The kinetics of the reaction of superoxide radical with Fe(III) complexes of EDTA, DETAPAC and HEDTA

Garry R. Buettner, Thomas P. Doherty and Larry K. Patterson*

Department of Chemistry, Wabash College, Crawfordsville, IN 47933 and **Radiation Laboratory, University of Notre Dame, Notre Dame, IN 46556, USA*

Received 3 March 1983

To gain an understanding of the mechanism by which the hydroxyl free radical can arise in superoxide generating systems and learn how different chelaters of iron can inhibit this reaction, a pulse radiolysis kinetic study of the reaction of $O_2^{\cdot -}$ with Fe(III)EDTA, Fe(III)HEDTA and Fe(III)DETAPAC (or DTPA) was undertaken. Superoxide reacts readily with Fe(III)EDTA and Fe(III)HEDTA with a pH-dependent second-order rate constant having values of $1.9 \times 10^6\ M^{-1}.s^{-1}$ and $7.6 \times 10^5\ M^{-1}.s^{-1}$ at pH 7, respectively. However, the rate constant for the reaction of $O_2^{\cdot -}$ with Fe(III)DETAPAC was found to be much slower, the upper limit for the rate constant being $10^4\ M^{-1}.s^{-1}$. These results in conjunction with spin-trapping experiments with Fe(II)EDTA, Fe(II)HEDTA, Fe(II)DETAPAC and H_2O_2 suggests that DETAPAC inhibits the formation of $^{\cdot}OH$ by slowing the reduction of Fe(III) to Fe(II) and not by inhibiting the Fenton reaction.

Superoxide *Fenton reaction* *Hydroxyl radical* *Haber–Weiss reaction* *Iron complex* *Pulse radiolysis* *Spin trapping*

1. INTRODUCTION

The superoxide radical, $O_2^{\cdot -}$, appears to be formed in all aerobic organisms, and has many potential deleterious effects [1–4]. While the reactivity of superoxide alone is quite limited, there is considerable evidence that in the presence of H_2O_2, it will give rise to the highly reactive hydroxyl free radical [5–8]. To date there has been no demonstration of a direct reaction between $O_2^{\cdot -}$ and H_2O_2 [9–11]. However, addition of small amounts of iron salts to superoxide-generating systems results in the formation of $^{\cdot}OH$ [5,12,13]. The mechanism is believed to be the 'iron-catalyzed Haber–Weiss reaction':

$$\text{Fe(III) complex} + O_2^{\cdot -} \longrightarrow \text{Fe(II) complex} + O_2 \qquad (1)$$

$$\text{Fe(II) complex} + H_2O_2 \longrightarrow \text{Fe(III) complex} + OH^- + {}^{\cdot}OH \qquad (2)$$

It has been shown that the iron-catalyzed production of hydroxyl radical by superoxide-generating systems can be inhibited by the metal chelator diethylenetriaminepentaacetic acid (DTPA or DETAPAC) [5,13,14], but not by the related ligands ethylenediaminetetraacetic acid (EDTA) and *N*-hydroxyethylenediaminetriacetic acid (HEDTA). This inhibition could result from a decrease in the rate of the reduction of Fe(III) by superoxide, eq.(1), or from a slowing the Fenton reaction, eq.(2). DETAPAC appears not to block the Fenton reaction, eq.(2) above [14,15]; in a rather complex superoxide-generating enzyme system no evidence for the reaction of eq.(1) in the presence of DETAPAC was found [14,15]. In [16] reaction (1) was shown to be slower with the complex Fe(III)DETAPAC than with Fe(III)EDTA.

To discriminate further between these two modes of action in the inhibition of $^{\cdot}OH$ production we have applied both pulse radiolysis and spin trapping techniques to the study of reactions (1) and (2). We have chosen a set of structurally

Published by Elsevier Science Publishers B.V.
00145793/83/$ 3.00

related chelates to investigate these processes. Here we show by pulse radiolysis that the reaction of $O_2^{\cdot-}$ with Fe(III)DETAPAC is indeed very slow, much slower than indicated in [16], compared to its reaction with Fe(III)EDTA or Fe(III)HEDTA. Further, spin trapping confirms the production of $^{\cdot}OH$ by Fe(II) chelates of DETAPAC, EDTA and HEDTA.

2. MATERIALS AND METHODS

HEDTA (*N*-(2-hydroxyethyl)ethylenediaminetriacetic acid, trisodium salt dihydrate), DETAPAC (diethylenetriaminepentaacetic acid), EDTA (ethylenediaminetetraacetic acid), and the spin trap, DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide), were from Aldrich Chemical Co. (Milwaukee WI). The DMPO was purified as in [17] and stored at 4°C in aqueous solution. The concentration of the stock solution was determined using an ϵ_{232} = 7700 $M^{-1}.cm^{-1}$ in ethanol [18]. Ferric ammonium sulfate was from Mallinckrodt Chemical Works (St Louis MO) and all other reagents were from J.T. Baker Chemical Co. (Phillipsburg NJ).

The pulse radiolysis studies were done with the Notre Dame Radiation Laboratory linear accelerator using a ~5 ns pulse of 8-MeV electrons. Data gathering and analysis techniques are described in [19,20]. The $(CNS)_2^-$ ion was used for dosimetry [21]. Phosphate-buffered (5 mM) solutions of Fe(III)chelate (0.1 mM) containing 20 mM sodium formate were prepared immediately before radiation. Solutions were bubbled with O_2 and maintained at room temperature, 22°C. Superoxide anion was monitored in the region around 250 nm while Fe(III) reduction was studied at 280–300 nm.

Electron spin resonance (ESR) spin-trapping experiments were carried out with a Varian E-4 spectrometer equipped with an E-231 cavity and aqueous sample cell accessory.

3. RESULTS

3.1. *Pulse radiolysis kinetic results*

The presence of formate in the radiolysis solution allows the production of $O_2^{\cdot-}$ from the various radiolysis products [22,23]. As the pK of $HO_2^{\cdot}$ is 4.88 [24], the reducing radical is principally $O_2^{\cdot-}$ in the pH range studied. The rate constants were obtained from pseudo first-order kinetic analysis. Our results with Fe(III)EDTA (table 1) are in agreement with those in [16,23]. Fe(III)HEDTA is very similar to Fe(III)EDTA in its reaction with $O_2^{\cdot-}$ at pH 6 and 7. However, at pH 8 an apparent complex with superoxide was formed, similar to that reported for the Fe(III)EDTA and $O_2^{\cdot-}$ system at pH 10–12 in [23]. We were not able to detect any reaction of $O_2^{\cdot-}$ with Fe(III)DETAPAC in this pH range. In fact, in the present experiments the apparent lifetime of superoxide increased in the presence of Fe(III)DETAPAC. As DETAPAC was in slight excess, it is conceivable that the excess DETAPAC chelated those trace metals introduced by the buffer salts and formate, rendering them inactive in the catalytic dismutation of $O_2^{\cdot-}$. With the conditions of our experiments we suggest that the rate constant for the reaction of $O_2^{\cdot-}$ with Fe(III)DETAPAC is $< 1 \times 10^4$ $M^{-1}.s^{-1}$ compared to $\sim 10^6$ $M^{-1}.s^{-1}$ for the two other complexes studied.

3.2. *Spin trapping of $^{\cdot}OH$*

When aliquots of N_2 purged Fe(II)chelate solutions (20 μM final conc.) were introduced into a solution of H_2O_2 (80 μM) and DMPO (50 mM) an ESR signal consistent with the spin trapping of the hydroxyl free radical was observed ($aN = aH$ = 15.0 G [25,26]). The intensity of the signal observ-

Table 1

Second-order rate constants for the reaction of $O_2^{\cdot-}$ with Fe(III)chelates ($M^{-1}.s^{-1}$)

Chelate	pH 6.0	pH 7.0	pH 8.0	[Ref.]
Fe(III) EDTA	3.1×10^6	1.9×10^6	5.0×10^5	Here
	5×10^6 (pH 5.8)	1.8×10^6	4.6×10^5 (pH 8.1)	[23]
	3.6×10^6	1.3×10^6	3.0×10^5	[16]
Fe(III) HEDTA	3.8×10^6	7.6×10^5	Complex formation	Here
Fe(III) DETAPAC	$< 10^4$	$< 10^4$	$< 10^4$	Here
	No data	$< 10^5$	No data	[16]

ed was a function of the chelate, holding all other conditions constant. The most intense signal was obtained with Fe(II)DETAPAC. Fe(II)HEDTA produced a DMPO/OH signal whose intensity was about 40% of that observed with Fe(II)DETAPAC while Fe(II)EDTA produced a signal whose intensity was about 8% of that observed with Fe(II)DETAPAC. No signal was observed in the absence of the Fe(II)chelate.

4. DISCUSSION

We find that the rate of reaction (1) decreases with increasing pH. This appears to be correlated to either the degree of hydrolysis of the complex, and/or the net overall charge of the complex. Fe(III)DETAPAC at pH 6–8 exists in a nonhydrolyzed form with a net charge of −2 [27], whereas Fe(III)HEDTA in this pH range exists principally in a nonhydrolyzed form with a net overall charge of −1 [28]. At pH 8, ~10% of the Fe(III)HEDTA is hydrolyzed to a form with a −2 net charge. Fe(III)EDTA in the pH range 6–8 has both a non-hydrolyzed form with a −1 net charge and a hydrolyzed form with a −2 net charge present [27]. An approximate 50–50 mixture of these forms exists at pH 7.5. Thus, it would appear that the overall charge of the complex may play a role in the pH dependence of the kinetics of reaction (1).

In the pH range employed here, reaction (1) is thermodynamically favored for all three Fe(III) chelates. At pH 7, the data of [27–29] yield the following midpoint reduction potentials (VS SHRE):

$$\text{Fe(III)EDTA} + e^- \longrightarrow \text{Fe(II)EDTA} \qquad E = +113\ \text{mV}$$

$$\text{Fe(III)HEDTA} + e^- \longrightarrow \text{Fe(II)HEDTA} \qquad E = +372\ \text{mV}$$

$$\text{Fe(III)DETAPAC} + e^- \longrightarrow \text{Fe(II)DETAPAC} \qquad E = +357\ \text{mV}$$

Coupling this data with the one electron reduction of oxygen at pH 7

$$O_2 + e^- \longrightarrow O_2^{\cdot -}$$

$$E^{o1} = -330\ \text{mV}$$

we see that in fact the reaction of $O_2^{\cdot -}$ with Fe(III)DETAPAC appear to be the most thermodynamically favorable reaction. Thus, thermodynamics appear not to govern the observed rates of reaction (1), but rather, steric factors may play an important role.

The observations [14] with $^{\cdot}OH$ scavengers and the results of our spin trapping experiments indicate that the Fe(II) forms of these chelates are quite capable of participating in the Fenton reaction, eq. (2). Surprisingly, our studies show that Fe(II)DETAPAC is far more efficient in catalyzing the Fenton reaction than the structurally related EDTA and HEDTA. These kinetic experiments indicate that DETAPAC inhibits the 'iron-catalyzed Haber–Weiss reaction' by slowing the rate at which the reaction (1) occurs. Thus, the use of DETAPAC to probe for the role of the iron-catalyzed Haber–Weiss reaction in various biochemical and biological processes as employed by many investigators [30–38] is justified on kinetic grounds. However, it should be noted that if iron complexes are introduced into systems to probe for this chemistry it would be much better to introduce the Fe(III) form rather than the Fe(II) form as the reaction (2) will proceed. This has not always been done.

ACKNOWLEDGEMENTS

We gratefully acknowledge NSF equipment grants PRM-8109079 and TFI 8019273 as well as the support of the Research Corporation and the Haines Fund. Additional support was provided by the Office of Basic Energy Sciences of the Department of Energy. This is document NO NDRL 2429 from the Notre Dame Radiation Laboratory.

REFERENCES

[1] Fridovich, I. (1975) Annu. Rev. Biochem. 44, 147–159.
[2] Halliwell, B. (1981) in: Age Pigments (Sohal, R.S. ed) pp.1–62, Elsevier, Amsterdam, New York.
[3] Fridovich, I. (1978) Science 201, 875–880.
[4] Bors, W., Saran, M., Lengfelder, E., Michel, C., Fuchs, S.C. and Frenzel, C. (1978) Photochem. Photobiol. 28, 629–638.
[5] Buettner, G.R., Oberley, L.W. and Leuthauser, S.W.H.C. (1978) Photochem. Photobiol. 28, 693–695.

[6] Halliwell, B. (1978) FEBS Lett. 92, 321–326.
[7] Fong, K.-L., McCay, P.B., Poyer, J.L., Misra, H.P. and Keele, B.B. (1976) Chem.-Biol. Interact. 15, 77–89.
[8] Cohen, G. (1977) in: Superoxide and Superoxide Dismutases (Michaelson, A.M. et al. eds) pp.317–321, Academic Press, London, New York.
[9] McClune, G.J. and Fee, J.A. (1976) FEBS Lett. 67, 294–298.
[10] Halliwell, B. (1976) FEBS Lett. 72, 8–10.
[11] Rigo, A., Stevanato, R., Finazzi-Agro, A. and Rotillio, G. (1977) FEBS Lett. 80, 130–132.
[12] McCord, J.M. and Day, E.D. (1978) FEBS Lett. 86, 139–142.
[13] Halliwell, B. (1978) FEBS Lett. 92, 321–326.
[14] Cohen, G. and Sinet, P.M. (1982) FEBS Lett. 138, 258–260.
[15] Cohen, G. and Sinet, P.M. (1980) Dev. Biochem. 11A, 27–37.
[16] Butler, J. and Halliwell, B. (1982) Arch. Biochem. Biophys. 218, 174–178.
[17] Buettner, G.R. and Oberley, L.W. (1978) Biochem. Biophys. Res. Commun. 83, 69–74.
[18] Hamer, J. and Macaluso, A. (1964) Chem. Rev. 64, 473–495.
[19] Patterson, L.K. and Lilie, J. (1974) Int.J. Radiat. Phys. Chem. 6, 129–141.
[20] Schuler, R.H. and Buzzard, G.K. (1976) Int. J. Radiat. Phys. Chem. 8, 563–574.
[21] Schuler, R.H., Patterson, L.K. and Janata, E. (1980) J. Phys. Chem. 84, 2088–2089.
[22] Matheson, M.S. and Dorfman, L.M. (1969) Pulse Radiolysis, MIT Press, Cambridge MA.
[23] Ilan, Y.A. and Czapski, G. (1977) Biochim. Biophys. Acta 498, 386–394.
[24] Behar, D., Czapski, G., Rabani, J., Dorfman, L.M. and Schwartz, H.A. (1970) J. Phys. Chem. 74, 3209–3218.
[25] Harbour, J.R., Chow, V. and Bolton, J.R. (1974) Can. J. Chem. 52, 3549–3554.
[26] Buettner, G.R. (1982) in: Superoxide Dismutase (Oberley, L.W. ed) vol.2, ch.4, CRC Press, Boca Raton FL.
[27] Vandegaer, J., Chaberek, S. and Frost, A.E. (1959) J. Inorg. Nucl. Chem. 11, 210–221.
[28] Skochdopole, R. and Chabarek, S. (1959) J. Inorg. Nucl. Chem. 11, 222–233.
[29] Schwarzenbach, G. and Heller, J. (1951) Helv. Chim. Acta 34, 576–591.
[30] Rosen, H. and Klebanoff, S.J. (1981) Arch. Biochem. Biophys. 208, 512–519.
[31] Halliwell, B. (1978) FEBS Lett. 96, 238–242.
[32] Robertson, P. jr and Fridovich, I. (1982) Arch. Biochem. Biophys. 213, 353–357.
[33] McLennan, G., Oberley, L.W. and Autor, A.P. (1980) Radiat. Res. 84, 122–132.
[34] Lesko, S.A., Lorentzen, R.J. and Ts'o, P.O.P. (1980) Biochemistry 19, 3023–3028.
[35] Powis, G., Svingen, B.A. and Degraw, C. (1982) Biochem. Pharmac. 31, 293–299.
[36] Fischer, L.J. and Hamburger, S.A. (1980) Diabetes 29, 213–216.
[37] Grankvist, K., Marklund, S. and Taljedal, I.-B. (1979) FEBS Lett. 105, 15–18.
[38] Brawn, K. and Fridovich, I. (1981) Arch. Biochem. Biophys. 206, 414–419.