An Alternative Mechanism of Bicarbonate-mediated Peroxidation by Copper-Zinc Superoxide Dismutase

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Hydrogen peroxide can interact with the active site of copper-zinc superoxide dismutase (SOD1) to generate a powerful oxidant. This oxidant can either damage amino acid residues at the active site, inactivating the enzyme (the self-oxidative pathway), or oxidize substrates exogenous to the active site, preventing inactivation (the external oxidative pathway). It is well established that the presence of bicarbonate anion dramatically enhances the rate of oxidation of exogenous substrates. Here, we show that bicarbonate also substantially enhances the rate of self-inactivation of human wild type SOD1. Together, these observations suggest that the strong oxidant formed by hydrogen peroxide and SOD1 in the presence of bicarbonate arises from a pathway mechanistically distinct from that producing the oxidant in its absence. Self-inactivation rates are further enhanced in a mutant SOD1 protein (L38V) linked to the fatal neurodegenerative disorder, familial amyotrophic lateral sclerosis. The 1.4 Å resolution crystal structure of pathogenic SOD1 mutant D125H reveals the mode of oxyanion binding in the active site channel and implies that phosphate anion attenuates the bicarbonate effect by competing for binding to this site. The orientation of the enzyme-associated oxyanion suggests that both the self-oxidative and external oxidative pathways can proceed through an enzyme-associated peroxycarbonate intermediate.

Copper-zinc superoxide dismutase (SOD1, CuZn-SOD) is a 32-kDa homodimeric protein that catalyzes the disproportionation of superoxide anion into dioxygen and hydrogen peroxide ($2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2$) through redox cycling of its catalytic copper ion (1, 2). Each subunit of the enzyme contains a progressively narrowing channel lined with charged residues that guide $\text{O}_2$ toward the active site (3, 4). Immediately adjacent to the copper ion, the channel constricts and the guanidinium group of Arg-143 and the side chain of Thr-137 together act to exclude large nonsubstrate anions (5). Small anions such as cyanide ($\text{CN}^-$) and azide ($\text{N}_3^-$) can proceed past this channel constriction and competitively inhibit the enzyme by binding directly to the copper ion (6). Certain larger anions such as hydrogen phosphate ($\text{HPO}_4^{2-}$) are also pulled into the active site channel but do not bind tightly to the copper. Instead, they remain associated with Arg-143 in the “anion-binding site” approximately 5 Å away (7).

In addition to its well known $\text{O}_2$ disproportionation activity, the active site of CuZn-SOD can interact with $\text{H}_2\text{O}_2$ to generate a powerful oxidant (8–10). Once formed, this oxidant can participate in one of two reaction pathways. In the first, designated herein as the self-oxidative pathway, it can inactivate CuZn-SOD by damaging nearby active site histidine copper ligands, resulting in copper loss (11–14). In the second, designated as the external oxidative pathway, the oxidant instead reacts with exogenous substrates, protecting the enzyme from inactivation (8, 10, 15, 16). The following reaction scheme has been proposed for these pathways as shown in Reactions 1–3.

$$
\text{SOD-Cu(II)} + \text{H}_2\text{O}_2 \rightarrow \text{SOD-Cu(I)} + 2\text{H}^+ + \text{O}_2^-
$$

\text{REACTION 1}

$$
\text{SOD-Cu(I)} + \text{H}_2\text{O}_2 \rightarrow \text{SOD-Cu(II)(OH)} + \text{OH}^-
$$

\text{REACTION 2}

$$
\text{SOD-Cu(II)(OH)} + \text{XH} \rightarrow \text{SOD-Cu(II)} + \text{H}_2\text{O} + \text{X}
$$

\text{REACTION 3}

where \text{XH} represents amino acids at the active site or an exogenous substrate (8, 17). Analogous to the Fenton reaction,
Reaction 2 generates a highly reactive hydroxyl radical (HO·). The observation that this HO· does not readily react with scavengers of free HO· such as ethanol led to the proposal that the HO· was “bound” to the catalytic copper ion. This hypothesis was supported by the observation that small anions such as formate (HCO₃⁻) and N₃⁻ that can traverse the active site channel constriction and gain close approach to the copper ion are able to protect the enzyme from inactivation by serving as sacrificial substrates (8–10) as shown in Reactions 4 and 5.

\[
\text{SOD-Cu(II)(OH)} + \text{HCO}_3^- \rightarrow \text{SOD-Cu(II)} + \text{H}_2\text{O} + \text{CO}_3^{2-}
\]

**REACTION 4**

\[
\text{SOD-Cu(II)(OH)} + \text{N}_3^- \rightarrow \text{SOD-Cu(II)} + \text{OH}^- + \text{N}_3
\]

**REACTION 5**

This single electron oxidation of substrates is referred to as the peroxidase function of SOD1 because of its similarity to the one-electron oxidation by horseradish peroxidase and H₂O₂ (18). The peroxidase activity of SOD1 is not strictly limited to small substrates that can gain direct access to the copper ion. In the presence of bicarbonate anion (HCO₃⁻), larger reporter molecules such as DMPO (5,5-dimethyl-1-pyrroline-N-oxide), ABTS (2,2'-azino-bis-[3-ethylbenzothiazoline-6-sulfonate]), PBN (N-tert-butyl-α-phenylnitrone), azulenyl nitrone, tyrosine, and DCFH (dichlorodihydrofluorescein) are also oxidized (10, 15–17, 19–23). Several studies (24–27) have implicated this expanded peroxidase activity of SOD1 in the toxic gain-of-function of SOD1 mutants associated with the progressive, fatal, neurodegenerative disorder, familial amyotrophic lateral sclerosis (FALS). This expanded FALS SOD1 peroxidase activity exerted either on substrates critical for motor neuron viability or on the SOD1 molecule itself could play a role in FALS etiology (see “Discussion”). It is important to note that the oxidation of substrates too large to traverse the active site channel constriction can occur only in the presence of HCO₃⁻ or structurally similar anions such as HSeO₃⁻ and HSO₃⁻. Other anions such as N₃⁻, HCO₃⁻, HPO₄²⁻, thiocyanate (SCN⁻), nitrate (NO₃⁻), and Cl⁻ do not appear to support the oxidation of these larger substrates (16). The relevance of this activity is underscored by the significant concentration of HCO₃⁻ found in vivo (∼25 mM) (28) and by recent studies showing that at physiological pH values (7.4) and low H₂O₂ concentrations (1 µM), HCO₃⁻ dramatically enhances DCFH oxidation in a SOD1/H₂O₂/DCFH system (23).

Several laboratories have sought to delineate the mechanistic role of HCO₃⁻ in the external oxidative pathway of SOD1. Sankarapandi and Zweier (16) propose that HCO₃⁻ bound to the SOD1 anion-binding site creates a hydrogen-bonding template for H₂O₂ near the copper ion that facilitates its partitioning into OH⁻ and OH· (see Reaction 2). Liochev and Fridovich (17) suggest that if this were true, then both the rate of endogenous SOD1 self-inactivation and the rate of oxidation of larger exogenous substrates in Reaction 3 should be enhanced by the presence of HCO₃⁻. To test this hypothesis, they (17) monitored the rate of self-inactivation of SOD1 in 100 mM phosphate buffer and observed no significant rate enhancement when 10 mM HCO₃⁻ was added. On this basis, they suggested that HCO₃⁻ does not facilitate H₂O₂ binding, but rather, HCO₃⁻ can itself be oxidized by the copper-bound HO· to carbonate radical anion (CO₃⁻), which in turn can diffuse from the active site channel to oxidize larger, bulky, exogenous substrates (Reactions 6 and 7) or remain associated with the anion-binding site to oxidize histidine copper ligands (Reactions 8 and 9) (17, 20, 22, 23).

SOD-Cu(II)(OH) + HCO₃⁻ → SOD-Cu(II) + H₂O + CO₃⁻ (free)

**REACTION 6**

CO₃⁻ (free) + XH (exogenous) → HCO₃⁻ + X (exogenous)

**REACTION 7**

or

SOD-Cu(II)(OH) + HCO₃⁻ → SOD-Cu(II)(CO₃⁻) + H₂O

**REACTION 8**

SOD-Cu(II)(CO₃⁻) + X (self) → SOD-Cu(II) + HCO₃⁻ + X (self)

**REACTION 9**

Building on this model, we reasoned that if “diffusible” CO₃⁻ is indeed formed in the active site channel, the presence of HCO₃⁻ in the reaction mixture must partially protect the enzyme from self-inactivation as is observed with formate or azide in Reactions 4 and 5 (8–10). Here, we test the effect of HCO₃⁻ on the rate of self-inactivation in the absence of other oxyanions that might compete for binding to the anion-binding site (e.g. phosphate). We find that the rate of self-inactivation of wild type SOD1 is significantly enhanced under these conditions than diminished. Thus, the strong oxidant produced in this experiment arises from a pathway that is mechanistically distinct from Reactions 2 and 6. We also show that the human Leu-38 to Val (L38V) FALS SOD1 protein demonstrates increased rates of self-inactivation relative to the wild type protein whether HCO₃⁻ is present or not. Finally, x-ray crystallographic analysis of the human Asp-125 to His (D125H) FALS SOD1 protein suggests a mechanism for both the self-oxidative and external oxidative pathways that proceeds through an enzyme-associated peroxycarbonate (HCO₃⁻) intermediate. This chemistry has direct relevance to the understanding of SOD1-mediated oxidative cellular damage and how members of the “wild-type-like” and “metal-binding region” mutant classes of FALS SOD1 proteins can be fused into a single class of molecules that are toxic to motor neurons (for review see Ref. 29).

**EXPERIMENTAL PROCEDURES**

**Materials**—All of the solutions were prepared using distilled water passed through a Millipore ultrapurification system. EDTA was purchased from Sigma. pH was adjusted by the addition of H₂SO₄ (double distilled from Vycor, GFC Chemical Co.) and NaOH (Puratronic, Baker Chemical Co.). Monobasic phosphate buffer (Ultrace, JT Baker Co.) at a concentration of 100 mM was used in all of the measurements requiring phosphate. Sodium bicarbonate (EM Science) at a concentration of 100 mM was used in all of the measurements requiring bicarbonate anion. Solutions buffered using 0.5 mM Tris were adjusted against iodate and by its absorbance at 230 nm (extinction coefficient 1 cm⁻¹ M⁻¹ cm⁻¹). Ethanol was purchased from Quantum Chemical Co.

**Expression and Purification of Wild type and L38V SOD1—Human** wild type and L38V SOD1 proteins were expressed in insect cells and purified as described previously (30). The metallation states of protein samples were not altered following purification. SOD1 protein concentrations were determined using an extinction coefficient of 1.08 × 10⁴ M⁻¹ cm⁻¹ for the purified enzyme. Purity was estimated using SDS-PAGE and electrospray mass spectrometry. Metal content analyses were performed using inductively coupled plasma mass spectrometry techniques.

**Pulse Radiolysis Experiments**—Pulse radiolysis experiments were performed using the 2 MeV Van de Graaff accelerator at Brookhaven National Laboratory. Dosimetry was established using the KSCN dosimeter, assuming that (SCN⁻)₂ is generated with a G value of 6.13 and has a molar absorptivity of 7950 M⁻¹ cm⁻¹ at 472 nm. Irradiation of
in the presence of SOD was extracted from the observed change in absorbance (at 290 nm) with respect to time. The reported rate constants for the studies were calculated by dividing the observed rate by the total concentration of copper bound to the enzyme in solution for CuZn-SOD. A set of self-inactivation experiments were carried out in the presence and absence of 100 mM phosphate at pH 7.2 and 25 °C to facilitate comparison with the studies of Linz and Fridovich (17) and Saagarapandi and Zweier (18). All of the other self-inactivation experiments were carried out in the absence of phosphate at pH 8.0 and 37 °C to stabilize the concentration of bicarbonate and to mimic in vivo temperatures. Previous work (9, 31) has demonstrated that SOD1 reacts almost exclusively with the peroxide anion and that the self-inactivation reaction has a large activation energy (data not shown). To account for both the increase in effective peroxide anion concentration at pH 8.0 and the increase in reaction rate because of the elevated temperature of 37 °C, a lower concentration of peroxide (relative to that in the self-inactivation at pH 7.2) was used. Conditions of the experiments performed to monitor the effect of bicarbonate anion in the presence of phosphate at 25 °C were as follows: 0.5 mM copper-bound D125H CuZn-SOD, 100 mM sodium phosphate, pH 7.2, 10 μM EDTA, 20 mM H2O2, with and without 10 mM sodium bicarbonate. Conditions of the experiments performed to monitor the effect of bicarbonate in the absence of phosphate at 37 °C were as follows: 0.4 mM copper-bound CuZn-SOD, 0.5 mM Tris, pH 8.0, 100 mM NaCl, 10 μM EDTA, either 4 or 8 mM H2O2, with and without 25 mM sodium bicarbonate. 1-mL aliquots were withdrawn at timed intervals, and a drop of EtOH was added just before pulsing to yield an approximate concentration of 0.25 mM EtOH in solution. The solutions were immediately pulse-irradiated, and their SOD activity was determined. SOD activity is known to be ionic strength-dependent, and the pK of ethanol is well above 9; therefore, variation in the final EtOH concentration would not be significant absorption, whereas zinc exhibited strong absorption of the experiments. The pulse radiolysis cell was thermostated to the same temperature as the water bath.

**D125H SOD1 Purification, Crystallization, and Structure Determination**—Recombinant human D125H CuZn-SOD was obtained as described previously through Stachycharomysis ceylonensis expression under control of the ySOD1 promoter in the strain EG118 (sold–1), which lacks the gene encoding the yeast CuZn-SOD polypeptide (30, 32). D125H SOD1 at 20 mg/ml in 2.25 mM sodium phosphate buffer, pH 7.0, 60 mM NaCl, crystallized as thick rectangular blocks in space group C2221, at 4 °C in 1–2 weeks with unit cell parameters a = 70.5 Å, b = 103.4 Å, c = 143.1 Å. As shown in Fig. 1A, when the self-inactivation of SOD1 is monitored in 100 mM phosphate buffer, pH 7.2, the addition of 10 mM bicarbonate has little effect. To determine the effect of bicarbonate anion on SOD1 mutant proteins found to cause familial amyotrophic lateral sclerosis, we compared the self-inactivation of wild type SOD1 and the FALS mutant L38V. L38V shows increased self-inactivation rates relative to those of wild type whether or not bicarbonate is present. Fig. 1C shows that the presence of HCO3− increases the rate of self-inactivation of both proteins to approximately the same extent, suggesting a common mechanistic pathway for this effect.

**Crystal Structure of D125H SOD1**—The x-ray crystal structure of the human FALS mutant D125H was determined to 1.4 Å resolution using single wavelength anomalous dispersion phasing methods (Table I). The as-isolated D125H SOD1 protein is nearly devoid of metal ions, binding only ~0.1 and ~0.4 equivalents of copper and zinc, respectively, per dimer (wild type = 2.0 equivalents) (30, 32). The D125H FALS protein crystallizes from a solution containing 10 mM ZnSO4 at pH 6.5. Zinc is found to occupy both metal binding sites, a fact confirmed through the analysis of fluorescence spectra that precede the x-ray data collection experiments and through single wavelength anomalous dispersion phasing of experimental electron density maps using zinc as the anomalous scatterer. Fig. 2A shows the zinc-occupied copper binding site of a D125H monomer superimposed on 1.4 Å electron density contoured at 1.2 σ. The Zn(II) ion is coordinated by the three copper ligands, His-46, His-48, and His-120, all at distances of ~2.0 Å. A sulfate anion (HSO4−) is observed in the active site channel with its OX1 atom acting as a fourth ligand to the zinc ion at a distance of ~1.9 Å. The zinc coordination geometry is best described as pseudo-trigonal planar with the zinc ion displaced ~0.4 Å from a plane formed by the nitrogen atoms of the three histidine ligands. In addition to its role as a metal ligand, the HSO4− OX1 atom receives a nearly ideal hydrogen bond donated by the NE2 atom of His-63, the bridging imidazolyl. The HSO4− OX2 atom participates in hydrogen-bonding interactions with the epsilon and guanidinium nitrogen atoms of Arg-143 and with the ND2 atom of the side chain of Asn-26 from a symmetry-related D125H molecule in the crystal lattice. The symmetry-related Asn-26 side chain also donates a hydrogen bond to the backbone oxygen atom of Gly-141, which forms part of the active site rim. Fig. 2A also shows HCO3− modeled into the SOD1 active site channel based on the position of the observed HSO4−, such that two of its oxygen atoms occupy the same positions as the OX1 and OX2 atoms of HSO4− in the process where suitable 3D difference electron density and reasonable hydrogen bond geometry were indicated.

**Modeling of Carbonate into the D125H SOD1 Structure**—HCO3− was modeled into the SOD1 active site channel based on the position of the observed HSO4− in the D125H FALS mutant SOD1 structure. The carbonate molecule was downloaded from the Hetero-compound Information Center (HIC-Up, Uppsala, Sweden) (website: x-ray.bmc.uu.se/hicup) (Release 6.1) (35). The anion was positioned in the molecular graphics program O, such that two of its oxygen atoms occupy the same positions as the OX1 and OX2 atoms of HSO4− in the...
As part of the normal disproportionation reaction as copper-bound HO• must protect the enzyme from self-inactivation (to some extent) in a way analogous to that observed for formate or azide (Reactions 4 and 5) (8–10). However, we find that the rate of self-inactivation of wild type SOD1 in 0.5 mM Tris buffer, pH 8.0, is significantly enhanced when 25 mM HCO₃⁻ is added (Fig. 1A). The strong oxidant produced in this experiment must therefore arise from a pathway distinct from that described in Reactions 2 and 6. When we repeat the self-inactivation reaction using conditions identical to those used previously (10 mM HCO₃⁻ in 100 mM phosphate, pH 7.2) (17), we do not observe this rate enhancement (Fig. 1B). We interpret this to mean that the (excess) HPO₄²⁻ anions present compete with HCO₃⁻ for binding to the anion-binding site. In support of this finding, previous studies have shown that at a fixed HCO₃⁻ concentration, the rate of oxidation of DMPO in the external oxidative pathway is significantly attenuated by increasing phosphate concentrations (16). Conversely, at a fixed phosphate concentration, the self-inactivation rates are enhanced by increasing HCO₃⁻ concentrations (40).

We next compared the self-inactivation rate of wild type SOD1 with that of the L38V FALS mutant in the presence and absence of HCO₃⁻ (Fig. 1C). The pathogenic human SOD1 mutant exhibits overall increased rates of self-inactivation compared with wild type. However, HCO₃⁻ does not increase inactivation of L38V to any greater extent than it does the wild type, suggesting a common mechanistic pathway of HCO₃⁻ enhanced self-inactivation for both proteins.

Insight into the mechanism of the HCO₃⁻ effect on both the self-oxidative and external oxidative pathways comes from the x-ray crystal structure of human FALS mutant D125H. Although there is substantial evidence of oxyanion binding to SOD1 in solution (7), the D125H structure presented here is the first high resolution crystal structure to reveal spatial details of how an oxyanion can be bound in the active site channel. A hydrogen sulfate anion (HSO₄⁻) is positioned at the anion-binding site between Arg-143 and Thr-137. The mode of HSO₄⁻ binding to this site provides an excellent template upon which to model the binding of both bicarbonate and phosphate anions. When HCO₃⁻ is modeled in the position of the enzyme-associated HSO₄⁻, we see that it is capable of simultaneously interacting with the metal ion, Arg-143, and an asparagine residue (Asn-26) from a symmetry-related SOD1 protein in the crystal lattice (Fig. 2A). That oxyanions bound at the SOD1 anion-binding site can be in close contact with a metal (in this case, zinc) at a position very nearly corresponding to that of Cu(I) in the wild type protein was unanticipated. The interaction with the side chain of Asn-26 is particularly intriguing, because it demonstrates that such a bound oxyanion can also simultaneously contact much larger molecules (in this case, another SOD1 protein) in the bulk solvent. Based on this structure and our chemical data, we now propose the following novel mechanism that can explain the HCO₃⁻ mediated enhancement in the rates of both the self-oxidative and external oxidative pathways but does not require that CO₃²⁻ act as a diffusible oxidant. This mechanism is illustrated schematically in Fig. 3 where the steps are labeled as i–vi in a counterclockwise direction. In step i, the Cu(II) ion is reduced to Cu(I). This can occur via O₂⁻ as part of the normal disproportionation reaction as shown in Reaction 10,

\[
\text{SOD-Cu(II) + O}_2^- \rightarrow \text{SOD-Cu(I) + O}_2
\]

**REACTION 10**

or via H₂O₂ as shown in Reaction 1. In step ii, HCO₃⁻ binds to the anion-binding site in the mode predicted by the D125H SOD1 crystal structure. In step iii, HCO₃⁻ is guided into the active site channel where it reacts with HCO₃⁻ to form peroxy-
Peroxycarbonate-mediated Oxidation by CuZn-SOD

| X-ray data | Native | Zinc |
|------------|--------|------|
| λ (Å)      | 1.0000 | 1.2811 |
| No. of observations | 658,344 | 143,764 |
| No. of unique reflections | 95,234 | 57,725 |
| Resolution range (Å) | 50–1.4 | 50–2.0 |
| (Last shell) | 1.45–1.4 | 2.07–2.0 |
| Completeness (%) | 94.7 | 85.2 |
| (Last shell) | 85.1 | 52.3 |
| \( R_{\text{sym}} \) (on L) (%) | 4.8 | 4.1 |
| (Last shell) | 42.6 | 6.6 |

**Phasing**

| No. of sites | 6 |
| Resolution range (Å) | 37.0–2.0 |
| Overall phasing power | 2.2 |
| Overall figure of merit | 0.43 |
| Figure of merit after density modification | 0.80 |

**Table 1**

| Crystallographic data, phasing, and refinement of human FALS mutant SOD1 D125H |
|---------------------------------|--------|------|
| Refinement | CNS | Shelx-97 |
| Final model | | |
| Resolution range (Å) | 35.8–1.4 | 10.0–1.4 |
| \( R_{\text{free}} \) (%) | 20.3 | 14.6 |
| \( R_{\text{sym}} \) (%) | 22.3 | 21.2 |
| \( F_{\text{obs}}/F_{\text{calc}} \) | >0 | >0 |

\( R_{\text{sym}} = \Sigma I - (I) / 2I, \) where I is the observed intensity and (I) is the average intensity of multiple symmetry-related observations of that reflection.

\( R_{\text{free}} = \Sigma |F_{\text{obs}}| - |F_{\text{calc}}| / \Sigma |F_{\text{obs}}|, \) where |F_{\text{obs}}| is from a test set not used in the structural refinement (2002 reflections).

\( \text{Density modification using solvent flipping implemented in CNS (34).} \)

\( \text{Fig. of merit represents the weighted mean of the cosine of phase error.} \)

\( \text{Overall figure of merit} \)

| No. of water molecules | 736 |
| No. of metal ions | 6 zinc |

The HO\(^{-}\) produced can directly attack histidine copper ligands or oxidize substrates exogenous to the active site channel, leaving HCO\(_3\)\(^{-}\) in the anion-binding site (\(\text{OH}^-\)) and completing the cycle. The salient feature of this mechanism is that a strong oxidant is generated \textit{in situ} that protrudes into the bulk solvent or reacts with residues in and around the active site.

Investigations of proteolized \(\text{H}_2\text{O}_2\)-treated SOD1 using mass spectrometry indicate that multiple amino acids in the vicinity of the catalytic copper ion can be oxidatively damaged (13, 14). These residues include His-46, His-48, Pro-62, His-63, and His-120 (human numbering). The positions of these residues relative to the enzyme-associated bicarbonate anion are shown in Fig. 2B. Uchida and Kawakishi (13) have reported that His-118 in the bovine enzyme (His-120 in the human) is selectively converted to 2-oxo-histidine at its C\(_\text{E}\) atom (13). As first proposed by Sankarapandi and Zweier (16), the examination of Fig. 2, A and B, suggests that there does indeed exist a pre-formed hydrogen-bonding template comprised of the OX2 atom of the enzyme-bound bicarbonate anion and the carbonyl oxygen of Gly-141. In the D125H crystal structure, this hydrogen-bonding position is occupied by the ND1 atom of Asn-26 coming from a symmetry-related molecule in the crystal lattice. It is tempting to speculate that the reason for selective self-oxidation at His-118 (His-120) is that \(\text{HO}_2^+\) (or \(\text{H}_2\text{O}_2\)) preferentially forms the peroxy carbonate moiety on the OX2 atom of the enzyme-bound bicarbonate anion where it is stabilized by hydrogen bonding interactions with the carbonyl oxygen of Gly-141. In either of the peroxy carbonate-partitioning pathways described above, the strong oxidant subsequently derived would be in close proximity to the C\(_\text{E}\) atom of His-120.

The potential relevance of this peroxidative chemistry to FALS is underscored by the fact that bicarbonate is normally present in tissue at relatively high concentration (\(\sim 25 \text{ mM})\) (28).
and that this activity has been measured at H₂O₂ concentrations as low as 1 μM at neutral pH (23). In pathological conditions of oxidative stress where H₂O₂ may persist in the cytosol long enough to react with SOD1, the external oxidative pathway could significantly increase tyrosine oxidation and nitration (22, 42). Such products are signs of oxidative damage that, in sufficient amounts, could potentially lead to apoptosis. This idea has received support from other studies. For example,
human neuroblastoma cells transfected with the G93A SOD1 mutant demonstrate increased DCFH oxidation relative to cells transfected with wild type SOD1 (43). In spinal cord extracts of G93A-expressing transgenic mice, increased oxidization of the spin trap azulenyl nitrore is observed when compared with those of nontransgenic animals or transgenic mice expressing wild type human SOD1 (44, 45).

Although pathogenic SOD1 might oxidatively damage neuronal cellular constituents directly through enhanced rates of peroxidation, perhaps the most enticing hypothesis on how the enhanced peroxidase activity in pathogenic SOD1 proteins could cause ALS is that this activity can facilitate SOD1 misfolding and aggregation. High molecular weight-insoluble protein complexes, composed in part of FALS SOD1, are now widely believed to play a role in ALS pathogenesis either by sequestering heat shock proteins (46, 47) and/or interfering with the neuronal axonal transport (48, 49) and protein degradation (50, 51) machineries. The H$_2$O$_2$-mediated oxidation of histidine residues that bind metals in the SOD1 active site has been shown to stimulate SOD1 aggregation relative to the oxidized protein in vitro (52). Moreover, recent results from our own laboratory demonstrate that, unlike the holo-wild type protein, two metal-deficient pathogenic SOD1 proteins, H46R and S134N, can form higher order filamentous assemblies through non-native SOD1-SOD1 protein-protein interactions (53). These non-native interactions occur only through sub-units of the SOD1 protein that are devoid of copper, zinc, or both. Thus, any chemistry that could result in an increase in the amount of metal-deficient SOD1 could lead to pathogenesis indirectly through the gradual accumulation of such higher order SOD1 assemblies and aggregates. Finally, if enhanced rates of self-inactivation are related to increased aggregation of SOD1 with itself or with other proteins, it is possible that sporadic ALS, which comprises 85–90% of all ALS cases, might also be triggered by oxidatively damaged wild type SOD1.

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An Alternative Mechanism of Bicarbonate-mediated Peroxidation by Copper-Zinc Superoxide Dismutase: RATES ENHANCED VIA PROPOSED ENZYME-ASSOCIATED PEROXYCARBONATE INTERMEDIATE

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