L. METALS IN REDOX CATALYSIS

Metals in Redox Reactions

While metals can assist a lot of important chemistry without themselves acting as reactants, they are uniquely able to serve a direct role in oxidation and reduction processes. While cysteine can be reversibly oxidized to cystine and there are organic cofactors such as NAD and FAD that perform redox chemistry, the range of reactions that are accessible through metal ion participation is remarkable. Likewise, metal ions perform redox tasks in the biochemical environment that would seem unusual to a chemist who is only used to observing that chemistry in solution. How metals are capable of having their chemical reactivity adjusted by the protein environment is the topic of this section.

Standard Reduction Potentials

Standard reduction potentials are typically given for the addition of "n" electrons to a given oxidant (X here) to produce a reduced species:

$$X + n\,e^- \rightarrow X^{n-} \qquad E^\circ = \text{xxx V}$$

Just as the pK_a is the measure of an acid's strength, so is the standard reduction potential (E° or in some sources E_m) the measure of an oxidant's strength. The more positive the value of the reduction potential, the stronger the oxidant. The reduction potential is typically measured in Volts, where 1 V is equal to 1 J/C (see Table A for a list of units and their meanings). By measuring the strength of oxidant in Volts, we are really describing the amount of energy carried by a Coulomb's worth of electrons, rather than by the amount of energy exchanged by a mole of reactant or product. Otherwise, the concepts remain the same as any other thermodynamic variable.

Table A. Units used in redox chemistry

Unit	Equivalence	Meaning
1 Joule	4.184 J = 1 cal	You've been corrupted by me all semester into using non-SI units
1 Coulomb	1 e = 1.60×10^{-19} C	The Coulomb is a unit of charge. The charge on an electron isn't much but…
1 Faraday	1 F = 96485 C	The charge on one mole of electrons, the Faraday, is 96485 Coulombs
1 Volt	1 V = 1 J/C	The volt is a measure of energy carried by some number of electrons

Also, like other thermodynamic variables, there is a reference point. All reduction potentials are measured vs. the reduction of hydrogen ions to produce H_2 gas, which is arbitrarily defined as 0.00 V:

$$2\ H^+_{(aq)} + 2\ e^- \rightarrow H_{2(g)} \qquad E^\circ = 0.00\ V$$

That is to say, adding electrons to H^+ is our reference. If an oxidant is stronger than H^+, then it will have a more positive reduction potential. If the oxidant is weaker, the reduction potential will be increasingly negative. For example, O_2 is a strong oxidant:

$$O_{2(g)} + 4\ H^+_{(aq)} + 4\ e^- \rightarrow 2\ H_2O_{(l)} \qquad E^\circ = 1.21\ V$$

and sodium ion is an extremely weak oxidant:

$$Na^+_{(aq)} + e^- \rightarrow Na_{(s)} \qquad E^\circ = -2.71\ V$$

However, just as weak acids have strong conjugate bases, weak oxidants are typically related to strong reductants. For a species like sodium, the negative reduction potential indicates that the reverse reaction, the oxidation of sodium metal, is quite favorable in comparison to the oxidation of H_2, and in fact we see that sodium will spontaneously reduce H^+ ions:

$$2\ Na_{(s)} + H^+_{(aq)} \rightarrow 2\ Na^+_{(aq)} + H_{2(g)}$$

We can evaluate the favorability of this process by calculating the reaction potential (ΔE°) as the difference between the reduction potential of the oxidant and the reductant:

$$2\ H^+_{(aq)} + 2\ e^- \rightarrow H_{2(g)} \qquad E^\circ(H^+) \qquad = 0.00\ V$$

$$\underline{2\ (Na_{(s)} \rightarrow Na^+_{(aq)} + e^-) \qquad -E^\circ(Na^+) \qquad = +2.71\ V}$$

$$2\ Na_{(s)} + H^+_{(aq)} \rightarrow 2\ Na^+_{(aq)} + H_{2(g)}$$

$$\Delta E^\circ = E^\circ(H^+) - E^\circ(Na^+) = 2.71\ V$$

The positive value indicates that the reaction is spontaneous. Note that we don't have to worry about stoichiometry. Since the reduction potentials are all defined as energy per unit of charge, that scales automatically to any stoichiometry between reactants.

In order to convert this reaction potential to a more usual measure of spontaneity, one can calculate ΔG° from ΔE° by the following relationship:

$$\Delta G^\circ = -nF\Delta E^\circ$$

Where **n** is the number of electrons being transferred in the balanced reaction and F is the Faraday, 96485 C, the number of Coulombs in a mole of electrons. For the reaction of sodium with hydrogen, the change in free energy is:

$$\Delta G^\circ = -(2)(96485\ C)(2.71\ J/C)(10^{-3}\ kJ/J) = 523\ kJ$$

Note that this can be converted to kcal by dividing by 4.184 kJ/kcal.

Reduction Potentials at pH 7

Note that the "standard" in standard reduction potential refers to the standard state in which all dissolved species are at 1 M. That means the pH for any of these reactions is 0. Since we're typically adverse to running biochemical reactions in 1 M acid, biochemistry tends to choose a slightly different standard state, the **physiological** standard state, in which all species are at 1 M concentration at pH 7. Reduction potentials measured at the physiological standard state are given as $E^{\circ\prime}$. Still not so realistic, but getting closer. To work between the two states, we need the Nernst equation, which permits recalculation of the reduction potential according to the non-standard state concentrations (Q), using the same form as the equilibrium expression.

$$\Delta E^{\circ\prime} = \Delta E^{\circ} - \frac{RT}{nF}\ln(Q)$$

For example, at the physiological standard state, the reaction of sodium with protons has a reaction potential of:

$$\Delta E^{\circ\prime} = \Delta E^{\circ} - \frac{RT}{nF}\ln\left(\frac{P_{H2}[Na^+]^2}{[H^+]^2}\right)$$

Since only H^+ is not at 1 M concentration (or 1 atm pressure), we can simplify this equation at 25°C to:

$$\Delta E^{\circ\prime} = \Delta E^{\circ} - \frac{RT}{nF}\ln\left(\frac{1}{\left(10^{-7}\right)^2}\right) = \Delta E^{\circ} - \frac{0.0592\ \text{V}}{n}\log_{10}\left(10^7\right)^2 = \Delta E^{\circ} - 2(0.42\ \text{V}/n)$$

Since n = 2,

$$\Delta E^{\circ\prime} = 2.71\ \text{V} - 0.84\ \text{V} = 1.87\text{V}.$$

In general, the adjustment is to multiply –0.42 V by the number of protons in the balanced reaction and divide by the number of electrons.

Reactive Oxygen Species (ROS) and Metals

One of the greatest challenges to life in an aerobic environment is the oxidizing nature of the surroundings. Oxygen is a remarkably strong oxidant and capable of doing severe damage to biological molecules, as can be observed in forest fires. In a healthy cell, a number of unstable and reactive oxygen-derived species can be generated by the reduction of O_2 (Table B). There are a number of ways in which these unstable species can be generated. For example, the ferrous ion (Fe^{2+}) in hemoglobin can react with molecular oxygen in to produce superoxide ($O_2^{\bullet -}$).

$$O_2 + Fe^{2+} \rightarrow O_2^{\bullet -} + Fe^{3+}$$

The superoxide anion is a particularly reactive oxidant and is capable of doing real damage in the cell by propagating radical mechanisms in otherwise stable biochemical species.

Table B. Reduction potentials for processes involving reactive oxygen species.

Reaction	Physiological Reduction Potential
$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$	$E^{\circ\prime} = +0.82$ V
$O_2 + 2\ H^+ + 2\ e^- \rightarrow H_2O_2$	$E^{\circ\prime} = +0.27$ V
$O_{2(g)} + e^- \rightarrow O_2^{\bullet -}$	**$E^{\circ\prime} = -0.33$ V**
$O_{2(g)} + e^- + H^+_{(aq)} \rightarrow HO_2^{\bullet}$	$E^{\circ\prime} = -0.13$ V
$O_2^{\bullet -} + e^- + 2\ H^+_{(aq)} \rightarrow H_2O_{2(aq)}$	**$E^{\circ\prime} = +0.89$ V**

In order to protect itself against oxidative damage from these oxidants, almost all cells contain and enzymatic activity called superoxide dismutase, which catalyzes the following spontaneous process, the disproportionation of superoxide:

$$O_2^{\bullet -} + e^- + 2\ H^+ \rightarrow H_2O_2 \qquad E^{\circ\prime} = +0.89 \text{ V}$$

$$O_2^{\bullet -} \rightarrow O_{2(g)} + e^- \qquad -E^{\circ\prime} = +0.33 \text{ V}$$

$$2\ O_2^{\bullet -} + 2\ H^+ \rightarrow H_2O_2 + O_2 \qquad \Delta E^{\circ\prime} = 1.22 \text{ V}$$

So, the decomposition of superoxide to hydrogen peroxide and oxygen is spontaneous, with a change in free energy of -56 kcal.[1] It is also a rapid second order process, with a rate constant of **$4 \times 10^5\ M^{-1}s^{-1}$**. The chief barrier to the reaction is the electrostatic repulsion of two anionic species at pH 7. However, the reaction becomes substantially more rapid when superoxide becomes protonated at low pH (pK_a is 4.8). Problem is that $[O_2^{\bullet -}]$ is only about 300 pM in the *E. coli* cell (without any

[1] $\Delta G^{\circ} = -(2)(96485\ C)(1.22\ V)(1\ kcal/4184\ J) = -56$ kcal

enzymatic activity to remove it)[2], so the overall rate of reaction works out to roughly 4 x 10^{-14} M/s. At a concentration of 300 pM, superoxide would have a half-life of 4200 s in the cell (70 min).

Thus protection of the cell requires catalysis of the disproportionation reaction. This is actually not too tough. In fact, it is possible to catalyze the reaction with hydrated ferric ion, which has a standard reduction potential of 0.77 V. Since metal ions tend to be cationic, they allow this reaction to bypass the anionic repulsion felt between two superoxide molecules interacting directly. The reaction takes place in a two-step mechanism, which is spontaneous at pH 7. Ferric ion oxidizes superoxide:

$$Fe^{3+}_{(aq)} + e^- \rightarrow Fe^{2+}_{(aq)} \qquad E^{\circ\prime} = 0.77 \text{ V}$$

$$\underline{O_2^{\bullet-} \rightarrow O_{2(g)} + e^-} \qquad \underline{-E^{\circ\prime} = +0.33 \text{ V}}$$

$$Fe^{3+}_{(aq)} + O_2^{\bullet-} \rightarrow Fe^{2+}_{(aq)} + O_{2(g)} \quad \Delta E^{\circ\prime} = 1.10 \text{ V}$$

followed by the re-oxidation of ferrous ion:

$$O_2^{\bullet-} + e^- + 2\,H^+ \rightarrow H_2O_2 \qquad E^{\circ\prime} = +0.89 \text{ V}$$

$$\underline{Fe^{2+}_{(aq)} \rightarrow Fe^{3+}_{(aq)} + e^-} \qquad \underline{-E^{\circ\prime} = -0.77 \text{ V}}$$

$$Fe^{2+} + O_2^{\bullet-} + 2\,H^+ \rightarrow H_2O_2 + Fe^{3+} \qquad \Delta E^{\circ\prime} = +0.12 \text{ V}$$

Thus, it can be seen that any metal capable of being reduced by one electron at a physiological standard reduction potential between –0.33 V and 0.89 V is capable of catalyzing the reaction. As a point of interest, it turns out that most biological catalysts of the reaction have a physiological reduction potential of about 0.3 V, roughly at the midpoint of the allowable range.

Given the relative simplicity of this reaction, it is perhaps not too surprising that enzymes (called **superoxide dismutases** – SODs) have managed to catalyze the superoxide disproportionation reaction not just with the ferric ion as an active metal cofactor, but also with Mn^{3+}, Cu^{2+} and Ni^{3+}. The relevant reduction potentials are:

Cu^{2+}/Cu^+ $\quad E^\circ = 0.15$ V

Fe^{3+}/Fe^{2+} $\quad E^\circ = 0.77$ V

Mn^{3+}/Mn^{2+} $\quad E^\circ = 1.51$ V

Ni^{3+}/Ni^{2+} $\quad E^\circ >> 1$ V

Note that of these metals, only copper and iron appear to have reduction potentials in the appropriate range to perform catalysis. However, the reduction potentials of metals are tunable by the coordination environment. As an example, Mn^{3+} has standard reduction potential of 1.51 V in 1 M HCl, but the potential drops to –0.25 V in 1 M NaOH as the anionic hydroxide ligand can

[2] With superoxide dismutase activity, the concentration of superoxide decreases roughly 10 fold to 30-40 pM. See Fridovich (1992) JBC **267**, 8757.

stabilize the higher charge of Mn^{3+}. In a similarly dramatic case, ligand choice can have a significant effect. Cobalt(III) in water has a reduction potential of 1.95 V, but when coordinated to ethylene diamine (an amine-containing bidentate ligand), the reduction potential drops to –0.2 V, again due to preferential stabilization of the +3 oxidation state of cobalt. Similarly, it's been shown that Ni^{3+} can be reduced spontaneously at a much lower potential when bound to thiolate ligands.

In the following sections (only some of which will be completed in 2006), I'll discuss the active sites of the four classes of SOD and how each has been specifically designed to accommodate the particular metal ion being used in that enzyme.

CuZn SOD

The first SOD enzyme to be found contains one atom each of copper and zinc. It may alternately be identified as SOD1 or CuZn SOD. Notable initially for its bright blue color upon isolation from red blood cells, where superoxide is produced via the oxidation of heme by molecular oxygen, it is broadly distributed in the cytoplasm of animal and plant cells, and is likewise found in may species of bacteria. Despite many years of study, it has been difficult to fully characterize the structural changes that take place during the catalytic cycle, which alternates between the oxidation of superoxide by the copper(II) form of the enzyme and the reduction of superoxide by the copper(I) form. The following is a brief summary that completely neglects years and years of elegant experimentation.

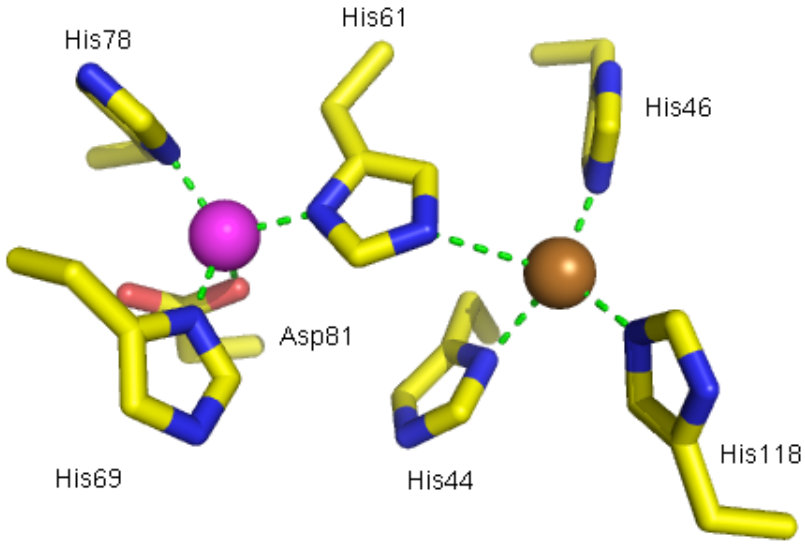

Figure 1. Active site of oxidized CuZn SOD. Note that His61 is a negatively charged bridging residue between the Zn^{2+} ion (magenta) and the Cu^{2+} ion (copper).

CuZn SOD is active as a homodimer and contains a copper/zinc pair bound in a "binuclear" (indicating two metal ions) complex (Figure 1). The two metals are bound jointly by six histidyl residues and one aspartyl residue. In the oxidized state, both metals are found in tetrahedral coordination geometry. The zinc ion is bound by three histidines and the one aspartate.

Intriguingly, His64 is bound both to zinc and copper in a "bridging" role, which requires that it carry a negative charge in the oxidized form. The copper atom is bound additionally by three other histidines. Thus, in oxidized CuZn SOD there is a total excess charge within the immediate coordination environment of +2. The negative charge on Asp** and His** is offset by the +2 charges on both zinc and copper. Note that this creates a destabilizing environment for copper and helps explain the fact that CuZn SODs tend to have a more positive reduction potential than free Cu^{2+}:

$$SOD{\bullet}Cu^{2+}/SOD{\bullet}Cu^{+} \quad E^{\circ\prime} = 0.42 \text{ V}$$

This still lies within the acceptable range for superoxide dismutase activity and so is consistent with the desired catalytic role.

For a long time, there was difficulty in obtaining a structure of the reduced form of the enzyme.

MnSOD and FeSOD

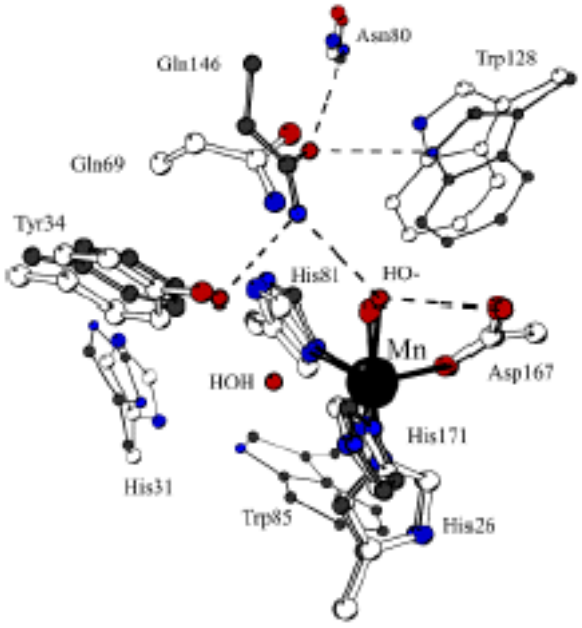

Figure 1. Overlay of the metal centers in FeSOD (light colored) and MnSOD (dark colored) in their oxidized forms. Note general homology, except for second sphere Gln (146 in MnSOD and 69 in FeSOD). Gln146 is better positioned to donate a hydrogen-bond to the metal-bound solvent in MnSOD. (copied w/o permission from Maliekal *et al.* (2002) *JACS* 124, 15065.)

Manganese-containing SOD (MnSOD, sometimes SodA) and iron-containing SOD (FeSOD, sometimes SodB) are the two most common forms of SOD found in bacteria. In addition MnSOD is found in mitochondria of eukaryotes. The stunning thing about these two enzymes is the similarity in their structures. MnSOD and FeSOD from *E. coli* possess sequence identity of 42% and a sequence similarity of 56% over their roughly 200 aa sequence. Likewise, they have extremely similar coordination environments around the relevant metal ion, which adopts a trigonal

bipyramidal geometry with three neutral histidine residues, an aspartate and a solvent molecule in the first coordination shell (Figure 1). The close similarity in active site structure bespeaks a generally similar mechanism of catalysis. Superoxide anion is believed to bind to form an octahedral complex with the metal, *trans* to the aspartate ligand (Figure 3). Thus reduction and oxidation take place through direct coordination.

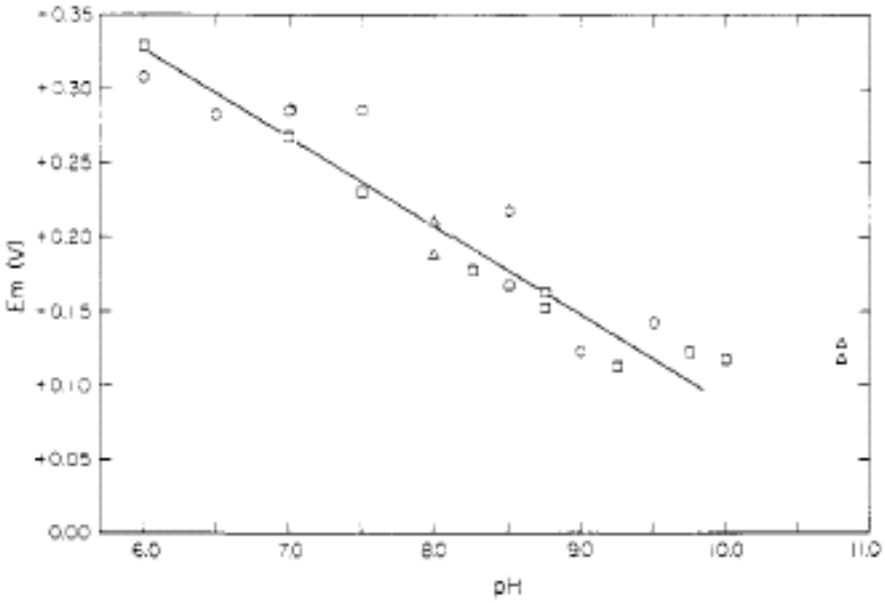

Figure 2. Plot of reduction potential of Fe^{3+}SOD vs. pH. The negative slope indicates that the metal ion is harder to reduce at high pH. The value of –60 mV in the slope is consistent with the uptake of one proton during the reduction. As protons become more scarce, the enzyme is harder to reduce. (copied w/o permission from Barrette *et al.*[3]

Interestingly, there is a strong pH dependence to the reduction potential of the FeSOD and MnSOD enzymes. It was observed by that the reduction potentials of both FeSOD and MnSOD drop by 60 mV with each increase of one pH unit between pH 6.0 and 10.0 (Figure 2)[3]. That indicates the following relationship:

$$E = E^{\circ} - \frac{0.0592\ \text{V}}{n} \log_{10}\left(\frac{1}{[H^+]}\right)$$

This implies that one proton is a reactant in the reduction process:

$$Fe^{3+}SOD + H^+ + O_2^{-}\bullet \rightarrow Fe^{2+}SOD\bullet H^+ + O_2$$

Essentially, the proton is used to maintain charge balance during the oxidation process so that a

[3] Barrette *et al.* (1983) *Biochemistry* **22**, 624.

fixed positive charge remains in the metal binding site. Another advantage of this co-reduction/protonation is that it simplifies the subsequent re-oxidation of the metal, since one would normally need to add two protons to $O_2^-\bullet$ to create H_2O_2:

$$Fe^{2+}SOD\bullet H^+ + H^+ + O_2^-\bullet \rightarrow Fe^{3+}SOD + H_2O_2$$

It is now generally accepted that the species being protonated during the reduction of the Fe^{3+} ion is the bound hydroxide ion, which becomes a bound water in the reduced Fe^{2+} structure (Figure 3). Thus the proton is close to the position in which the second, oxidizing equivalent of superoxide will bind and can easily be transferred during the oxidation process.

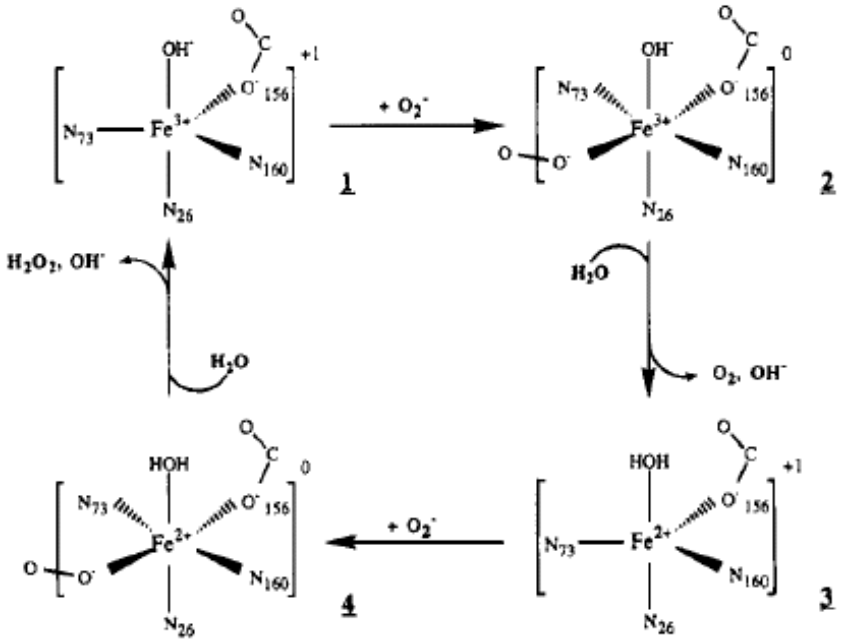

Figure 3. Catalytic cycle catalyzed by FeSOD. Note that protonation is required from **2** to **3**, concurrent with the transfer of an electron from $O_2^-\bullet$ to Fe^{3+}. That proton is lost again during the reduction of superoxide to hydrogen peroxide as **4** goes to **1**.

Despite the similarities in structure and mechanism, these enzymes are individually designed to function <u>only</u> with one metal. Manganese-substituted FeSOD (Mn_{sub}FeSOD) and iron-substituted MnSOD (Fe_{sub}MnSOD) are incapable of catalyzing the SOD reaction.[4] A quick look at the reduction potentials of each enzyme explains why (Table C). Note that the MnSOD stabilizes the +3 oxidation state of the metal more effectively than the FeSOD. This is important since Mn^{3+} has a higher reduction potential in solution and must be brought down from 1.51 V to the 0.3 V range, while Fe^{3+} must only be stabilized enough to lower the reduction potential from 0.77 V to the midpoint range of superoxide dismutation.

[4] Vance and Miller (2001) *Biochemistry* **40**, 13079.

Table C. Reduction potentials of MnSOD and FeSOD substituted with either Mn^{3+} or Fe^{3+}. Note that MnSOD gives a lower reduction potential for both metals, indicating relative destabilization of the +3 oxidation state (from Vance and Miller)[4].

Enzyme	w/ Mn^{3+}	w/ Fe^{3+}
MnSOD	0.29 V	-0.24 V
FeSOD	0.96-1.170 V*	+0.22 V

*The range is due to the impossibility of performing the titration in water, which oxidizes to O_2 at 0.82 V. Vance and Miller saw no oxidation with $IrCl_6^{2-}$ (E° = 900 mV) but full oxidation by MnO_4^- (1230 mV)

This is a surprising result, because MnSOD and FeSOD are so close structurally. Why is it that one (MnSOD) stabilizes the oxidized metal so much more effectively than the other. The answer appears to be related to the one significant difference visible in their metal binding site (Figure 1). Note that Gln146 forms a hydrogen to the bound hydroxide in the Mn^{3+} form of MnSOD, while Gln69 is outside of hydrogen-bonding distance to the hydroxide in Fe^{3+}SOD. Note that hydrogen bond donation stabilizes the hydroxide form of the bound solvent molecule, which in turn provides electrostatic stabilization to the +3 form of the metal, which otherwise only accepts a ligand bond from one other anionic group, Asp167. If Fe^{3+} is placed in the MnSOD enzyme, it too will receive a stabilizing H-bond which will reduce its reduction potential relative to that observed in FeSOD (a stable Fe^{3+} is a weaker oxidant). To confirm this hypothesis, Miller's lab created a Gln69→Glu mutation in FeSOD. Glutamate will be, at best, a hydrogen bond acceptor, instead of a donor like glutamine in this enzyme, and will thus stabilize the presence of a protonated water bound to Fe^{3+} either through direct hydrogen bonding or through electrostatic stabilization of a neutral, bound water (Figure 5). The results of their study indicate that this mutation substantially raises the E° of the ferric form of FeSOD, and in fact they can only isolate the reduced, Fe^{2+}, form. Thus, a small variation in active site structure, moving an H-bond donor to within 2.8 Å of a metal-bound hydroxide as in MnSOD, can stabilize the oxidized form of the enzyme by roughly 0.5 to 0.7 V (Table C).

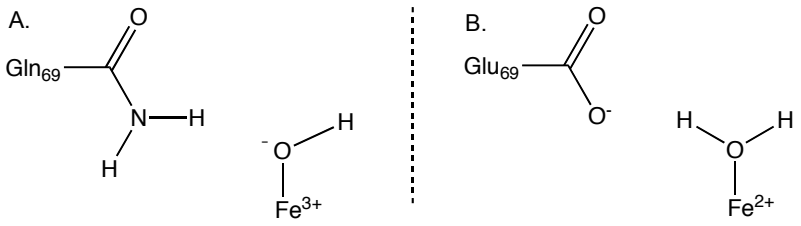

Figure 5. Active site of (A) wild type Fe^{3+}SOD and (B) the Gln69→Glu mutant of Fe^{3+}SOD. The glutamate side chain promotes a bound water molecule, which destabilizes the +3 form of iron and increases its reduction potential.