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Variability of the Fenton reaction characteristics of the EDTA, DTPA, and citrate complexes of iron

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Abstract

The common metal chelation agents, DTPA and EDTA are often used as models for physiological low-molecular weight iron complexes in biochemical studies, or for common biochemical protocols. In the biochemical literature there are apparent conflicts as to whether EDTA and DTPA are pro-oxidant or antioxidant additives. This apparent conflict is puzzling since in chemical systems Fe^{II}EDTA and Fe^{II}DTPA are well known Fenton reaction reagents. In this investigation we examined the voltammetric characteristics of the iron complexes of EDTA, DTPA, and citrate and the effect of the ligand:metal ratio (L:M) on the electrocatalytic (EC') waves that result from reduction of H_2O_2 by this complex. At a ratio of 1:1, the cyclic voltammetric waves of the complexes indicate the presence of a reversible species corresponding to the $Fe^{II/III}$L couple, along with a second irreversible reduction peak. The second irreversible voltammetric peak decreases at higher L:M ratios for EDTA and citrate. The 1:1 iron complexes of EDTA, DTPA, and citrate clearly induce the catalytic reduction of H_2O_2. In the presence of a greater than 100 fold excess of H_2O_2 relative to iron, higher L:M ratios greatly reduced the catalytic EC' wave compared to the 1:1 ratios. At H_2O_2:Fe ratios less than 50, the L:M ratio has very little effect of the EC' current. These observations may explain the apparent discrepancies in the biochemical literature. Addition of EDTA or DTPA may enhance oxidative processes if the L:M is low (less than unity), whereas rates of on-going oxidative processes may decrease if that ratio, along with the relative amount of H_2O_2, are both high (excess ligand). The impact of this study is of particular importance given the widespread use of these ligands in biochemical studies.

Introduction

There is much confusion on the topic of the pro-oxidant and the anti-oxidant physiological characteristics of both ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) (Egan *et al.* 1992). Various biological and biologically-related investigations of EDTA, and DTPA have indicated that both ligands form metal complexes that are seemingly able to either encourage (Yamazaki & Piette 1990; Egan *et al.* 1992; Ansari *et al.* 1996; Peskin 1996; Kachur *et al.* 1998; Cantini-Esnault *et al.* 2000; Foijta *et al.* 2000; Stolze *et al.* 2000) or prevent (Burkitt & Gilbert 1990; Berman *et al.* 1996; Ramakrishna & Cederbaum 1996; Zho *et al.* 1996; Yoo *et al.* 1999; Evans *et al.* 2000; Sayer *et al.* 2000; Yoon *et al.* 2000; Lee *et al.* 2001) pro-oxidant processes depending on the specific study. In many cases the cited studies may indicate that EDTA is a pro-oxidant, and DTPA is an antioxidant, or vice versa. Such conflicts formed the impetus for this study.

The oxidative damage to biological systems is precipitated by the by-products of the metabolites of dioxygen (Crichton 1987; Koppenol 1987; Gutteridge 1994; Pierre & Fontacave 1999) which, as many investigators have hypothesized, lead to physiological damage and the pathogenesis of many diseases (Berry *et al.* 2001; Chan 2001; Kovacic & Jacintho 2001; Kowald 2001; Penta *et al.* 2001; Wattanapitayakul & Bauer 2001). The most likely species are the one and two electron products of superoxide ion ($O_2^{\cdot -}$), and hydrogen peroxide, respectively. These reactive oxygen species participate in two pro-oxidant reactions:

$$Fe^{II}L + H_2O_2 \rightarrow Fe^{III}L + HO^- + HO^{\cdot} \quad (1)$$

$$O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + HO^- + HO^{\cdot} \quad (2)$$

The Fenton (1) and the Haber-Weiss (2) reactions both produce the highly oxidizing hydroxyl radical ($E^0 > 1.8$ V S.H.E.). The hydroxyl radical, along with the ensemble of the reactive oxygen and nitrogen species is responsible for age-related ailments, such as cancers, Alzheimer's, Parkinson's, and circulatory diseases. The hydroxyl radical oxidizes most physiological organic components with diffusion limited kinetics (Stadtman & Berlett 1991).

The Fenton reaction requires a suitable $Fe^{II/III}L$ complex. Many reviews are available on this subject (Crichton 1987; Koppenol 1987; Pierre & Fontacave 1999). Briefly, the $HO^{\cdot}/HO^-$ redox couple requires a $Fe^{II/III}$ potential negative of 0.32 volts S.H.E. at pH 7.4. Within biological systems there is a plethora of iron-containing enzymes involved in oxygen activation schemes.

The vast majority of these enzymes are capable Fenton reaction centers. The Haber-Weiss reaction is metal catalyzed and may actually be a form of the Fenton reaction where the superoxide ion is responsible for the reduction of $Fe^{III}L$ to $Fe^{II}L$ (Buettner & Jurkiewicz 1996; Patruta & Horl 1999; Kehrer 2000). Under normal circumstances the physiological iron pool is well regulated so as to avoid the consequences of Reactions 1 and 2 (Kaim & Schwerderski 1996). However, under conditions of oxidative stress elevated quantities of reactive oxygen species are produced as a pathogen defense and during inflammatory responses (Babior 2000; Hensley *et al.* 2000; Lavrosky *et al.* 2000; Lum & Roebuck 2001). The oxidative damage by ROS on the organic Fe ligands may cause mobilization of the iron into a low-molecular weight pool (Jacobs 1977; Gutteridge 1986; Gordan & Wietzman 1988; Halliwell & Gutteridge 1989; Halliwell & Arouma 1991; Dabbagh *et al.* 1993; Novellino *et al.* 1999; Jung *et al.* 2000; Alayash *et al.* 2001). Such species, in conjunction with reactive oxygen species may give rise to a cascade of damage to physiological components.

Previous voltammetric studies of the $Fe^{II/III}EDTA$ complex have clearly indicated that it is Fenton reaction active (Kaneko *et al.* 1978; Aoki *et al.* 1988; Zhuang 1993). Such evidence is available in the form of EC' voltammetric waves resulting from the reduction of H_2O_2. In the absence of a suitable $Fe^{II/III}L$ couple, H_2O_2 experiences little or no electrochemical activity. The electrochemically mediated reduction of H_2O_2 is possible by an EC' sequence which follows as:

$$E : Fe^{II}IEDTA + e = Fe^{II}EDTA \quad (3)$$

$$C' : Fe^{II}EDTA + H_2O_2 \rightarrow Fe^{III}EDTA + HO^- + HO^{\cdot} \quad (4)$$

Regeneration of $Fe^{III}EDTA$ within the vicinity of the electrode causes amplification of its cyclic voltammetric reduction wave (Bard & Faulkner 2001). The redox potentials of the $Fe^{II/III}L$ complexes of DTPA, and citrate ($COH(COO^-)CH_2(COO^-)2$) indicate that they are thermodynamically suitable Fenton reaction agents (Stulikova & Vidra 1972; Cox & Cummings 1973; Escot *et al.* 1989; Sohn *et al.* 1993). Given these data it is surprising that many biomedical, and biochemical related studies have indicated these ligands as having antioxidant properties. However, the concentration of chelatable iron in these studies is for the most part unknown. It is estimated that this concentration ranges from 37 μM to 10 nM for sepsis to healthy tissue, respectively (Crichton 1992; Galley & Webster 1996; Gutteridge 1996) It is therefore reasonable to expect that the ratios of metal to ligand vary greatly from study to study.

It is often assumed that given the high affinity and hexa-coordinate nature of chelates such as EDTA and DTPA that the iron-chelate complex, once formed, is fully coordinated by the chelate to the exclusion of all other ligands present. However, at least in the well-studied case of $Fe^{III}EDTA$, the hexadentate chelate will allow coordination of aqua-, hydroxo-, and peroxo- Lewis donors to form $Fe^{III}EDTA$-L mixed ligand complexes. Such complexes are heptacoordinate crystalline solids and are generally accepted to be hepta-coordinate in solution as well (Walling *et al.* 1970; Neese & Solomon 1998). The Fenton reaction characteristics of these possible mixed ligand complexes vary, so it reasons that if the abundances of these species are a function of the relative ratio of Fe:L:H_2O_2, that the EC' current will vary both on the absolute amount of H_2O_2 present as well as the speciation of Fenton complexes. The peroxo-$Fe^{III}EDTA$ complex results in the most extensive decomposition of H_2O_2. The characterization and stability constant for this complex is well known, (Walling *et al.* 1970; Neese & Solomon 1998) and it can be shown through speciation modeling that its relative abundance at pH 7.4 is highly dependent on the concentration of H_2O_2.

Experimental

Chemicals

Diethylenetriaminepentaacetic acid (DTPA, 98%+) and ethylenediaminetetraacetate, tetrasodium salt, (EDTA, 99%) were both obtained from Acros Organics (Pittsburgh, Pennsylvania) and used without further purification. Citric acid, ferric nitrate, nitric acid, hydrogen peroxide (30%), tris buffer, were supplied by Fisher Scientific (Pittsburgh, Pennsylvania) and used as received. Nitrogen (99.99%) was used a purge gas and was obtained from Oxarc (Spokane, Washington).

Solutions

Solutions were prepared by adding approximately 10 ml of Tris Buffer to a volumetric flask. A drop of diluted nitric acid was added to prevent hydrolysis of the Iron. The chelator was dissolved in this solution and the ferric nitrate was added. All components were dissolved and the volumetric flask was brought to capacity with the tris buffer, hydrogen peroxide and metal/chelator stock solutions. The pH of the solution was measured and adjusted to 7.4. Final tris buffer concentration was 80 mM. Prior to voltammetric experiments the solution was purged for 10 min with nitrogen gas. Each solution was run 3–5 times to obtain an average peak current.

Cyclic voltammetry

All cyclic voltammetric experiments were conducted on a Bioanalytical Systems (BAS) CV-50W (West Lafayette, Indiana). The reference electrode was a Ag/AgCl BAS MF2052, and the working electrode was a glassy carbon disk BAS MF2012. The glassy carbon electrode was polished with an aqueous slurry of 1 μm alumina powder between each voltammogram to ensure reproducibility.

Results

Cyclic voltammetry and speciation of the $Fe^{II/III}$ complexes of EDTA, DTPA, and citrate

Figure 1 illustrates the cyclic voltammetric behavior of each complex at various ligand:metal (L:M) ratios at a scan rate of 5 mV/s. The 1:1 $Fe^{II/III}$EDTA complex exhibits a set of reversible coupled cyclic voltammetric peaks ($E_{1/2} \sim -0.1$ V) and a second irreversible reduction peak (Ep ~ -0.4V).

As the L:M ratio increased to 1:10 that second peak was reduced to zero and only the set of reversible CV peaks were observed (ΔEp $\sim$ 60 mV). With DTPA, there was not a significant change as the L:M ratio changed from 1:1 to 5:1, the ligand was insoluble at higher M:L ratios (1.0 mM Fe^{II}). At a L:M ratio of 1:1 citrate exhibited no reversible cyclic voltammetric peaks. A set of irreversible cathodic peaks are apparent from -0.05 to -0.45 V, with one anodic peak at $+0.15$ V. At a 10:1 L:M ratio a clear set of reversible cyclic waves are apparent with a $E_{1/2}$ of $+0.25$ V. A significant irreversible cathodic peak is also present at -0.4 V.

Electrocatalytic reduction of H_2O_2 by the $Fe^{II/III}$ complexes of EDTA, DTPA, and citrate

In the absence of an intermediary redox couple, the direct reduction of 25.2 mM H_2O_2 is insignificant at a sweep rate of 5 mV/s (Voltammograms C of Figure 2). A catalytic EC' wave appears in the presence of 0.1 mM Fe^{III}EDTA (Voltammograms A of Figure 2). This wave is attributable to the sequence of steps previously outlined in Reactions 3 and 4. Iron complexes of DTPA and citrate also exhibited EC' characteristics via the redox cycling of the Fenton Reaction.

The voltammograms labeled B in Figure 2 illustrates the loss of that current as the ligand concentration increases relative to Fe beyond a ratio of 1:1. Figure 3 summarizes the examination of the importance of the L:M ratios with respect to the catalytic H_2O_2 reduction by the Fe complexes. Maximum peak currents for Fe complexes of EDTA and DTPA occurred at ratios of 0.25:1 to 1:1. At L:M ratios of 1.5:1 and beyond the catalytic currents experience a drop greater than 75% for DTPA and EDTA. The L:M range in which Fe-citrate complexes were able to effectively promote the Fenton reaction was much broader than other examined ligands. It can be seen from Figure 3 that this L:M range is from 0.5:1 to 15:1 for Fe-citrate.

The effect of H_2O_2 concentration on the EC' current was also investigated. These studies focused on the complexes of Fe^{III}EDTA due to the larger body of stability constant data available on this system. The combined effects of the relative H_2O_2 and EDTA concentrations on the iron Fenton chemistry and resulting measurable EC' current are best illustrated by the series of overlaid voltammograms of Figure 4. It

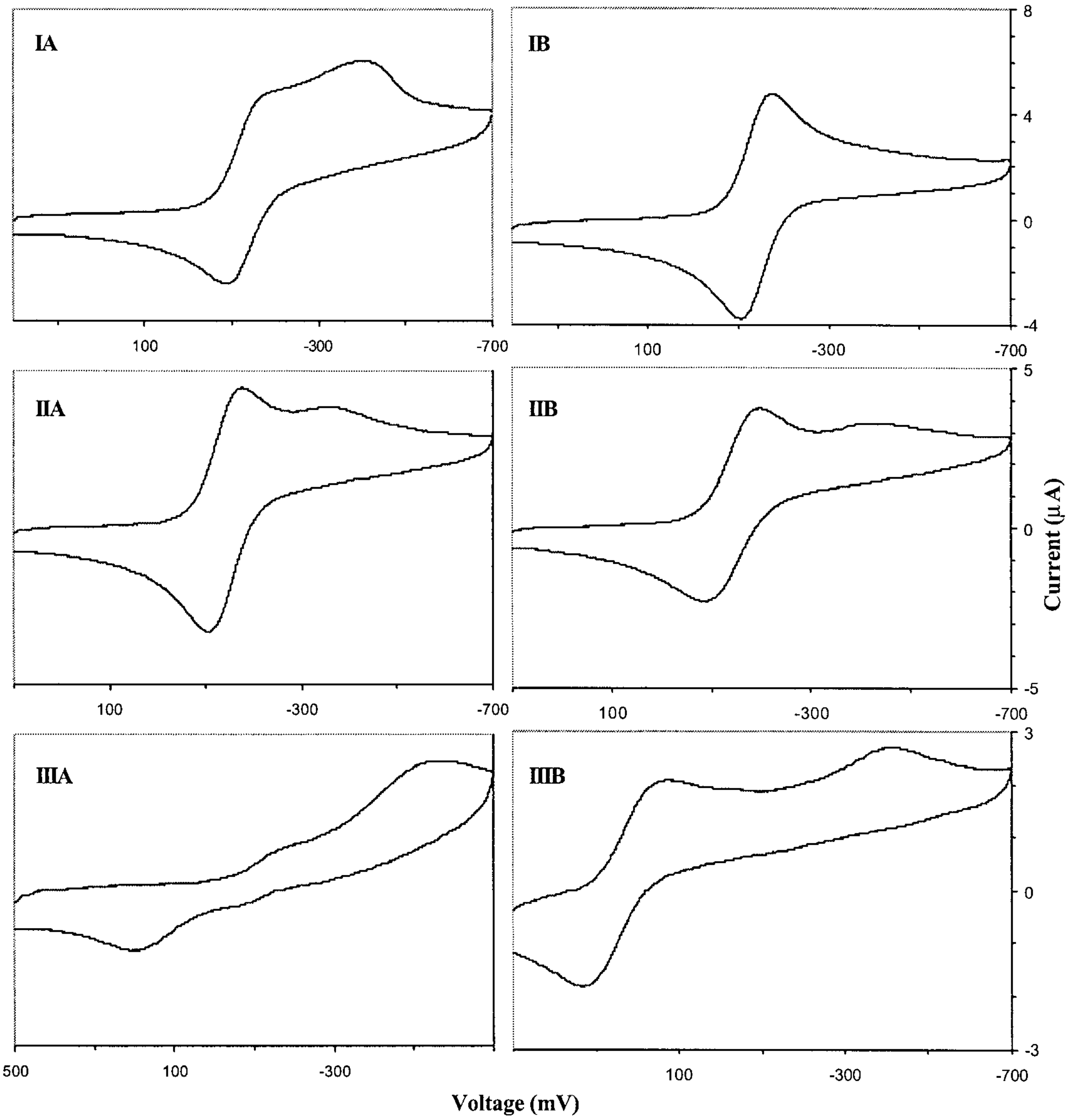

Figure 1. Cyclic voltammograms of Fe(III) complexes of EDTA(I), DTPA(II), and citrate(III) at metal to ligand ratios of 1:1 (IA-IIIA), 1:5 (IIB), and 1:10 (IB and IIIB). The pH was 7.4 (0.08 M Tris) and the scan rate was 5 mV/s for all voltammograms.

was found that at a 1:10 Fe:EDTA ratio, the measured EC' current increased steadily up to a relative H_2O_2 concentration of ~150 times the Fe concentration, then began to level off (Figure 4A). In contrast, a 1:1 Fe:EDTA ratio experienced a period of drastic increase in measure EC' current over the range of ~50–150 fold excess of H_2O_2 relative to iron (Figure 4B). At greater than a 150-fold excess the current once again leveled off. At low relative H_2O_2 concentrations, the Fe:EDTA ratio has little effect on the observed EC' current (Figure 4C). However, at relatively high H_2O_2 concentrations, the effect of the Fe:EDTA ratio is quite large (Figure 4D). Clearly, the data collected on the DTPA, and citrate iron complexes at H_2O_2:Fe ratios of 250:1 presented in Figures 2 and 3 are in agreement with these findings.

Discussion

The possibility of the transient irreversible waves of Figure 1 resulting from competition between the ligand and the Tris buffer was considered and modeled

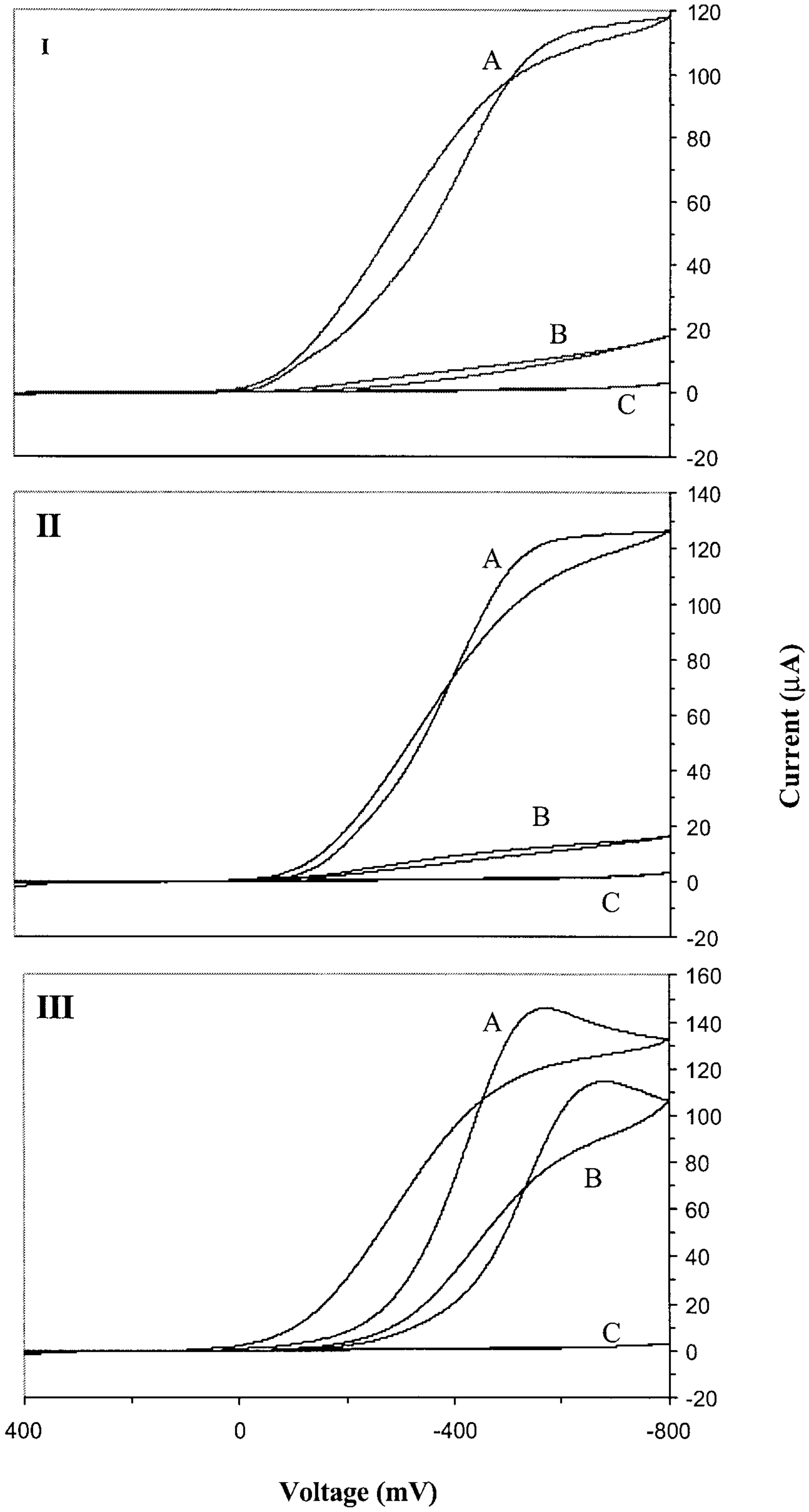

Figure 2. Electrocatalytic reduction of H_2O_2 by iron complexes of EDTA (I), DTPA (II), and citrate (III). Each figure subset contains waves corresponding to iron/ligand ratios of 1:1 (IAIIIA), 1:10 (IB, IIB), 1:15 (IIIB), and a blank (IC-IIIC). The pH was 7.4 (0.08 M Tris) and the scan rate was 5 mV/s for all voltammograms.

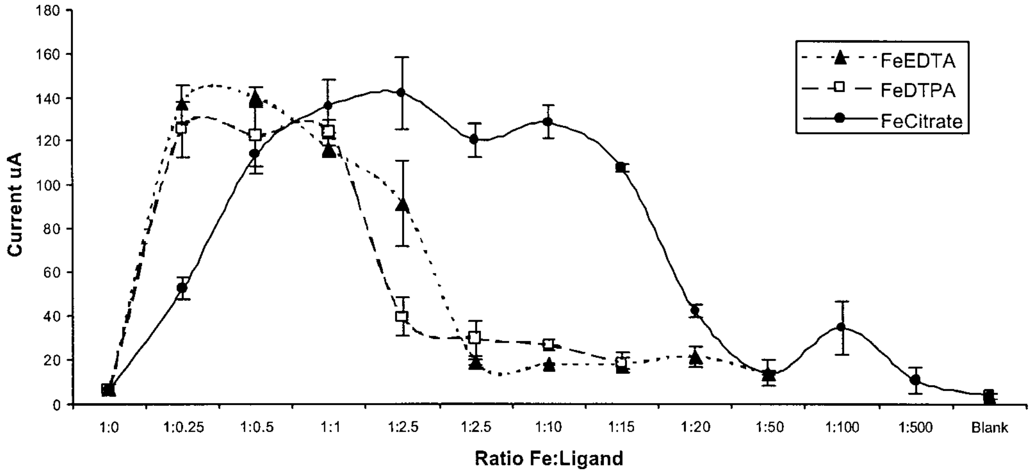

Figure 3. Effect of M:L ratio on the electrocatalytic reduction of H_2O_2. Maximum ligand ratios were 15, 50, and 500 times the metal concentration for DTPA, EDTA and citrate respectively; the 'blank' label on the x-axis corresponds to all three experiments and is the measured current for H_2O_2 reduction in the absence of the complex. Error bars are +/− the standard deviation for three replicate measurements.

using HySS speciation and simulation modeling software (Gans *et al.* 2000). Formation constants were obtained from Smith and Martel (1975). The modeling results indicate that the entire mass balance of iron is present as a 1:1 complex in the presence of the DTPA ($\log\beta = 28.0$), and a roughly 50/50 mix of FeEDTA and HO-FeEDTA in the presence of EDTA ($\log\beta = 25.1$) at L:M ratios of unity and greater. In the case of citrate ($\log\beta = 11.5$) it is not until L:M ratios of greater than 10:1 that the iron is entirely bound by the citrate. At lower L:M ratios, the remaining mass balance of iron is present as soluble hydroxide complexes. Models that considered the formation of solid precipitates predicted that the iron would eventually precipitate as a solid hydroxide at the operational pH of 7.4, but this reaction is kinetically slow, and no solid formation was observed during the timescale of the cyclic voltammetric experiment. Thus, solid iron hydroxide formation was omitted from the model.

There are no literature values available for the formation of a Fe^{III}TRIS complex suggesting that the formation constant is weak to non-existent. None-theless, the possibility of such a species was considered, and in the case of iron and citrate, the hypothetical Fe^{III}TRIS complex would not be present in an appreciable amount (greater than 1% of the iron mass balance) at $\log\beta$ values less than 9.0. In the case of iron and DTPA and EDTA, the $\log\beta$ values for the supposed Fe^{III}TRIS complex would need to be substantially greater than the case of iron and citrate. It is highly unlikely that the TRIS buffer presents much in the way of competition for the iron.

The observed influence of Fe:L:H_2O_2 ratio on the resulting Fenton chemistry is best explained by modeling the speciation of possible complexes as a function of the relative analytical reagent concentrations of iron, ligand and hydrogen peroxide. Figure 5 compares speciation models with the corresponding EC' voltammograms for systems of iron, EDTA, and hydrogen peroxide at relative peroxide concentrations above and below the period of sharp EC' current increase. It can be concluded that the peroxy-FeEDTA is key in producing maximum H_2O_2 decomposition, and that this complex is only present in appreciable amounts at relative peroxide concentrations in excess of ~100:1 relative to iron. It could also be concluded that the Fe:EDTA ratio may also affect the presence of the peroxy-FeEDTA complex, but speciation modeling with known stability constants do not support this finding. This leads to the somewhat dissatisfying conclusion that the L:M ratio exerts an effect either through a sort of kinetic Fenton deactivation or that a completely accurate model describing all species present has not yet been attained.

Of the three ligands examined in this investigation only citrate is produced by physiological systems. The Fe-citrate complex has ubiquitous biological relevance to many types of organisms (Pierre & Gautier-Luneau 2000). Given this it is reasonable to expect that this complex along with other low-molecular weight species contribute to oxidative damage under conditions of oxidative stress. Furthermore, it is evident that the L:M window in which citrate acts as a Fenton reaction center is large. On the other hand, that

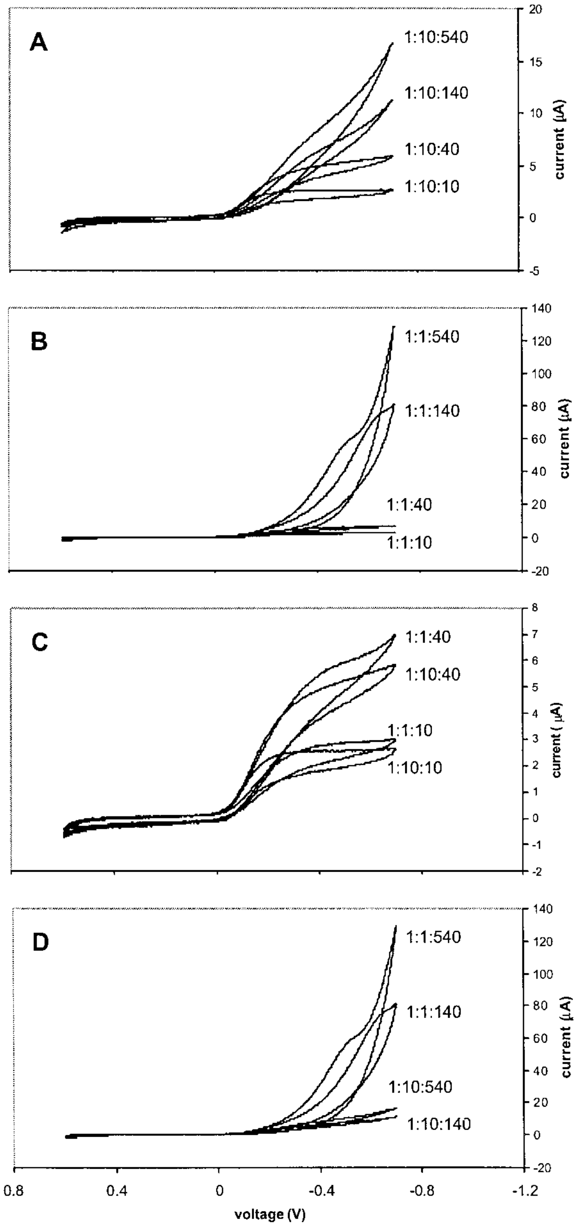

Figure 4. Combined effects of Fe:EDTA:H_2O_2 ratio on the electrocatalytic reduction of H_2O_2. The Fe:EDTA:H_2O_2 ratios are indicated to the right of each voltammogram. The Fe^{III} analytical concentration was 0.10 mM and the scan rate was 20 mV/s in all instances.

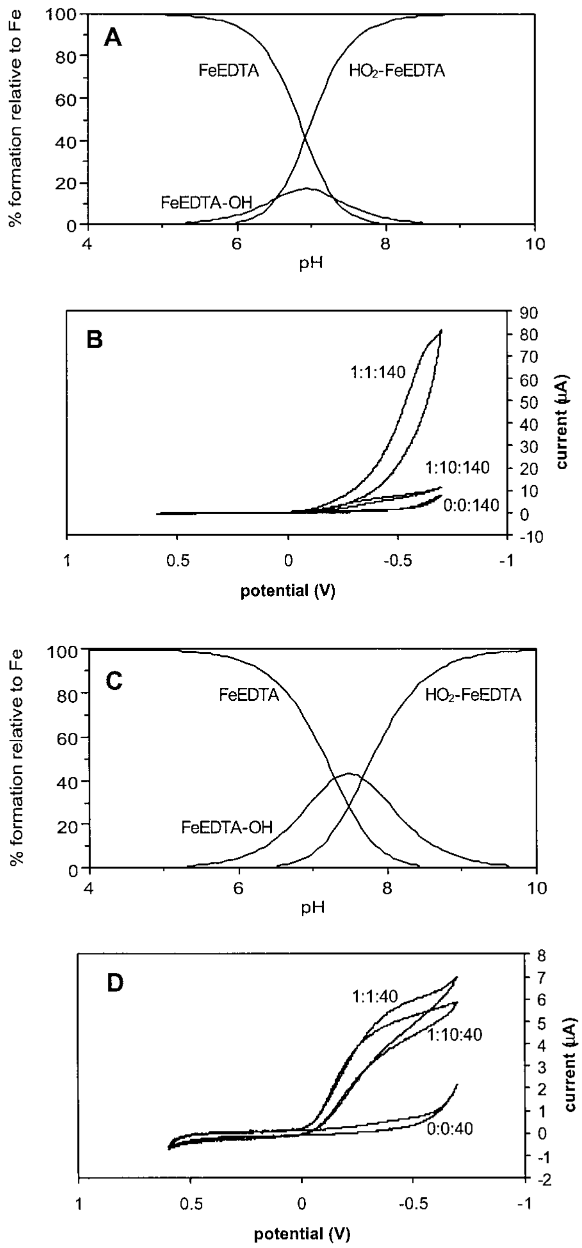

Figure 5. Comparison of Fe/EDTA/H_2O_2 speciation diagrams with corresponding EC' voltammograms at relative H_2O_2 concentrations of 140 (A & B) and 40 (C and D) fold excess to iron. The Fe:EDTA:H_2O_2 ratios are indicated to the right of each voltammogram (B & D), and the species are labeld in the speciation diagrams (A & C). The Fe^{III} analytical concentration was 0.10 mM and the scan rate was 20 mV/s in all instances.

window is small for both DTPA, and EDTA. This feature of a narrow L:M range for these ligands may form the basis of the seemingly contradictory observations cited in the *Introduction*. Both anthropogenic compounds, DTPA and EDTA are used as additives to biological specimens as part of a protocol to either encourage or discourage pro-oxidant processes. As it can be seen from this investigation it is the question as to whether this ligand induces or inhibits biological oxidative damage rests on the L:M ratio and relative concentration of H_2O_2. Those studies in which EDTA/DTPA encourage oxidations may have high L:M ratios, such ratio would depend on the quantity of chelatable iron within those biological systems. Studies in which EDTA/DTPA are observed to decrease the rate of oxidations may have ligand concentrations in excess of 2:1 L:M for chelatable metals.

It has been suggested that direct coordination of H_2O_2 on the metal center is necessary for the facilitation of Reaction 1 (Berlett *et al.* 1990). If this is true, than the most likely mode of deactivation, at least in the case of EDTA is indeed a shift in the speciation favoring the aqua- and hydroxy- over the peroxy- complexes of iron. It is also likely that DTPA and citrate follow similar trends. Studies involving varied relative concentrations of H_2O_2, along with the corresponding speciation models help lend support to this hypothesis by clearly illustrating the dependence of the observed EC' current on presence of the peroxy-FeEDTA complex.

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