydrogen peroxide (30% reagent, Fisher). All experiments were carried out in 50 mM phosphate buffer at pH 7.0.

Colorimetric assays for Fe^{2+} and Fe^{3+} were performed with a Gilford Stasar III flow-through spectrophotometer. Fe^{2+} were measured as the *o*-phenanthroline complex at 510 nm. Fe^{3+} were measured as the thiocyanate complex at 480 nm after acidification of an aliquot of the reaction mixture with trichloroacetic acid. For experiments performed under helium, the buffer containing DTPA was brought to a light boil to remove entrapped gas, and then it was cooled by continuous bubbling with helium; the stock solutions of Fe^{2+} and hydrogen

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peroxide were prepared in similarly treated water, which was then rebubbled with helium. In all experiments, hydrogen peroxide was added last, at 1 min after the addition of Fe^{2+} solution.

Ethylene gas formed from KTBA in sealed Erlenmeyer flasks was assessed by sampling with a plastic syringe followed by gas chromatography with flame ionization detection [8]. Phenol was assayed after extraction from NaCl-saturated solution with benzene followed by back-extraction into 0.1 N NaOH; assays were conducted with a Hewlett-Packard Model 1084 B liquid chromatograph equipped with an RP-8 column and an electrochemical detector (Bioanalytical Systems).

3. Results

Preliminary experiments showed that Fe^{2+}–DTPA was stable to autoxidation in the pH 7.0 buffer in the presence of air [6,9]. Subsequently, the loss in Fe^{2+} was measured with *o*-phenanthroline reagent after the addition of hydrogen peroxide to Fe^{2+}–DTPA and, in a second set of experiments, the appearance of Fe^{3+} was measured with thiocyanate reagent. The results in table 1 show that Fe^{2+} disappears and Fe^{3+} appears upon the addition of hydrogen peroxide to the ferrous chelate. Hydrogen peroxide is itself consumed during the reaction [6]. These results are consistent with the occurrence of a Fenton-type reaction [eq. (1)].

Two typical ·OH scavengers were tested to see if they would be transformed to the appropriate product upon the addition of hydrogen peroxide to Fe^{2+}–DTPA. KTBA forms ethylene gas upon attack by hydroxyl radicals [7,8]. Benzene is a traditional substrate for hydroxyl radical attack and it forms phenol as a product [10,11]. Both ethylene and phenol were formed in good yields during the Fenton-type reaction between hydrogen peroxide and Fe^{2+}–DTPA (table 2).

Table 2
Production of metabolized products of two hydroxyl radical scavengers upon addition of hydrogen peroxide to Fe^{2+}–DTPA[a]

Expt.	Fe^{2+}–DTPA	H_2O_2	Substrate	Product
1	50 μM	1.0 mM	KTBA	Ethylene 39 ± 1 μM
2	200 μM	0.4 mM	Benzene	Phenol 63 ± 2 μM

[a] In expt. 1, DTPA was present at 100 μM (100% molar excess over Fe^{2+}), KTBA at 10 mM and the atmosphere was air. In expt. 2, DTPA was present at 240 μM (20% molar excess over Fe^{2+}), benzene at 19 mM (saturated solution) and the atmosphere was helium:oxygen (9:1). Products were measured at 5 min after the addition of hydrogen peroxide. No significant yields of products were observed if either hydrogen peroxide or Fe^{2+} were omitted. The amount of ethylene gas is expressed as if the product were all present in solution rather than in the gas phase in the flask. Results are the mean ± SEM ($N = 6$, expt. 1; $N = 4$, expt. 2)

4. Discussion

The results show that Fe^{2+}–DTPA is oxidized to Fe^{3+}–DTPA, and that ·OH is produced when hydrogen peroxide is added to a solution of Fe^{2+}–DTPA in phosphate buffer at neutral pH. Therefore, Fe^{2+}–DTPA does participate in a Fenton-type reaction.

Table 1
Oxidation of ferrous–DTPA by hydrogen peroxide[a]

Expt.	Fe^{2+}	H_2O_2 Added	ΔFe	Reagent used
1	100 μM	12.5 μM	21.0 ± 1.0 μM	*o*-Phenanthroline (Fe^{2+} reagent)
2	100 μM	50 μM	91.2 ± 1.4 μM	Thiocyanate (Fe^{3+} reagent)

[a] DTPA was present at 200 μM, which is twice the concentration of Fe^{2+}: expt. 1 was conducted in a helium atmosphere; expt. 2 was conducted in air. The Fe^{2+}–DTPA chelate is stable to autoxidation in phosphate buffer at neutral pH [9]. Control experiments showed that the ferrous chelate with *o*-phenanthroline was not significantly oxidized by addition of hydrogen peroxide directly to this chelate. Results are the mean ± SEM ($N = 13$, expt. 1; $N = 5$, expt. 2)

The original studies which showed suppression of ·OH formation by DTPA were performed with the enzyme, xanthine oxidase, which generates both hydrogen peroxide and superoxide. Failure to form ·OH was assessed by the absence of an electron spin resonance signal for the adduct of ·OH with spin-trapping agents [12]. Subsequently, DTPA was shown to block formation of products expected from the reaction between generated ·OH and known scavengers [4]. DTPA has since been used effectively to block oxidative phenomena in systems where both hydrogen peroxide and superoxide are generated [7,13–17]; some of these observations concern intact cells [13, 16,17].

Since Fe^{2+}–DTPA readily engages in a Fenton-type reaction, another explanation is required to explain the ability of DTPA to block oxidative phenomena. A likely explanation is that DTPA suppresses the reduction of Fe^{3+} by superoxide [eq. (3)]. We attempted to assess this possibility directly by studying the formation of Fe^{2+}–DTPA from Fe^{3+}–DTPA by a superoxide-generating system (xanthine oxidase); catalase was added in order to remove the hydrogen peroxide as it was formed and, thereby, prevent the oxidation of Fe^{2+}–DTPA in a Fenton-type reaction. No accumulation of Fe^{2+}–DTPA was observed, a result consistent with inhibition of eq. (3). However, it would be prudent to confirm this result with a pure source of superoxide and with better control over minimizing the presence of hydrogen peroxide.

The inhibiting actions of DTPA on oxidative phenomena in biological systems imply the presence of free iron or iron chelates as either 'contaminants' in buffers or chemical reagents, or as endogenous and natural components of cellular systems. Since Fe^{2+}–DTPA is highly reactive in generating hydroxyl radicals when hydrogen peroxide is present it appears that an inhibitory action of DTPA may derive from the stabilization of iron in the ferric state. Recent studies have shown that bacterial killing by hydrogen peroxide is enhanced when bacteria (*Staphylococcus aureus*) are grown for several days in an iron-rich environment in order to raise intrinsic iron concentrations [18]; since bacterial killing appears to involve hydrogen peroxide and derived oxy-radicals, these data imply that iron can mediate peroxide-dependent toxicity in intact cellular systems.

At the current time, it can be asserted that Fe^{2+}–DTPA does participate in a Fenton-type reaction. Therefore, either DTPA blocks the reduction of Fe^{3+} by superoxide as well as by other reducing agents in cells, or the 'iron-catalyzed Haber-Weiss reaction' is not correctly described by eq. (2) and (3).

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References

[1] Weinstein, J. and Bielski, B. H. J. (1979) J. Am. Chem. Soc. 101, 58–62.
[2] Ferradini, C., Foos, J., Houee, C. and Puchealt, J. (1978) Photochem. Photobiol. 28, 697–700.
[3] McCord, J. M. and Day, E. D. jr (1978) FEBS Lett. 86, 139–142.
[4] Halliwell, B. (1978) FEBS Lett. 92, 321–326.
[5] Fee, J. A. (1980) Dev. Biochem. 11B, 41–48.
[6] Cohen, G. and Sinet, P. M. (1980) Dev. Biochem. 11A, 27–37.
[7] DiGuiseppi, J. and Fridovich, I. (1980) Arch. Biochem. Biophys. 205, 323–329.
[8] Cohen, G. and Cederbaum, A. I. (1980) Arch. Biochem. Biophys. 199, 438–447.
[9] Cohen, G., Lewis, D. and Sinet, P. M. (1981) J. Inorganic Biochem. 15, 143–151.
[10] Dorfman, L. M. and Adams, G. E. (1973) NSRDS Natl. Bureau Stand. 46, Washington DC.
[11] Lindsay Smith, J. R. and Norman, R. O. C. (1963) J. Chem. Soc. 3, 2897–2905.
[12] Buettner, G. R., Oberley, L. W. and Leuthauser, S. W. H. C. (1978) Photochem. Photobiol. 28, 693–695.
[13] Halliwell, B. (1978) FEBS Lett. 96, 238–242.
[14] Rosen, H. and Klebanoff, S. J. (1981) Arch. Biochem. Biophys. 208, 512–519.
[15] Halliwell, B. and Gutteridge, J. M. C. (1981) FEBS Lett. 128, 347–352.
[16] Fischer, L. J. and Hamburger, S. A. (1980) Diabetes 29, 213–216.
[17] Grankvist, K., Marklund, S. and Taljedal, I.-B. (1979) Febs Lett. 105, 15–18.
[18] Repine, J. E., Fox, R. B., Berger, E. M. and Harada, R. N. (1981) Infect. Immun. 32, 407–410.