ment of the UV-vis spectra and the molecular weights.

Supplementary Material Available: Experimental procedures for the synthesis of TST, TSTBr, BrTSTBr, TTSTT, $(TST)_2$, $(TST)_3$, and $(TST)_n$, listings of their spectral and analytical data, and details of the X-ray structure, crystal data, atomic coordinates, thermal parameters, and full data of bond distances and angles for TST (10 pages). Ordering information is given on any current masthead page.

Structural Characterization of the Binuclear Mn Site in *Lactobacillus plantarum* Manganese Catalase

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There is presently substantial interest in multinuclear Mn proteins. In addition to the photosynthetic oxygen evolving complex (OEC), which contains four Mn atoms,[1] Mn clusters have been found in several non-heme catalases[2-4] and in ribonucleotide reductase.[5] Mn catalases[2-4] contain a binuclear Mn site and have at least four accessible oxidation states.[6-9] We report here the results of an EXAFS study of the reduced, Mn(II)/Mn(II), and the superoxidized, Mn(III)/Mn(IV), derivatives of the *Lactobacillus plantarum* Mn catalase.

Mn catalase is inactivated by treatment with NH_2OH + H_2O_2,[6a] giving a superoxidized species having a 16-line EPR signal.[7,9] The temperature dependence and the hyperfine coupling constants[10] are consistent with a strongly coupled Mn(III)/Mn(IV) dimer, and X-ray edge spectra confirm this assignment.[9] Unfortunately, these give no direct information regarding the Mn-Mn separation. EPR spectroscopy shows that the Mn ions in reduced catalase are weakly coupled,[7] but again provides no information on the Mn-Mn distance. Preliminary crystallographic results[4,11] suggest an Mn-Mn distance of ca. 3.6 Å, although the oxidation state was not given. Knowledge of the Mn-Mn distances could be used to define the Mn-bridging ligands,[12] since $(\mu\text{-O})_2$ structures have Mn-Mn ≈ 2.7 Å, $(\mu\text{-O})(\mu\text{-OH})$ structures have Mn-Mn ≈ 2.8 Å,[13] $(\mu\text{-O})(\mu\text{-carboxylato})_2$ structures have Mn-Mn ≈ 3.0-3.3 Å,[12] and other geometries (e.g., hydroxo and carboxylato or only carboxylato bridges) have longer, weaker Mn-Mn interactions that may not be detected with EXAFS.

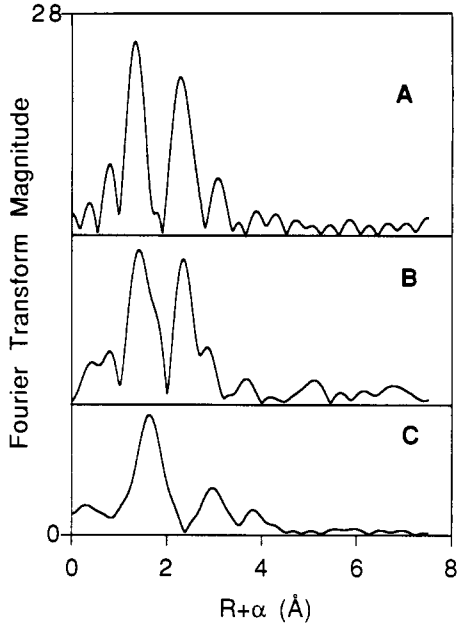

Figure 1. Fourier transforms (k^3 weighted, k = 3.5-11.8 Å^{-1}) of the EXAFS spectra for (A) S_1 state of the OEC, (B) superoxidized catalase, and (C) reduced catalase. Spectra offset vertically for clarity. OEC data from ref 14b.

Table I. Manganese Catalase EXAFS Fitting Results[a]

	superoxidized			reduced			Mn(II)-(imidazole)$_6$[b]
	N	R (Å)	$\Delta\sigma^2 \times 10^3$	N	R (Å)	$\Delta\sigma^2 \times 10^3$	R (Å)
O	2	1.82	2.1				
N/O	4	2.14	-1.9	6	2.19	0.0	2.27
Mn	1	2.67	1.4				
C	4	3.00[c]	0.0	8	3.16	6.9	3.27
N/C	4	4.33[c]	-0.8	4	4.42	-1.9	4.43

[a] Best fit to unfiltered data. Coordination numbers were fixed at integer values giving the best fits. Debye-Waller factors (σ^2) given as Å^2 × 10^3. Absolute σ^2 is reported for Mn-Mn; other values are $\Delta\sigma^2$ relative to reference compound. [b] Crystallographic data from ref 19. [c] Shell is poorly defined. An alternate minima exist at 3.3 Å (C) and 3.9 Å (N/C).

Catalase was isolated as described previously.[9] X-ray absorption spectra were measured and analyzed using conventional methods.[14,15] The Fourier transform for superoxidized catalase (Figure 1B) has two principal peaks at $R + \alpha \approx 1.4$ and 2.3 Å, corresponding to Mn-(O,N) nearest neighbor and Mn-Mn scattering, respectively. Perhaps the most striking feature of this spectrum is its similarity to the Fourier transform for the OEC[14,17] (Figure

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(15) Catalase solutions were frozen in lucite cells having 6-μm polypropylene windows. Data were measured at 8 K using NSLS beamline X19A (150 mA, 2.5 GeV), with an Si(111) double crystal monochromator detuned 50% for harmonic rejection. Fluorescence excitation spectra were recorded using a 13-element Ge detector array (courtesy of Prof. S. P. Cramer). Model compounds were $KMnO_4$ and Mn(III)(urea)$_6$ for Mn-O and Mn(II)(imidazole)$_6$ for Mn-N and Mn-C. Mn-Mn EXAFS data were analyzed using theoretical Mn phase and amplitude functions[16] calibrated by fitting models of known structure.

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1A). The catalase Mn site is dramatically altered on reduction, with longer Mn-(O,N) distances and no 2.7-Å Mn-Mn interaction (Figure 1C). The outer-shell peaks ($R + \alpha \approx 3.0$ and 3.8 Å) are typical of, but smaller than, those observed for Mn-imidazole complexes.

Curve fitting results are summarized in Table I (and in Table S1 in the supplementary material). The data for the superoxidized enzyme cannot be fit without including two shells of nearest-neighbor scatterers and an Mn-Mn interaction at 2.7 Å. The 1.82-Å Mn-O distance is typical of Mn-bridging oxo distances. Both the Fourier transform and the curve fitting suggest additional low-Z scatterers, consistent with second and third shell N/C atoms in coordinated imidazoles.[18] The apparent coordination numbers suggest an average of 1-2 imidazoles per Mn. However, this number is not well defined due to the limited k range of the data and interference from the strong Mn-Mn scattering.

As suggested by the Fourier transform, the EXAFS for reduced catalase is dominated by a single shell of low-Z scatterers at 2.19 Å. There is no evidence for a shell of scatterers at ca. 1.8 Å (i.e., a bridging oxo ligand); however, the fit quality is improved significantly if additional shells of C and N/C scatterers are included at ca. 3.2 and 4.4 Å. The apparent coordination numbers suggest an average of 2-4 imidazoles per Mn, although once again this number is not well defined. There is a *small* improvement in the fit if a shell of Mn is added at 3.55 Å. However, equivalent improvements are observed if instead the Mn is added at 3.99 Å or if a shell of C is added at 3.64 Å. In no case is the improvement sufficient to support the conclusion that the reduced catalase contains an EXAFS detectable Mn-Mn interaction. All of the Mn-scatterer distances in reduced catalase are slightly but significantly shorter than those in Mn(II)(imidazole)$_6$. This may be due to a coordination number less than six for one or both of the Mn atoms, the presence of oxygen ligands (e.g., from carboxylate bridges), or a combination of these effects. Overall, the Mn ligation appears very similar to that of Fe in deoxy hemerythrin.[20]

On the basis of ESEEM spectra, Dikanov et al. suggested[21] that one or more imidazoles are coordinated to the Mn in superoxidized catalase. The EXAFS data confirm this ligation for *both* superoxidized and reduced catalase. Although not well defined, the apparent coordination numbers suggest that imidazole ligands are lost on forming the superoxidized enzyme. This could occur if one or more imidazoles are replaced by bridging oxo groups.

The short Mn-Mn distance demonstrates that there are two oxo bridges in superoxidized catalase.[12] Although there are few examples, it appears that unsupported $(\mu\text{-O})_2$ bridges[22] have Mn-Mn distances of ≥2.70 Å, while additional bridging ligands lead to shorter Mn-Mn distances.[23] The 2.67-Å Mn-Mn distance thus suggests an additional bridge, e.g., $(\mu\text{-O})_2(\mu\text{-carboxylato})$. A carboxylate bridge is consistent with the proposal[12b,24] that the oxidized Mn(III)/Mn(III) enzyme has a $(\mu\text{-O})(\mu\text{-carboxylato})_2$ bridged core.

The EXAFS data for the reduced enzyme do not permit unambiguous definition of an Mn-Mn distance. Similar difficulties in defining metal-metal distances in binuclear iron proteins[25] have been attributed to the loss of bridging ligands. In the present case, the absence of readily detectable Mn-Mn EXAFS allows us to rule out $(\mu\text{-O})_2$ or $(\mu\text{-OH})_2$ bridged structures. Bridging structures consistent with the data include $(\mu\text{-carboxylato})_n$ and $(\mu\text{-OH})(\mu\text{-carboxylato})_n$, where $n = 1\text{-}3$.

Recently we have shown that the superoxidized catalase is inactive, due to its inability to oxidize H_2O_2.[9] The present results suggest an explanation for this observation. If the oxidized enzyme has an [Mn(III)$(\mu$-O$)(\mu$-carboxylato$)_2$Mn(III)] core,[12,24] conversion to the superoxidized derivative involves addition of an oxo bridge. This is expected to stabilize Mn with respect to reduction, thus converting the Mn(III)/Mn(III) derivative, which is a good oxidant, into a species that, although formally more oxidized, is in fact a poor oxidant.[9] The similarity between the EXAFS for superoxidized catalase and for the OEC suggests that they contain similar, probably di-μ-oxo-bridged, Mn structures. The relatively poor oxidizing power of this unit may play an important role in stabilizing the OEC against premature oxidation of water. With the findings that the *L. plantarum* Mn catalase possesses structural, as well as chemical and spectroscopic, similarities to the OEC, Mn catalase takes on added significance as a well-characterized example of a high oxidation state biological Mn dimer.

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Supplementary Material Available: Table S1 and Figures S1 and S2 showing the quality of fit for different models and the EXAFS spectra (3 pages). Ordering information is given on any current masthead page.

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Nature of the Frontier Orbitals of Tungsten Benzylidyne Complexes

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Transition-metal alkylidyne (or carbyne) complexes[1,2] differ electronically from metal-oxo and nitrido complexes,[2] their first-row, triply bonded relatives, in that they tolerate a much broader spectrum of ancillary ligands, ranging from strong π-acceptors, for which Fischer's group VI M(≡CR)(CO)$_4$X complexes are the archetypes,[3] to strong π-donors, as exemplified by

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