Fenton Reagents (1:1 $Fe^{II}L_x$/HOOH) React via [$L_xFe^{II}OOH(BH^+)$] (1) as Hydroxylases (RH → ROH), not as Generators of Free Hydroxyl Radicals (HO•)

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Most[1-5] regard Fenton chemistry as synonymous with the in situ production of free hydroxyl radical (HO•) from the one-to-one combination of iron(II) and hydrogen peroxide (HOOH) (Fenton reagent, usually in aqueous media at pH 2),

$$Fe^{II}(OH_2)_6^{2+} + HOOH \xrightarrow{k_1} (H_2O)_5^{2+}Fe^{III}OH + HO^{\bullet} + H_2O \quad (1)$$

$$k_1 = 41\ M^{-1}\ s^{-1}$$

With this assumption, subsequent reactions have been based on the primary chemistry of HO• (usually generated via pulse radiolysis),[6] which reacts with iron(II)

$$HO^{\bullet} + Fe^{II}(OH_2)_6^{2+} \xrightarrow{k_2} (H_2O)_5^{2+}Fe^{III}OH + H_2O \quad (2)$$

$$k_2 = 3 \times 10^8\ M^{-1}\ s^{-1}$$

and hydrocarbons (RH)[6]

$$HO^{\bullet} + RH \xrightarrow{k_3} R^{\bullet} + H_2O \quad (3)$$

$k_3 = 1.8 \times 10^9\ M^{-1}\ s^{-1}$ (ethane, C_2H_6), $3.7 \times 10^9\ M^{-1}\ s^{-1}$ (c-C_5H_{10}), $7.5 \times 10^9\ M^{-1}\ s^{-1}$ ($PhCH_2CH_3$)

The resultant carbon radical (R•) can (a) dimerize to R_2, (b) react with a second HO• to form ROH, and (c) in the presence of air, couple to O_2 to form ROO•. The ROO• radicals are unreactive with saturated hydrocarbons and couple to give an unstable intermediate [ROOOOR] that homolytically dissociates to ROOR and O_2 ($k_4 = 10^3$–$10^7\ M^{-1}\ s^{-1}$).[3]

The kinetics for substrate reactivities with Fenton-generated "HO•" usually are determined via the relative rate of disappearance of iron(II) (eq 2) to that of the substrate.[1] However, if Fenton reagents generate reactive intermediates (X) other than free HO•, the reactivity of X with iron(II) and organic substrates will be different and may not produce free carbon radical (R•).

Table I summarizes the reactivities and product profiles for several Fenton reagents [$Fe^{II}(PA)_2$, $Fe^{II}(bpy)_2^{2+}$, $Fe^{II}(OH_2)_6^{2+}$/HOOH, *t*-BuOOH] with cyclohexane (c-C_6H_{12}), ethylbenzene ($PhCH_2CH_3$), $Me_2CHCH_2CH_3$, and pyridine[5,7-9] and compares

Table I. Comparison of Hydrocarbon (RH) Reactivities for 1:1 $Fe^{II}L_x$/HOOH(Bu) (10 mM/10 mM) Fenton Reagents [$Fe^{II}(PA)_2$,$Fe^{II}(bpy)_2^{2+}$, $Fe^{II}(OH_2)_6^{2+}$] with Those for Hydroxyl Radical (HO•)

	Fenton reagents (yield, mM ± 5%)[a]									
	$Fe(PA)_2$/(py)$_2$HOAc			$Fe^{II}(PA)_2$/MeCN		$Fe^{II}(bpy)_2^{2+}$/MeCN		$Fe^{II}(OH_2)_6^{2+}$/H_2O^b Ar, pH 2	hydroxyl radical: HO•/H_2O^c
substrate (RH)	Ar	PhSeSePh[d]	O_2, 1 atm	Ar	O_2	Ar	O_2		
c-C_6H_{12}, primary product	R(py) (4)	RSePh (9)	c-C_6H_{10}(O) (2)	ROH (3)	c-C_6H_{10}(O) (1)	nr[e]	c-C_6H_{10}(O) (1)	ROH	R•
$k_{c\text{-}C_6H_{12}}/k_{c\text{-}C_6D_{12}}$ (KIE)[e]	1.7 (HOOH)[f]	2.4 (HOOH)	2.1 (HOOH)				2.7 (HOOH)[g]	1.1 (HOOH)	1.0
	4.6 (*t*-BuOOH)		8.2 (*t*-BuOOH)				10 (*t*-BuOOH)		
$PhCH_2CH_3$, primary product	PhC(O)Me (2)	RSePh (2)	PhC(O)Me (4)	PhC(O)Me (6)	PhC(O)Me (7)	nr	PhC(O)Me (2)	PhCH(OH)Me	HOPh•Et
c-C_6H_{12}/$PhCH_2CH_3$, rel reactivity	2.0	3.9	0.6	0.5	0.1		0.5		0.6
$(Me)_2CHCH_2Me$, primary product	R(py)	RSePh (5)	$Me_2CHC(O)Me$	ROH		nr	$Me_2CHC(O)Me$	ROH	R•
relative reactivity per CH (normalized, 1°/2°/3°)		0.07/0.44/1.0	0.0/1.0/0.0					0.10/0.48/1.0	0.41/0.50/1.0
pyridine (py), primary product	3-HO-py	py-SePh		3-HO-py					2-HO-py• 4-HO-py•

[a] Substrate (1 M) and $Fe^{II}L_x$ (10 mM) combined in 3.5 mL of solvent, followed by the slow addition of HOOH (50%, in H_2O) or *t*-BuOOH (in 2,2,4-trimethylpentane) to give 10 mM HOOH(Bu-*t*). The product solutions were analyzed by capillary-column gas chromatography and GC–MS after a reaction time of 3 h at 24 ± 2 °C (essentially the same product profiles, reactivity ratios, and KIE values were observed for reaction times of 15 min). [b] Data from refs 5–7. [c] Reference 6. In the presence of O_2 the primary product is ROO• (unreactive with saturated hydrocarbons), which dimerizes to [ROOOOR] prior to homolytic dissociation to give ROOR and O_2 (ref 3). [d] Reference 7. Reaction run in the presence of 5 mM PhSeSePh. [e] Kinetic isotope effect. [f] In (py)$_4$HOAc the (py)$_4$$Fe^{II}(OAc)_2$/HOOH/c-$C_6H_{12}$ Fenton system produces (c-C_6H_{11})py (KIE, 1.5) (ref 9). [g] The $Fe^{II}(OPPh_3)_4^{2+}$/HOOH/MeCN and $Cu^{I}(bpy)_2^{+}$/HOOH/(MeCN)$_4$(py) systems are unreactive under Ar, but in the presence of O_2 they yield c-C_6H_{10}(O) (KIE = >10 and 2.4, respectively) (ref 10). [h] No reaction.

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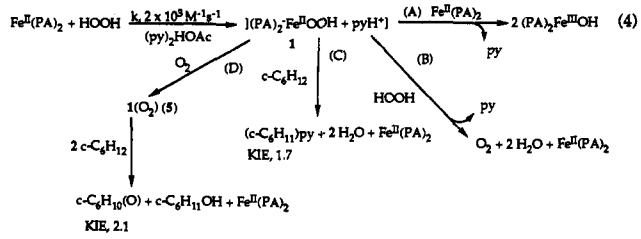
these with those for free HO•. The kinetic isotope effect for cyclohexane (KIE, $k_{c\text{-}C_6H_{12}}/k_{c\text{-}C_6D_{12}}$) in relation to its major products is listed for the various Fenton reagents and solution conditions (absence and presence of O_2) and for free HO•.[6] The product profiles and reactivity for the several Fenton reagents are compared when (a) *t*-BuOOH is substituted for HOOH and (b) O_2 (1 atm) is present in the reaction matrix. In no case is substrate dimer (R–R) (dominant product for HO•/saturated-hydrocarbon reactions in the *absence* of O_2) or ROOR (dominant product for HO•/saturated-hydrocarbon reactions in the *presence* of O_2) detected in the product solutions. In the absence of O_2 the 1:1 $Fe^{II}(bpy)_2^{2+}$/HOOH system is unreactive with c-C_6H_{12} [also true for $Fe^{II}(OPPh_3)_4^{2+}$/HOOH and $Cu^{I}(bpy)_2^{+}$/HOOH].

In the *absence* of O_2, (a) the Fenton systems yield R(py) or ROH [KIE = 1.1–1.7 (HOOH) and 4.6 (*t*-BuOOH)] with reaction efficiencies up to 80% [product per HOOH(Bu-*t*)], and for $Fe^{II}(PA)_2$/HOOH the relative reactivity of c-C_6H_{12}/$PhCH_2CH_3$ is 2.0 (reaction exclusive with the alkyl side chain of $PhCH_2CH_3$), and (b) HO• yields R• (KIE = 1.0) with a reaction efficiency of one R• (or $^1/_2$ R_2) per HO• and has a relative reactivity for c-C_6H_{12}/$PhCH_2CH_3$ of 0.6:1.0 (85% aryl addition). In the *presence* of O_2, (a) the Fenton systems yield ketones [KIE = 2.1–2.7 (HOOH) and 8.2–>10 (*t*-BuOOH)] and have a relative reactivity for c-C_6H_{12}/$PhCH_2CH_3$ from 0.1 [$Fe^{II}(OPPh_3)_4^{2+}$/*t*-BuOOH, O_2] to 0.5 [$Fe^{II}(PA)_2$/HOOH, O_2] to 1.6 [$Cu^{I}(bpy)_2^{+}$/HOOH, O_2], and (b) HO• yields ROO• (KIE = 1.0) with a reaction efficiency of one ROO• (or $^1/_2$ ROOR) per HO•.[3]

Although HO• reacts with CH_4 ($k = 0.11 \times 10^9$ M^{-1} s^{-1} vs 1.8×10^9 M^{-1} s^{-1} for C_2H_6),[6] Fenton reagents are unreactive. When pyridine is the substrate (or present in the solvent matrix), HO• reacts to give 2-HO-py and 4-HO-py (2:1 ratio; $k = 3 \times 10^9$ M^{-1} s^{-1}).[6] However, with Fenton reagents the dominant product is 3-HO-py. Hydroxyl radical reacts with $Fe^{II}(bpy)_3^{2+}$ via aryl addition to give $(bpy)_2^{2+}Fe^{III}$(bpy-OH) ($k = 9 \times 10^9$ M^{-1} s^{-1}),[6] but the 1:1 combination of $Fe^{II}(bpy)_2^{2+}$ and HOOH in MeCN is unreactive (Table I).

In spite of the common belief that Fenton reagents ($Fe^{III}L_x$/HOOH) produce free HO• (eq 1), recent studies[11,12] provide clear evidence that free HO• is not the dominant reactant, and that with highly stabilized iron(II) complexes [Fe^{II}(DETAPAC) and Fe^{II}(EDTA)] a nucleophilic adduct [(EDTA)$Fe^{II}OOH + H_3O^+$, **1**; "bound HO•"] reacts directly with substrates.[12] Another study finds product profiles that are inconsistent with free HO• as the dominant reactive intermediate for a biological Fenton reagent.[13]

The $Fe^{II}(PA)_2$ complex in combination with HOOH is an effective Fenton reagent for organic substrates[7] and has reactivities and product profiles that are within the same mechanistic framework as those for traditional aqueous Fenton reagents.[1] The initial nucleophilic addition of HOOH to $Fe^{II}(PA)_2$ yields the primary reactive intermediate (**1**),[8,14] which reacts with (a)



excess $Fe^{II}(PA)_2$ via path A, (b) excess HOOH via path B to give O_2, (c) excess c-C_6H_{12} via path C to give (c-C_6H_{11})py [aqueous Fenton systems produce c-C_6H_{11}OH with a KIE of 1.1,[5] and free HO• (pulse radiolysis) produces c-C_6H_{11}• with a KIE of 1.0],[6] and (d) with O_2 and excess c-C_6H_{12} via path D and **5** to give c-C_6H_{10}(O). Although radical traps (e.g., PhSeSePh, $BrCCl_3$, DMSO)[7,11,12] often are used to "prove" that free carbon radicals are formed by "free HO•" from Fenton reagents, these also react with nonradicals (e.g., the intermediate of path C, eq 4; Table I).

The results of Table I provide compelling evidence that Fenton reagents *do not produce* (a) free HO•, (b) free carbon radicals (R•), or (c) aryl adducts (HO–Ar•). Early work[15] has demonstrated that the primary chemistry of HOOH is nucleophilic addition, even in matrices as weakly basic as water at pH 2. Hence, Fenton reagents with electrophilic transition-metal complexes ($Fe^{II}L_x$, $Cu^{I}L_x$, and $Mn^{III}L_x$) must have a primary step of nucleophilic addition to the metal center to give **1** (the reactive intermediate of Fenton reagents). The efficient and selective reactivity of **1** (Fenton chemistry) and **5** (*oxygenated Fenton chemistry*) makes them more reasonable cytotoxic agents than free HO• within the oxy-radical theory of aging and heart disease.[16,17]

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Supplementary Material Available: Table comparing hydrocarbon (RH) reactivities for Fenton reagents with those for free hydroxyl radical (HO•) (1 page). Ordering information is given on any current masthead page.

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