Investigation of the Highly Active Manganese Superoxide Dismutase from Saccharomyces cerevisiae

Kevin Barnese,†‡§ Yuewei Sheng,†‡ Troy A. Stich,∥ Edith B. Gralla,‡ R. David Britt,‖ Diane E. Cabelli,*‡ and Joan Selverstone Valentine*†§

Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, Department of Bioinspired Chemistry, EWHA Womans University, Seoul 120-750, Korea, Department of Chemistry, University of California, Davis, California 95616, and Department of Chemistry, Brookhaven National Laboratory, Upton, New York 11973

Received May 14, 2010; E-mail: cabelli@bnl.gov; jsv@chem.ucla.edu

Abstract: Manganese superoxide dismutase (MnSOD) from different species differs in its efficiency in removing high concentrations of superoxide (O$_2^-$), due to different levels of product inhibition. Human MnSOD exhibits a substantially higher level of product inhibition than the MnSODs from bacteria. In order to investigate the mechanism of product inhibition and whether it is a feature common to eukaryotic MnSODs, we purified MnSOD from Saccharomyces cerevisiae (ScMnSOD). It was a tetramer with 0.6 equiv of Mn per monomer. The catalytic activity of ScMnSOD was investigated by pulse radiolysis and compared with human and two bacterial (Escherichia coli and Deinococcus radiodurans) MnSODs. To our surprise, ScMnSOD most efficiently facilitates removal of high concentrations of O$_2$ among these MnSODs. The gating value $k_2$/ $k_3$ that characterizes the level of product inhibition scales as ScMnSOD > D. radiodurans MnSOD > E. coli MnSOD > human MnSOD. While most MnSODs rest as the oxidized form, ScMnSOD was isolated in the Mn$^3+$ oxidation state as revealed by its optical and electron paramagnetic resonance spectra. This finding poses the possibility of elucidating the origin of product inhibition by comparing human MnSOD with ScMnSOD.

Manganese superoxide dismutase (MnSOD) enzymes catalyze superoxide (O$_2^-$) disproportionation by a mechanism that is more complex than those of the other SODs. In particular the reduction of superoxide can proceed via one of two pathways. One pathway dominates when the O$_2^-$ concentration is low relative to the enzyme concentration (reaction 2), and the other pathway dominates when the ratio [O$_2^-$]/[MnSOD] is high (reactions 3 and 4). The paradoxical finding is that MnSOD is a less effective SOD catalyst when O$_2^-$ levels are elevated.$^1$

\[
\begin{align*}
\text{Mn}^{3+}\text{SOD} + O_2 & \rightarrow \text{Mn}^{3+}\text{SOD} + O_2 & (1) \\
\text{Mn}^{2+}\text{SOD} + O_2 & \rightarrow \text{Mn}^{2+}\text{SOD} + H_2O_2 & (2) \\
\text{Mn}^{2+}\text{SOD} + O_2 & \rightarrow \text{Mn}^{3+}\text{SOD} & (3) \\
\text{Mn}^{3+}\text{SOD} + O_2 & \rightarrow \text{Mn}^{3+}\text{SOD} + H_2O_2 & (4)
\end{align*}
\]

The depressed catalytic activity at high O$_2^-$ concentrations is known to be due to the formation of a product-inhibited Mn$^{3+}$.

Table 1. Rate Constants for the Different MnSODs

<table>
<thead>
<tr>
<th>Organism</th>
<th>$k_0$ (nM s$^{-1}$)</th>
<th>$k_1$ (nM s$^{-1}$)</th>
<th>$k_2$ (nM s$^{-1}$)</th>
<th>$k_3$ (s$^{-1}$)</th>
<th>$k_2/k_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human$^{ab}$</td>
<td>1.4</td>
<td>0.6</td>
<td>0.5</td>
<td>130</td>
<td>1.6</td>
</tr>
<tr>
<td>E. coli$^{bc}$</td>
<td>1.1</td>
<td>0.9</td>
<td>0.17</td>
<td>60</td>
<td>5.3</td>
</tr>
<tr>
<td>D. radiodurans$^{ab}$</td>
<td>1.2</td>
<td>1.1</td>
<td>0.07</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>S. cerevisiae (this work)</td>
<td>1.1$^\pm$1.5</td>
<td>0.8</td>
<td>0.04$\pm$0.05</td>
<td>90$\pm$140</td>
<td>16$\pm$20</td>
</tr>
</tbody>
</table>

Superoxide concentrations are known to be variable in cells; for example, it has recently been shown that transient O$_2^-$ bursts, termed “superoxide flashes”, are formed in human mitochondria,$^2$ creating the possibility of even greater variability in H$_2$O$_2$ formation rates. However, a slower product-inhibited pathway in human MnSOD would allow for more constant H$_2$O$_2$ formation even when O$_2^-$ concentrations vary.

Low levels of H$_2$O$_2$ play an important role in signaling in mammalian cells, regulating numerous processes including rates of cell growth and division.$^3$ It has been proposed that the slower pathway for human MnSOD appeared in response to an evolutionary pressure to control more tightly intracellular H$_2$O$_2$ levels,$^4$ to reduce H$_2$O$_2$ mediated oxidative damage, and to optimize its signaling function.$^3$ The $k_2/k_3$ values determined for human and bacterial MnSODs (Table 1) are consistent with this hypothesis. However, structural studies of different MnSODs, both wild type and mutant, have yet to reveal why $k_2/k_3$ differs so dramatically for this enzyme.$^1$

The budding yeast Saccharomyces cerevisiae is widely used as a single-cell model for higher eukaryotic organisms because it is remarkably similar to mammalian cells. S. cerevisiae also appears to be less sensitive to H$_2$O$_2$ than human cells, and the only currently known H$_2$O$_2$ sensing proteins in S. cerevisiae (YAP1p and Skn7p) are involved in regulating oxidative stress protection; a more general signaling role has yet to be found.$^{5,9}$ Both human MnSOD and S. cerevisiae MnSOD (ScMnSOD) are tetramers$^8$ and localized to the mitochondrial matrix,$^7$ while most bacterial MnSODs are dimers.

$^1$ These authors contributed equally.
$^2$ University of California, Los Angeles.
$^3$ Ewha Womans University, Korea.
$^4$ University of California, Davis.
$^5$ Brookhaven National Laboratory.

10.1021/ja104179r © 2010 American Chemical Society
Moreover, human MnSOD shares greater sequence similarity with ScMnSOD than with *Escherichia coli* or *Deinococcus radiodurans* MnSODs (62.2%, 52.9%, and 54.5% respectively). Published reports of the activity of ScMnSOD do not include a determination of the degree of product inhibition. We therefore turned our attention to characterizing the catalytic mechanism of ScMnSOD with the expectation that the high contribution from the product-inhibited pathway would prove to be a property common to eukaryotic MnSODs. Surprisingly, we found instead that ScMnSOD is even less product-inhibited than the bacterial MnSODs characterized to date, surpassing even the high activity of MnSOD from the radiation resistant bacterium *D. radiodurans*.

The gene of ScMnSOD, which includes the mitochondrial targeting sequence, was inserted into the plasmid YEp352. The enzyme was overexpressed in *E. coli* and initial O2− concentrations (2−48 μM) using pulse radiolysis, conditions that, while not physiological, are necessary to study kinetics by pulse radiolysis. As described above, human MnSOD already shares the electronic absorption spectrum of as-isolated MnSOD with O2− oxidation state. (A) EPR spectrum of our as-isolated ScMnSOD also indicates that the enzyme is reduced, since the perpendicular-mode EPR spectrum is similar to those of other Mn2+ SODs, with the usual six-line hyperfine splitting from the 55Mn nucleus (I = 5/2) seen at g∥ = 6.0 (Figure 2B). We looked for evidence of the integer spin Mn2+ (S = 2) by parallel-mode EPR, but our spectrum lacked the sextet hyperfine pattern typically displayed by Mn3+ SOD (Supporting Information).13

The only other MnSOD enzymes that have been isolated in the Mn2+ oxidation state are mutant MnSODs, most notably the Gln143 mutant. However, the factors that determine the resting oxidation state are unknown.

Increased levels of MnSOD activity have been shown to slow tumor growth in cultured human cells and in animal studies, and it has been proposed that this effect is related to cellular H2O2 levels. For that reason and to improve our understanding of the basis of the observed product inhibition, human MnSOD has been repeatedly mutated in attempts to make its activity resemble that of the bacterial proteins, but with limited success. As described above, human MnSOD already shares greater sequence similarity with ScMnSOD than with the bacterial enzymes, and the two eukaryotic proteins are tetramers while the bacterial ones are dimers. Also, ScMnSOD resembles the bacterial ones in that k2 is small but is similar to the human enzyme in that k4 is large. Thus, to improve our understanding of what causes the unusual kinetic properties of human MnSOD, it may be more productive to compare/contrast it with ScMnSOD than with the bacterial MnSODs. Investigation of the slight structural differences between the enzymes may provide a key to understanding the chemical mechanism of product inhibition. We will also continue to study the evolutionary significance of product inhibition by studying MnSOD from other organisms.

**Acknowledgment.** This work was supported by Grant DK46828, KOSEF/MEST through WCU project (R31-2008-000-10010-0) to J.S.V. and National Institutes of Health and Grant GM48242 to R.D.B. Radiolysis studies were carried out at the Center for Radiation Chemistry Research at BNL, which is funded under Contract DE-AC02-98CH10886 with the U.S. Department of

![Figure 2](image-url)
Energy and supported by its Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences.

Supporting Information Available: ScMnSOD isolation details, pulse radiolysis, complete ref 2, and parallel mode EPR. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(5) Stone, J. R.; Yang, S. P. Antioxid. Redox Signaling 2006, 8, 243.
(9) Schrank, I. S.; Sims, P. F. G.; Oliver, S. G. Gene 1988, 73, 121.

JA104179R