Inorganica Chimica Acta, *152* (1988) 53–54

Structural Change in the One-electron Oxidation–Reduction at the Copper Site in Nitrite Reductase. Evidence from EXAFS

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(Received October 28, 1987)

Abstract

The structural change of the nitrite reductase in the one-electron reduction at the copper center has been studied using the EXAFS technique. In the one-electron reduction, the Cu–S(cysteine) distance is elongated by about 0.1 Å, and Cu–N(imidazole) distances are slightly changed.

Denitrification, which is the anaerobic respiration process in denitrifying bacteria, is the only biological process to release N_2 and/or N_2O from nitrate and nitrite [1]. Nitrite reductases found in denitrifying bacteria catalyze the reaction of $NO_2^- \rightarrow NO$ and are classified into two groups: copper-containing and heme-containing enzymes [1]. Masuko *et al.* [2] studied the spectral properties for the oxidized form of the copper-containing nitrite reductase (NiR) of *Alcaligenes* sp. We have also reported the EXAFS study for the copper(II) site of the freeze-dried NiR of the same strain [3]. The features of the absorption and EPR spectra [2] and the bond length [3] for the NiR are similar to those of type I copper [4], but the magnetic circular dichroism [5] and resonance Raman [6] spectra are different. The most popular type I copper-containing proteins are azurin and plastocyanin, whose functions are electron transfer agents. It is important to clarify the structural changes at the copper center on one-electron oxidation–reduction to understand the relationship between the function and structure of NiR. We report the structural study at the copper site of the oxidized and reduced forms of NiR using the EXAFS technique.

Nitrite reductase was isolated from *Alcaligenes* sp. NCIB 11015 as described previously [2]. The purity index (A_{280}/A_{594}) was 14.1. A purified and concentrated NiR (oxidized form) solution (*ca.* 6 mM in 0.15 M potassium phosphate buffer pH 7.0) was used for the EXAFS experiment. The reduced form was obtained by the addition of a minimum amount of sodium dithionite. The X-ray absorption measurements in transmission mode were carried out at the Photon Factory of the National Laboratory of High-energy Physics (beam energy 2.5 GeV, beam current 70–120 mA). The EXAFS spectra were analyzed by standard procedures [7].

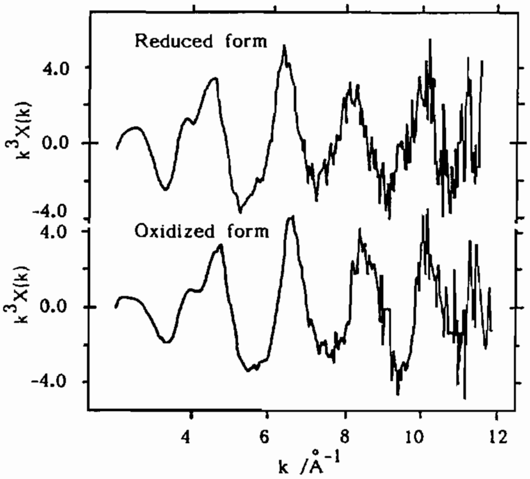

Fig. 1. EXAFS spectra near the Cu edge of the reduced and oxidized forms.

Figure 1 shows the EXAFS spectra, $k^3\chi(k)$, of both the reduced and oxidized forms of NiR. Fourier transformation was applied to the $k^3\chi(k)$ values over a k range of 3.5–11.5 Å^{-1}. Both radial distributions (Fig. 2) resulting from the Fourier transformation show a main peak centered at about 1.7 Å and two satellite peaks at about 2.6 and 3.5 Å (not corrected phase-shift). The main peaks of both forms are similar and should correspond to the 2N and S atoms of the coordinated imidazole and cysteine ligands [3]. Two satellite peaks are characteristic for the copper–imidazole scattering [8].

To obtain metal–ligand bond distances, a curve-fitting analysis [9] was performed for the main peak. Reference compounds, $[Cu(Im)_4]SO_4$ (Im = imidazole) [10] and $[(n\text{-}C_4H_9)_4N]_2[Cu(mnt)_2]$ (mnt =

0020-1693/88/$3.50

TABLE I. Curve-Fitting Results for the First-Shell of the Cu-Edge EXAFS[a]

Form	Cu–N (A)	Cu–S (A)
Oxidized	1.99 ± 0.02 (1.8×10^{-2})	2.11 ± 0.03 (5.7×10^{-2})
Reduced	2.05 ± 0.02 (2.0×10^{-2})	2.21 ± 0.03 (5.7×10^{-2})

[a] The values in parentheses are the c_1 parameters in ref. 9.

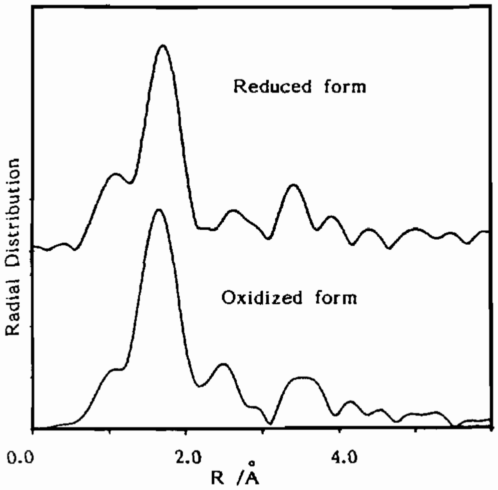

Fig. 2. Fourier transforms of Fig. 1.

maleonitriledithiolate) [11] were prepared according to the cited references and were diluted by 20% with boron nitride. Their EXAFS spectra were measured to obtain the parameters needed for a curve-fitting analysis.

Two-shell fits with fixed values for coordination numbers of 2 Cu–N and 1 Cu–S were carried out for both forms of NiR (Table I). The Cu–N and Cu–S bond distances for the oxidized form are 1.99 Å and 2.11 Å, respectively, which are roughly consistent with the previous results [3] when the experimental errors, the different curve-fitting[§] and the different sample forms are considered. The Cu–N and Cu–S for the reduced form are found to be 2.05 and 2.21 Å, respectively. In an one-electron reduction, the copper–ligand distances are elongated, but the differences in the atomic distances are smaller than that of copper ionic radii (Cu^{2+}; 0.72 and Cu^{+}; 0.95 Å) [12]. Solomon and Gray [4] reviewed the structural change of type I copper protein in an oxidation-reduction in which the Cu–S distances of the reduced form are 2.22 Å and 2.25 Å for plastocyanin and stellacyanin. Murata *et al.* [13] reported the elongation of 0.1 and 0.2 Å for two Cu–imidazoles and that of 0.1 Å for Cu–S distances in the one electron–reduction of plastocyanin by X-ray crystal analysis. Our present result also shows the elongation of 0.1 Å for the Cu–S distance. It is important, however, to point out that the elongation of Cu–S is larger than that of Cu–N in NiR. The larger elongation of the Cu–S distance than the Cu–N on the reduction may be an important clue in understanding the function and structure of NiR.

Acknowledgements

We thank the staff of the Photon Factory at KEK for providing beam time and for kindly giving us useful advise and assistance. We also thank Mr. Tetsuya MARUO for help in XAS data collection. This research was partially supported by Ishida Fundation 59-289 and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture (M.S.).

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[§] The previous curve-fitting [3] was made with a variable parameter for the Cu–S coordination number.