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Reactions of iron(II) nucleotide complexes with hydrogen peroxide

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The rate constants for the reactions of hydrogen peroxide with ferrous complexes of ATP, ADP, UTP, citrate and pyrophosphate were measured at pH 7.2. These ligands are potential chelators of iron(II) in the low-molecular weight iron pool that may catalyze oxidative degradation of tissues. The second-order rate constants range from 5.5×10^3 $M^{-1}s^{-1}$ (UTP) to 1×10^5 $M^{-1}s^{-1}$ (pyrophosphate) at pH 7.2. The kinetic dependences of the ATP reaction are consistent with a mechanism involving a 'ferryl' (Fe^{IV}) transient which decays to the hydroxyl radical and ferric ATP.

Fenton reaction; Adenosine triphosphate; Hydrogen peroxide; Ferrous; Nucleotide; Pyrophoshate; Ferryl intermediate

1. INTRODUCTION

Recently, evidence has been presented [1] that ATP ligates the small amount of mobile or 'free' iron in the biological iron pool [2,3]. It has been argued that these trace amounts of redox-active iron catalyze processes that result in oxidative damage in tissues [4,5]. Deleterious reactions may involve both ferrous and ferric oxidation states [6], or perhaps iron(IV) species [7–10], though the most certain cause of free-radical promoted peroxidation is the Fenton reaction [11]. It has been shown [12,13] that ferrous ATP is oxidized by hydrogen peroxide to produce an intermediate that reacts with amino acids, and with the free-radical trap DMPO to produce its hydroxyl radical adduct. In this communication we describe the kinetics of the reactions of hydrogen peroxide with ferrous ATP, reaction 1,

$$Fe^{II}ATP^{2-} + H_2O_2 \longrightarrow Fe^{III}(OH^-)ATP^{2-} + {}^{\cdot}OH \qquad (1)$$

and some related polyphosphate complexes. We show that the rates of these reactions are comparable to those of Fe^{II}-EDTA and -nitrilotriacetate (NTA) [7,8].

2. MATERIALS AND METHODS

[ATP, the disodium salt (98%) from Sigma, was dissolved in a deaerated phosphate buffer and ferrous ions were added as ferrous ammonium sulphate. A binding constant larger than 5×10^4 M^{-1} was assumed, based on literature data for the manganous complex [14]. Usually, a 4-fold excess of ATP over the ferrous ion concentration was employed, sufficient to ensure at least 95% complexation. These solutions were mixed in a stopped-flow spectrometer (Kinetics Instruments/On Line Instrument Systems) with a deaerated hydrogen peroxide solution under conditions of either an excess of Fe^{II}ATP or of hydrogen peroxide. Rates of Fe^{II}ATP formation, measured at 300 and 330 nm, were exponential. An exactly similar procedure was used for uridine-5′-triphosphate (UTP) and adenosine-5′-diphosphate (ADP). The reactions of ferrous pyrophosphate and ferrous citrate with hydrogen peroxide were measured in 10 mM solutions of the ligand, also at pH 7.2 and 25°C.

3. RESULTS

Observed rate constants for the formation of iron(III), obtained in the presence of excess iron(II) complex, are shown in fig.1 for the ligands ATP, UTP, and pyrophosphate. The rates are proportional to the concentration of ferrous complex. Calculated second-order rate constants, listed in table 1A 1, vary from 5×10^3 $M^{-1}s^{-1}$ for ferrous citrate to 1×10^5 $M^{-1}s^{-1}$ for ferrous pyrophosphate. The rates of reactions of ferrous ATP and UTP are virtually independent of the base. The rate of the Fe^{II}ATP reaction increases with pH. At neutral pH the second-order rate constant is 6.0×10^3 $M^{-1}s^{-1}$ by interpolation. The ADP complex is approximately twice as reactive as the ATP system. Removal of the organic component of the ligand causes a nearly 10-fold increase in the rate of reaction of ferrous pyrophosphate in comparison with ferrous ADP. This trend-increased reactivity when the ferrous ion is more accessible has also been observed for aminopolycarboxylate ligands [7,8] and is to be expected for an inner-sphere reaction.

If hydroxyl radicals formed in eq.1 reacted with excess ATP to form a radical incapable of further reaction with ferrous ATP, then graphs of the rates of ferric chelate formed vs excess reagent, either Fe^{II}ATP or H_2O_2, would be identical. The reaction of the hydroxyl radical with hydrogen peroxide proceeds slowly (2×10^7 $M^{-1}s^{-1}$, [15]) and can be neglected. The dependence of Fe^{III}ATP formation upon hydrogen peroxide concentration is non-linear as shown in fig.1. At low

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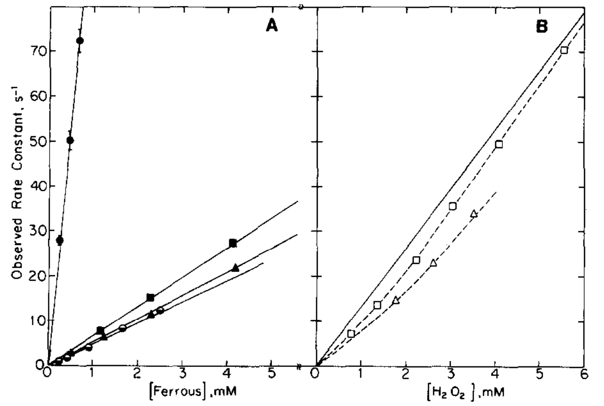

Fig.1. (A) Kinetic data for the reactions of $Fe^{II}L$ with H_2O_2 under conditions of excess ferrous complex. L = pyrophosphate (●); Uridine-5′-triphosphate (▲); Adenosine-5′-triphosphate (■); and citrate (⊖). (B) Data for ATP (□) and UTP (△) were obtained under conditions of excess hydrogen peroxide. The dashed lines through these ATP and UTP data were calculated with the help of eq. 6. The slope expected for a reaction in which two ferrous ATP molecules are oxidized per hydrogen peroxide (R = 2 in eq. 6) is shown as the solid diagonal.

(<0.5 mM) hydrogen peroxide concentrations the observed rates are approximately identical to those obtained under excess ferrous complex conditions, but the rates are nearly twice as large at hydrogen peroxide concentrations in excess of 4 mM. The following mechanism is consistent with these observations.

$$Fe^{II}ATP + H_2O_2 \xrightarrow{k_2} Fe^{IV}OATP + H_2O \quad (2)$$

$$Fe^{IV}OATP + H^+ \xrightarrow{k_3} Fe^{III}ATP + {}^{\cdot}OH \text{ or } Fe^{III}ATP^{\cdot} \quad (3)$$

$$Fe^{IV}OATP + H_2O_2 \xrightarrow{k_4} Fe^{III}O_2^{\cdot}\,ATP + H_2O \quad (4)$$

$$Fe^{III}O_2^{\cdot}\,ATP + Fe^{II}ATP + 2H^+ \xrightarrow{k_2} 2\,Fe^{III}ATP + H_2O_2 \quad (5)$$

In eq. 3 $ATP^{\cdot}$ indicates an unspecified ligand radical, the result of the reaction of the hydroxyl radical with ATP. The hydrogen peroxide dependent rate constant and the stoichiometry of the reaction are consistent with a ferryl ATP transient capable of further reaction with hydrogen peroxide (reaction 4) *and* competitive decomposition to the hydroxyl radical and $Fe^{III}ATP$ (reaction 3). The rate law below was derived from eqs. 2–5 and was used to fit the rate dependence on excess hydrogen peroxide concentration:

$$\frac{d[Fe^{III}]}{d(t)} = Rk_2[Fe^{II}ATP]\,[H_2O_2];$$

$$R = 1 + \frac{(k_4/k_3)\,[H_2O_2]}{1 + (k_4/k_3)\,[H_2O_2]} \quad (6)$$

The fitted curve in fig.1B is obtained when $k_2 = 6.6 \times 10^3\ M^{-1}s^{-1}$ and $k_4/k_3 = 1.33 \times 10^3\ M^{-1}$. A similar dependence applies to the ferrous UTP reaction.

Table 1

(A) Summary of second-order rate constants for reactions of $Fe^{II}L$ with H_2O_2

Reaction	Conditions[a]	Rate constant ($M^{-1}s^{-1}$)
$Fe^{II}ATP^{2-}$	pH 7.2	$6.6(\pm 0.3) \times 10^3$
	pH 4.5	$\sim 2.4 \times 10^3$
	pH 5.5	$\sim 3.8 \times 10^3$
	pH 6.6	$\sim 4.4 \times 10^3$
	pH 7.8	$\sim 7.2 \times 10^3$
	pH 8.9	$\sim 2.6 \times 10^4$
$Fe^{II}ADP^-$	pH 7.2	$1.1(\pm 0.2) \times 10^4$
$Fe^{II}UTP^{2-}$	pH 7.2	$5.2(\pm 0.3) \times 10^3$
$Fe^{II}P_2O_7^{2-}$	pH 7.2	$1.0(\pm 0.2) \times 10^5$
$Fe^{II}citrate^-$	pH 7.2	$4.9(\pm 0.3) \times 10^3$

(B) Rate data for the reaction of $Fe^{II}ADP$ with H_2O_2

$[Fe^{II}ADP]$ (mM)	Observed rate constant (s^{-1})
0.3	2.3
0.75	8.8
1.5	18
3.0	34

[a] All experiments were carried out at 25°C and in the presence of 0.1 M NaCl and 5–10 mM phosphate buffer

This interpretation of the hydrogen peroxide dependence was supported by other experiments. The involvement of a second molecule of hydrogen peroxide does not involve its oxidation to free superoxide by either hydroxyl radical or $Fe^{IV}OATP$, since bovine copper-zinc superoxide dismutase added to the hydrogen peroxide solution immediately before reaction did not affect the rate. Methanol at a final concentration of 100 mM similarly had no influence when hydrogen peroxide was in excess. This confirms that the hydroxyl radical does not play a part in the oxidation of ferrous ATP. A direct determination of R in eq. 6 was made by analysis of the hydrogen peroxide concentration before and after its reaction with various concentrations of ferrous ATP. These results and the stoichiometries estimated from eq. 6 are given in table 2.

Table 2

Dependence of the $Fe^{III}ATP/H_2O_2$ stoichiometry upon iron (II) and H_2O_2

Initial concentrations (mM)		Stoichiometries $d[Fe^{III}]/d[H_2O_2]$[a]	
$[Fe^{II}ATP]$	$[H_2O_2]$	Experiment	Calculated
2.3	5.0	1.5	1.75
2.3	8.2	1.5	1.80
1.1	5.0	1.5	1.75
1.1	2.6	1.6	1.64
1–4	0.1	1(±0.1)	1.00

[a] Hydrogen peroxide was determined before and after reaction as the titanium (IV) peroxide complex (ε at 408 nm = 470 $M^{-1}\cdot cm^{-1}$) in 2 N H_2SO_4 with a correction for the small absorbance of Fe^{III}. When excess iron(II)ATP was present, the amount of iron(III)ATP was determined from the absorbance change in the stopped-flow cell

4. DISCUSSION

The ferrous ATP and UTP complexes react at rates comparable to that of ferrous EDTA and ferrous NTA [7,8]. These reactions are sufficiently fast that reaction with hydrogen peroxide will be significant, even at low concentrations. Preliminary experiments suggest that oxygen oxidizes ferrous ATP and ADP slowly near pH 7.4 ($k \cong 1\ M^{-1}s^{-1}$ for both complexes). Therefore, these divalent metal complexes will be long-lived, especially under conditions of reduced oxygen pressure as found in the cell.

Under biological conditions, reaction 3 will be the most probable course of the ferrous nucleotide/ hydrogen peroxide interaction. We confirmed the presence of a hydroxylating agent by detecting 2,3- and 2,5-dihydroxybenzoate by HPLC when salicylate is present during the anaerobic hydrogen peroxide reaction (not shown). The ratio of these products was consistent with the production of hydroxyl radicals [16], in agreement with the literature [17,18]. The kinetic evidence for $Fe^{IV}OATP$ is not conclusive but it is not surprising that it would be detected in a polyphosphate ligand system. Pulse radiolysis experiments have shown that short-lived Fe(IV) species can be formed by the hydroxyl radical oxidation of ferric pyrophosphate and hydroxo complexes at higher pH [19], and that such species react rapidly with hydrogen peroxide (B.H.J. Bielski, personal communication).

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REFERENCES

[1] Weaver, J. and Pollack, S. (1989) Biochem. J. 261, 787–792.
[2] Mulligan, M., Althaus, B. and Linder, M.C. (1986) Int. J. Biochem. 18, 791–798.
[3] Bakkeren, D.L., De Jeu-Jaspars, C.M.H., Van der Heul, C. and Van Eyck, H.G. (1989) Int. J. Biochem. 17, 791–798.
[4] Dunford, H.B. (1987) Free Radical Biol. Med. 3, 405–421.
[5] Halliwell, B. and Gutteridge, J.M.C. (1986) Arch. Biochem. Biophys. 246, 501–514.
[6] Minotti, G. and Aust, S.D. (1987) J. Biol. Chem. 262, 1098–1104.
[7] Rush, J.D. and Koppenol, W.H. (1987) J. Inorg. Biochem. 29, 199–215.
[8] Rush, J.D. and Koppenol, W.H. (1988) J. Am. Chem. Soc. 110, 4957–4963.
[9] Rahhal, S. and Richter, H.W. (1988) J. Am. Chem. Soc. 110, 3126–3133.
[10] Sutton, H.C. and Winterbourn, C.C. (1989) Free Radical Biol. Med. 6, 53–60.
[11] Fenton, H.J.H. (1884) J. Chem. Soc. 65, 899–910.
[12] Floyd, R.A. (1983) Arch. Biochem. Biophys. 225, 263–270.
[13] Floyd, R.A. and Lewis, C.A. (1983) Biochemistry 22, 2645–2649.
[14] Martell, A.E. and Smith, R.M. (1988) in: Critical Stability Constants. Volume 5: First Supplement, Plenum Press, New York.
[15] Buxton, G.V., Greenstock, C.L., Helman, W.P. and Ross, A.B. (1988) J. Phys. Chem. Ref. Data 17, 513–886.
[16] Maskos, S., Rush, J.D. and Koppenol, W.H. (1990) Free Radical Biol. Med., in press.
[17] Floyd, R.A., Henderson, R., Watson, J.J. and Wong, P.K. (1986) J. Free Radical Biol. Med. 2, 13–18.
[18] Floyd, R.A., Watson, J.J. and Wong, P.K. (1984) J. Biochem. Biophys. Methods 10, 221–235.
[19] Rush, J.D. and Bielski, B.H.J. (1986) J. Am. Chem. Soc. 108, 523–525.