Supplementary information for the manuscript “Ascorbate Oxidation by Iron, Copper and Reactive Oxygen Species: Review, Model Development, and Derivation of Key Rate Constants”

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1. Additional Mechanisms for Cu Reactions with Ascorbic Acid Proposed in the Literature

Jameson and Blackburn[1]

$$\mathrm{Cu^{2+} + AH^{-} \rightleftharpoons [Cu(AH)]^{+}} \qquad \text{Eq(7)}$$

$$\mathrm{2[Cu(AH)]^{+} \rightleftharpoons [Cu_2(AH)_2]^{2+}} \qquad \text{Eq(8)}$$

$$\mathrm{[Cu_2(AH)_2]^{2+} + O_2 \rightleftharpoons [Cu_2(AH)_2(O_2)]^{2+}} \qquad \text{Eq(9)}$$

$$\mathrm{[Cu_2(AH)_2(O_2)]^{2+} \rightarrow [CuA(O_2H)]^{\cdot} + Cu^{2+} + A^{\cdot -} + H^{+}} \qquad \text{Eq(10)}$$

$$\mathrm{A^{\cdot -} + [Cu_2(AH)_2(O_2)]^{2+} \rightarrow [CuA(O_2H)]^{\cdot} + [Cu(AH)]^{+} + DHA} \qquad \text{Eq(11)}$$

$$\mathrm{[CuA(O_2H)]^{\cdot} \rightarrow A^{\cdot -} + HO_2^{-} + Cu^{2+}} \qquad \text{Eq(12)}$$

$$\mathrm{2[CuA(O_2H)]^{\cdot} \rightarrow termination\ products} \qquad \text{Eq(13)}$$

$$\mathrm{H^{+} + HO_2^{-} \rightleftharpoons H_2O_2} \qquad \text{Eq(14)}$$

Scheme C Shtamm et al.[2]

$$2Cu^{2+} + AH^{-} \rightarrow 2Cu^{+} + DHA + H^{+} \qquad Eq(15)$$

$$Cu^{+} + O_2 \rightarrow CuO_2^{+} \qquad Eq(16)$$

$$CuO_2^{+} + AH^{-} \rightarrow Cu^{+} + DHA + HO_2^{-} \qquad Eq(17)$$

$$CuO_2^{+} + Cu^{+} + 2H^{+} \rightarrow 2Cu^{2+} + H_2O_2 \qquad Eq(18)$$

2. Fluorescence Measurement

The fluorescent product 3-(1,2-dihydroxyethyl)-fluoro[3,4-b]quinoxaline-1-one (DFQ) is excited at λ = 365 nm by a high-power UV LED, with a strong peak emission at λ_{max} = 430 nm measured using optical fibers (Thorlabs M93L01) coupled to a femtowatt photoreceiver (Thorlabs type PDF10A) with a cyan fluorescent protein excitation filter (Thorlabs type MF434-17), which only transmits light between 423 and 445 nm. For pH 7 the mixture containing DFQ is measured with a spectrometer (Ocean Optics QE Pro) with an integration time of 1000 ms and an integration interval from 425 to 435 nm.

3. Copper – ROS Reactions

OH· production from Cu(II) is very sensitive to Cu(II) + H_2O_2 (R77) which produces Cu(I) and the reactions between Cu(I) and H_2O_2 (R80-81) which together control the formation of OH·. Pham et al.[3] found a high rate constant for R77 of 460 $M^{-1}s^{-1}$, but most studies agree that the rate constant for copper and copper hydroxide species is < 1 $M^{-1}s^{-1}$ [4-7] and 70 $M^{-1}s^{-1}$ for the Cu(II)-chloro complex;[5] we adopt these values here (Tab. 2). Similar to the iron Fenton reaction, Cu(III) is also thought to be a possible product of the reaction between Cu(I) and H_2O_2 with a rate constant of 61 $M^{-1}s^{-1}$;[3] only an upper limit rate constant (100 $M^{-1}s^{-1}$) could be found for the reaction of Cu(I) and $H_2O_2 \rightarrow$ OH· (R80[8]). Relatively larger uncertainties exist in the copper Fenton reaction than iron.

Other potential sources of error can be the uncertainties of the rate law associated with the background ions (see manuscript).

Table S1. Rate constant for $OH\cdot + AH_2/AH^-$

pH	Rate constant ($M^{-1}\ s^{-1}$)	Reference
1.0	$7.9 \times 10^{9*}$	Redpath and Willson[9]
7.0	10^9	
7.4	$1.1 \times 10^{10*}$	Buettner and Schafer[10]
1.0	7.2×10^9	Bielski[11]
1.5	4.5×10^9	Bielski[11]
7.0	7.0×10^9	
7.0	1.1×10^{10}	Bielski[11]
11.0	4.1×10^9	Bielski[11]
7.4	1.1×10^{10}	Lakey et al.[7]

* Rate constants adopted in this study.

Table S2. Reaction conditions

Final reaction conditions: reaction coil-1				
	pH 2.8		**pH 7**	
Ascorbic acid concentration	100	µM	200	µM
Flow rates:				
Ascorbic acid	1.1	ml/min	0.5	ml/min
DHA/Cu(II)/Fe(II)/Fe(III)	1.1	ml/min	0.5	ml/min

Temp:	37	°C	37	°C
Reaction time:	20	min	20	min
Final reaction conditions: reaction coil-2				
Ascorbic acid concentration	67	µM	100	µM
oPDA concentration	15	mM	10	mM
Flow rates:				
Ascorbic acid	1.1	ml/min	0.5	ml/min
DHA/Cu(II)/Fe(II)/Fe(III)	1.1	ml/min	0.5	ml/min
oPDA	1.1	ml/min	1	ml/min
Temp:	20	°C	37	°C
Reaction time:	10	min	5	min

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