Thermochromic Conformational Change of *Methanobacterium thermoautotrophicum* Iron Superoxide Dismutase

Jean Philippe Renault and Irène Morgenstern-Badarau*

Laboratoire de Chimie Bioorganique et Bioinorganique, Institut de Chimie Moléculaire d'Orsay, Université Paris-Sud XI, 91405 Orsay Cedex, France

Mario Piccioli*

Laboratoire de Chimie Structurale Organique et Biologique, CNRS UMR 7613, Université Pierre et Marie Curie (Paris VI), 4 Place Jussieu, 75005 Paris, France

Received October 2, 1998

The temperature dependence of the structural and electronic properties of metalloenzyme active sites is an important point from which to address the thermal control of enzymatic reactions. In this respect, a relevant example is provided by iron- and manganese-containing superoxide dismutases (FeSOD and Mn-SOD) whose spectroscopic properties have been studied by various techniques,[1−5] and for which a variability in coordination number has been reported, spanning from the four-coordinated FeSOD from *Pseudomonas ovalis* [6] to the six-coordinated azide adduct of FeSOD from *Escherichia coli*.[7]

Although there is evidence that the redox potential of the metal ion is driven substantially by the protein environment,[8] the role of coordination properties and of metal specificity in driving catalysis is not yet fully understood. In the oxidized form of MnSOD from *E. coli* it has been recently shown that a low-temperature thermochromism occurs in the presence of an azide.[9] A chromophore switch with a transition temperature T_c of about 220 K has been interpreted as evidence of an unstable six-coordinated structure that acts as an intermediate in the enzymatic reaction.[9] The same equilibrium has been observed, but with a T_c of 305 K, in the analogous adduct of MnSOD from the thermophilic bacterium *Thermus thermophilus*, thus supporting the role of the above transition for catalysis.[10]

Here we report the first evidence of a room temperature thermochromism for the reduced form of the tetrameric FeSOD from the archaebacterium *Methanobacterium thermoautotrophicum*.[11,12] The challenge of this study is to address a structural change, located in the active site of a paramagnetic tetrameric enzyme, whose molecular weight is about 100 kDa, using variable temperature NMR. To the best of our knowledge, this is one of the largest biological systems that can be characterized by paramagnetic NMR.[13]

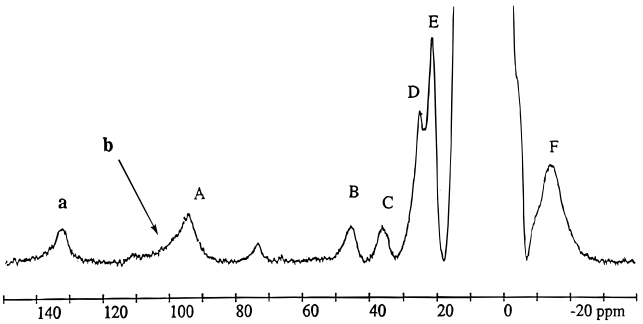

Figure 1. 200 MHz ^{1}H NMR spectrum of *M. thermoautrotrophicum* FeSOD at 283 K. Experimental conditions are described in ref 14. Labeled signals are discussed in the text.

We explored the temperature dependence of the one-dimensional spectrum of *M. thermoautotrophicum* FeSOD, which is shown in Figure 1.[14] In the NMR spectrum of this tetrameric protein, reported here for the first time, five signals (A, B, C, a, and b) are observed in a very far downfield-shifted region, in the range 150−30 ppm.[15] Many paramagnetically shifted signals (D, E, and other signals in the 20−10 ppm region) are also observed in the less downfield-shifted region and in the highfield part up to about −20 ppm (F). The five most downfield-shifted signals arise from protons of iron-coordinated histidines,[16] their shift properties accounting for their assignment to metal-bound resi-

* Corresponding authors. I.M.-B.: tel., +33 1 6915 7831; fax, +33 1 6915 7281; e-mail, imorgens@icmo.u-psud.fr. M.P.: tel., +33 1 4427 3112; fax, +33 1 4427 3843; e-mail, piccioli@ccr.jussieu.fr.

(1) Slykhouse, T. O.; Fee, J. A. *J. Biol. Chem.* **1976**, *251*, 5472−5477.
(2) Villafranca, J. J.; Yost, F. J.; Fridovich, I. *J. Biol. Chem.* **1974**, *249*, 3532−3536.
(3) Whittaker, J. W.; Whittaker, M. M. *J. Am. Chem. Soc.* **1991**, *113*, 5528−5540.
(4) Renault, J. P.; Verchère-Béaur, C.; Morgenstern-Badarau, I.; Piccioli, M. *FEBS Lett.* **1997**, *401*, 15−19.
(5) Sorkin, D. L.; Miller, A. F. *Biochemistry* **1997**, *36*, 4916−4924.
(6) Stoddart, B. L.; Howell, P. L.; Ringe, D.; Petsko, G. A. *Biochemistry* **1990**, *29*, 8885−8893. PDB access number 3sdp.
(7) Lah, M. S.; Dixon, M. M.; Pattridge, K. A.; Stallings, W. C.; Fee, J. A.; Ludwig, M. L. *Biochemistry* **1995**, *34*, 1646−1660. PDB access number 1isc.
(8) Fisher, C. L.; Chen, J.-L.; Li, J.; Bashford, D.; Noodleman, L. *J. Phys. Chem.* **1996**, *100*, 13498−13505.
(9) Whittaker, M. M.; Whittaker, J. W. *Biochemistry* **1996**, *35*, 6762−6770.
(10) Whittaker, M. M.; Whittaker, J. W. *J. Biol. Inorg. Chem.* **1997**, *2*, 667−671.
(11) Takao, M.; Yasui, A.; Oikawa, A. *J. Biol. Chem.* **1991**, *266*, 14151−14154.
(12) Takao, M.; Oikawa, A.; Yasui, A. *Arch. Biochem. Biophys.* **1990**, *283*, 210−216.
(13) Bertini, I.; Turano, P.; Vila, A. J. *Chem. Rev.* **1993**, *93*, 2833−2932.
(14) Sample concentration was about 0.3 mM protein (1.2 mM iron) in 50 mM Tris buffer, pH 7.8. The iron was reduced to the +II state using sodium dithionite. All samples were extensively dialysed prior to measurements. NMR experiments were recorded on a Bruker MSL 200, operating at 4.7 T. Experiments were collected using the superWEFT pulse sequence (Inubushi, T.; Becker, E. D. *J. Magn. Reson.* **1983**, *51*, 128−133). Recycle and interpulse delay were 60 and 50 ms, respectively. A range of 3×10^5 to 1×10^6 scans was collected for each one-dimensional experiment. Experimental temperature dependence was collected over the range 283−303 K. T_1 experiments were performed at 293 K, using the inversion recovery methodology. A 30 Hz line broadening has been used to obtain the spectrum in Figure 1.
(15) The minor signal observed at 75 ppm arises from free iron released upon reduction.
(16) Observed values of chemical shifts and relaxation rates are consistent with Hδ1 (Hπ in IUPAC notation) imidazole protons. As no nonexchangeable signals are observed in the same range, this is evidence that histidine binds the iron ion through the Nϵ2 (Nτ), as expected on the basis of the available X-ray structures of Fe- and Mn-containing SODs.

10.1021/ic981172o CCC: $18.00
Published on Web 01/28/1999

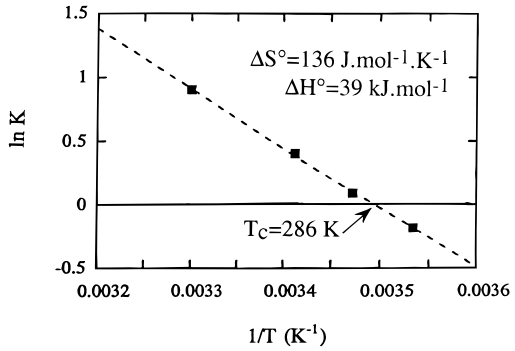

Figure 2. Relative temperature dependence intensities of the two species of *M. thermoautrotrophicum* FeSOD. Van't Hoff equation was used to estimate the standard reaction parameters $\Delta H°$ and $\Delta S°$. The equilibrium constant K was obtained from the intensity ratio between two well isolated signals B and a.

dues, and their disappearance when the spectrum is recorded in D_2O accounting for their assignment to exchangeable protons. They are markers of the active site addressing the chemical properties of the enzyme as a function of temperature.

Two sets of signals are identified from the relative temperature-dependent intensities of the signals A, B, and C versus those labeled as a and b. Increasing temperature up to 303 K causes a significant decrease in the intensities of signals a and b and a consequent increase of the intensities of signals A, B, and C. These changes in intensities, which were quantified from signal integrations, imply two distinct structures associated with high- and low-temperature forms of the active site, respectively. A thermal two-state transition is shown in Figure 2, as the temperature dependence of the equilibrium constant *K* between the two species defined from the intensity ratio between signals B and a. These two signals were used since they are well isolated. In addition, as they have similar line widths, their intensity is expected to be equally affected by the use of window functions.[17] Data analysis provides thermochemical parameters for the equilibrium which is characterized by a standard reaction enthalpy variation $\Delta H°$ equal to 39 ± 4 kJ mol^{-1} and a standard reaction entropy variation $\Delta S°$ equal to 136 ± 20 J mol^{-1} K^{-1}. The T_c midpoint transition is found to be equal to 286 ± 5 K.

The high-temperature species (signals A, B, and C) is highly analogous to the active site of FeSOD and of the iron-substituted MnSOD from *E. coli*.[18] The most downfield signal A has a shorter relaxation rate (3 ms) with respect to signals B (8 ms) and C (9 ms).[19] This can be interpreted as deriving from two similar histidines in equatorial position and to the axial histidine with markedly different properties in terms of the ligand centered unpaired spin density. The temperature dependence of the chemical shift for signal A compared to the ones for signal B and for signal C (data not shown) supports the above considerations. T_1^{-1} and T_2^{-1} relaxation rates are increased by about a factor of 2 with respect to the dimeric FeSOD from *E. coli*, as expected on the basis of an increased τ_r due to the tetrameric form of the present protein.[20] Thus we have firm evidence that the high-temperature species has the same five-coordinated active site as shown by X-ray studies on *E. coli* FeSOD.[7] On the other hand, the two signals indicative of the lower-temperature species, which still belong to histidine ligands, experience larger hyperfine coupling constants, suggesting that the chromophore evolves to a different coordination environment. However, a third signal of the minor species could have been lost because of line broadening and because of overlaping with other signals of the spectrum, due to the large molecular weight of the enzyme which provides very large and barely detectable signals. Still, both NMR and thermodynamic parameters are consistent with different coordination spheres of the metal ion, though the nature of the conformational change cannot be precisely assessed. The thermodynamic properties observed for the equilibrium of the two species account for a noncooperative process, typically a coordination change or a solvation/desolvation process.[10,21] $\Delta H°$ and $\Delta S°$ values that were obtained are comparable to the previously reported thermodynamic values for MnSOD azide adducts that have been accounted for on the basis of six-to-five coordination change in the active site.[9,10] If the observed conformational change is similar to the six-to-five switch of metal coordination proposed in the case of azide-MnSOD, it is likely that a water molecule can play the role of an additional ligand. The fact that an additional water molecule could be trapped in the first coordination sphere at temperatures lower than optimal temperature activity would be, however, an indication of the conformational flexibility of this active site. Although present experimental data do not rule out the possibility of such a five-to-six switch, they point out a possible alternative interpretation based on five-to-four change in coordination. Due to the detachment of one of the three histidines from metal coordination, only two signals from metal-coordinated histidines are observed when decreasing temperature.

The relevance of these results relies on the following findings: (1) the same effects, observed from the oxidized $Mn^{III}SOD$ can be observed on the reduced $Fe^{II}SOD$; (2) despite the fact that no exogenous ligands occur in the presently investigated derivative, a flexibility in the SOD active site is nevertheless observed; (3) unlike in former cases, thermochromism is here observed as a property of an iron enzyme.

Finally, reference should be made to the fact that, although we are dealing with an FeSOD system, *M. thermoautotrophicum* FeSOD is classified as belonging, in an evolutionary point of view, to the class of MnSODs.[22] This is confirmed by the fact that, at variance with most of FeSODs and similarly to MnSODs, it is not inhibited by hydrogen peroxide.[11]

From all the experimental data available up to now, thermochromism in SOD is a peculiar feature of MnSOD or of Mn-like FeSOD enzymes, as in the present investigation. These peculiar properties of MnSOD enzymes must be related to the already considered role of the protein in driving the coordination chemistry of active sites in metalloenzymes.

Acknowledgment. We thank M. Takao, A. Yasui, and A. Oikawa (Tohoku University, Japan) for their generous gift of the plasmid harboring the *M. thermoautotrophicum* FeSOD gene. We are grateful to I. Bertini and C. Luchinat (University of Florence, Italy) for allowing us to access the PARABIO European Union Large Scale Facilities (Contract No. ERBFMGECT950033).

IC981172O

(17) The recycle delay (60 ms) was longer than $5T_1$ for the signals A−C. Therefore, the signal intensity is not affected by the superWEFT sequence.

(18) (a) Ming, L.-J.; Lynch, J. B.; Holz, R. C.; Que, L., Jr. *Inorg. Chem.* **1994**, *33*, 83−87. (b) Vance, C. K.; Miller A.-F. *Biochemistry* **1998**, *37*, 5518−5527

(19) Signals D, E, and F are also observed in the NMR spectrum of *E. coli* FeSOD. In that case, signal D is attributed to the iron bound aspartate, whereas signals E and F arise from the second coordination sphere.

(20) Banci, L.; Bertini, I.; Luchinat, C. *Nuclear and Electron Relaxation*; VCH: Weinheim, 1991.

(21) Blomberg, M. R. A.; Siegbahn, P. E. M. *Theor. Chem. Acc.* **1997**, *97*, 72−80.

(22) Smith, M. W.; Doolittle, R. F. *J. Mol. Evol.* **1992**, *34*, 175−184.