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EXAFS investigation on nickel substituted Cu,Zn bovine superoxide dismutase

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Abstract

The analysis of the EXAFS spectrum of nickel substituted Cu,Zn superoxide dismutase excludes tetrahedral coordination for the nickel ion. The results are better in agreement with a five-coordinated stereochemistry which is probably obtained through a bidentate coordination of Asp 81 Our findings are in complete agreement with the spectral data reported in the literature.

Key words· EXAFS; Copper; Nickel; Superoxide dismutase

Introduction

Metal substitution in Cu,Zn superoxide dismutase (SOD) has been widely used for spectroscopic investigation of the structure of the active site [1, 2]. Co^{2+} or Ni^{2+} substitution at the zinc site has proved particularly suitable for NMR investigations [3, 4]. The activity of the cobalt substituted SOD is about 90% of the native, whereas the nickel substituted enzyme shows 30% activity [2].

Native SOD is a dimeric enzyme, with a copper and a zinc ion in each of the two active sites [5]. The zinc ion is tetrahedrally coordinated by three nitrogen atoms of His 61, His 69 and His 78 and by one oxygen atom of Asp 81 acting as monodentate ligand. However, the second oxygen atom of Asp 81 is only at 3.2 Å from the metal [5]. In fact the electronic spectra of the Ni-SOD derivative have been interpreted in terms of a five-coordinated stereochemistry for the nickel atom, Asp 81 probably acting as a bidentate ligand [4].

Nickel substitution at a zinc site has also been performed for carboxypeptidase A with retention of complete enzymatic activity. Spectral, magnetic and X-ray data have shown also in this case slight changes in the stereochemistry of the metal atom towards a five-coordinate square pyramidal geometry [6–8].

Recent X-ray crystallographic results on yeast and bovine cobalt substituted SOD have shown that the structure of the active site appears essentially preserved [9, 10]. However, no X-ray structural information is presently available for nickel substituted SOD. Therefore we have undertaken an EXAFS investigation on bovine Cu,Ni SOD at the nickel absorption edge in order to elucidate the structural changes at the active site due to the replacement of zinc with nickel.

Experimental

Bovine Cu_2Zn_2 SOD was purchased from Sigma Chemical Company and used without further purification. The Ni(II) substituted derivative Cu_2Ni_2 SOD was prepared in 50 mM phosphate buffer at pH 6.5 as previously reported [3]. Bis(*N*-isopropylsalicylaldiminato)nickel(II) (Niprop hereafter) was prepared according to described procedures [11].

X-ray absorption measurements on the protein sample and on the standard were performed on the PULS X-ray beam line at the ADONE storage ring in Frascati (Rome). During data collection the synchrotron was operating at 1.5 GeV with ring current between 60 and 25 mA. The protein spectra have been recorded between 8180 and 8990 eV in steps of 1 eV in the edge region and 2 eV in the EXAFS region. The spectra had to

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be cut at 8990 eV because of the appearance of the Cu edge. The X-ray absorption of Cu_2Ni_2SOD was monitored by fluorescence with a solid state detector equipped with a cobalt foil filter 10 μm thick to reject background photons. The protein solution 5×10^{-3} M in SOD at pH 6.5 was held in a teflon cell covered with Kapton windows. A series of 16 spectra of the protein were run under the same conditions at room temperature and averaged. EXAFS spectra of the model compound have been collected in the absorption mode on finely ground powder. The X-ray absorption data for $Ni(H_2O)_6{}^{2+}$ in solution were kindly provided by Professor G. Pinna (University of Cagliari) [12]. During the experiment the protein sample did not show any sign of damage and its electronic absorption spectrum measured after the exposure to the X-rays was identical to that of the unexposed protein.

The data were processed as already reported [13]. The sixteen scans were averaged and the EXAFS spectrum was extracted by subtracting the atomic background and by normalizing the edge step. The experimental energy threshold was chosen at the inflection point of the edge jump and the energy of the EXAFS data weighted by k^3 (Fig. 1(a)) were Fourier transformed over the range $k = 3.5$–12.5 Å^{-1} to obtain the radial distribution function. The first coordination shell of the nickel atom was filtered by using a smooth window from 1.1 to 2.2 Å. The back-transformed data were used for a curve-fitting procedure in which the four parameters R (neighbor distance), N (number of neighbors), $\Delta\sigma^2$ (difference in the Debye-Waller factors) and ΔE_0 (change in the threshold energy) were allowed to vary. The first coordination shells of the Niprop and $Ni(H_2O)_6{}^{2+}$ models were used as standards in the curve-fitting procedure. A measure of the quality of our fit is represented by a fit index F^2, which depends on the difference between the EXAFS of the model compound $\chi_m(k)$ and that of the protein, $\chi_u(k)$:

$$F^2 = \frac{\Sigma[k^3\chi_m(k) - k^3\chi_u(k)]^2}{\Sigma[k^3\chi_u(k)]^2}$$

Results and discussion

Figure 1 shows the k^3-weighted EXAFS spectrum of Cu_2Ni_2SOD and its Fourier transform uncorrected for the phase shift. Table 1 and Fig. 2 show the results of the curve-fitting analysis for the first coordination shell of Cu_2Ni_2SOD. In Niprop the nickel atom is four-coordinated by two nitrogen and two oxygen atoms of the ligand at the apexes of a distorted tetrahedron. The average Ni–donor atom distance is 1.90 Å [11]. In $Ni(H_2O)_6{}^{2+}$ the nickel ion is octahedrally coordinated by six water molecules, the average Ni–O distance being 2.06 Å [12].

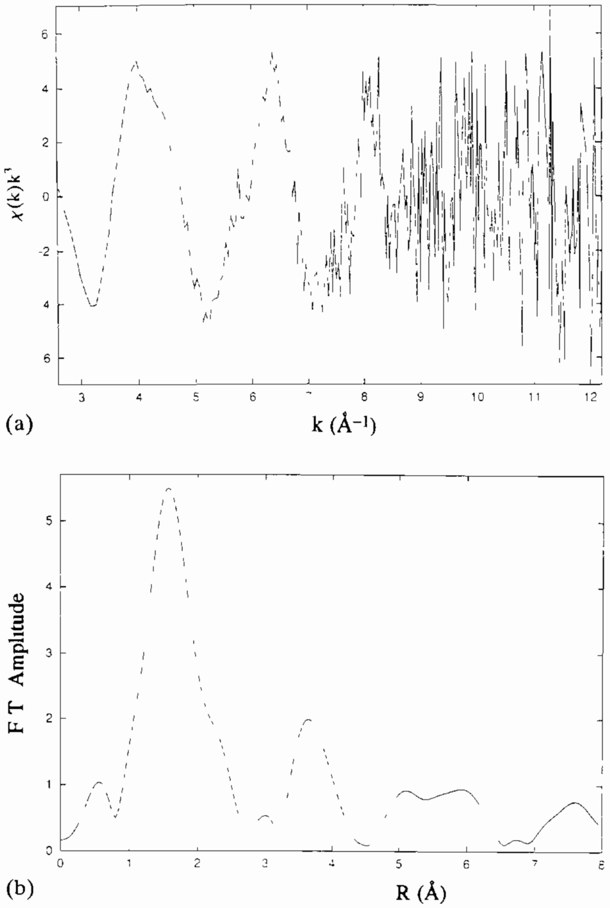

Fig 1 k^3-weighted EXAFS spectrum of Cu_2Ni_2SOD (a) and its Fourier transform uncorrected for the phase shift (b)

TABLE 1 EXAFS results for the first shell of Cu_2Ni_2SOD, nickel edge, from curve fitting, using Niprop and $Ni(H_2O)_6{}^{2+}$ as model compounds[a]

	N	R (Å)	$\Delta\sigma^2$ (Å$^2\times10^3$)	ΔE_0 (eV)	F^2
$Ni(H_2O)_6{}^{2+}$	0.9(2)	2.08(3)	0.0(1)	−0.8(3)	8.5×10^{-2}
Niprop	1.4(2)	2.08(3)	0.0(1)	−0.9(3)	2.3×10^{-2}

[a] N is the ratio between coordination numbers of the model and unknown compounds, R is the bond length from the nickel ion, σ^2 is the Debye–Waller factor, F^2 is a fit index defined as $\Sigma[k^3\chi_m(k) - k^3\chi_u(k)]^2/\Sigma[k^3\chi_u(k)]^2$, χ_m and χ_u being the EXAFS function of model and unknown compounds

The results of the EXAFS analysis performed with the curve-fitting procedure with amplitude and phase functions from the two model compounds are consistent and suggest for Ni^{2+} in Cu_2Ni_2SOD a quite homogeneous distribution of 5.0 ± 1 ligands at 2.08(3) Å (Table 1). This distance falls in the range of distances observed in the crystal structures of nickel complexes with imidazole ligands [14].

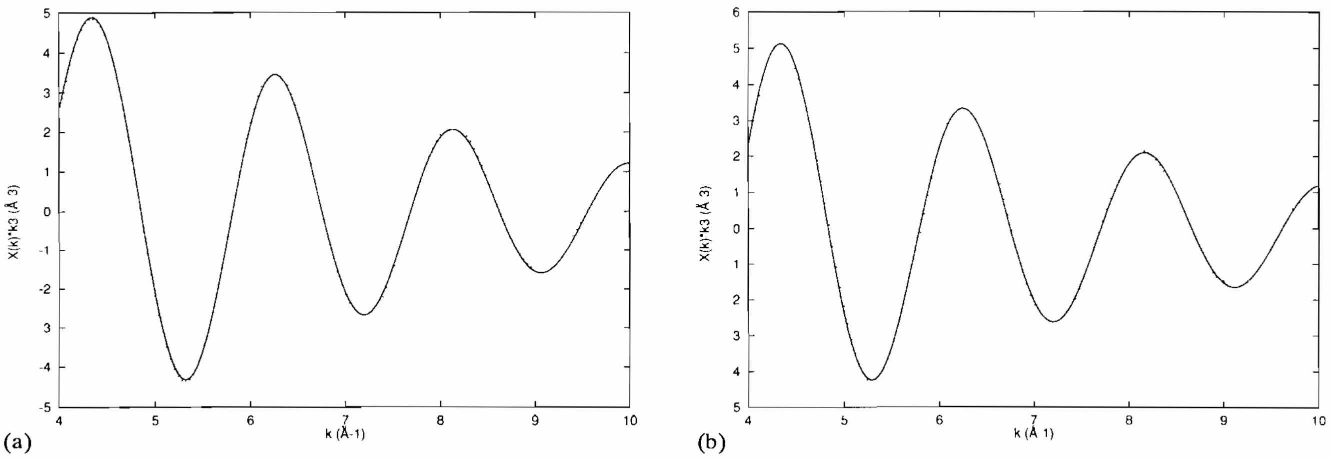

Fig. 2. Best fit of the back-transformed first shell of Cu_2Ni_2SOD with the amplitude and phase of Niprop (a) and $Ni(H_2O)_6^{2+}$ (b). experimental data (——), best fit (· · · ·)

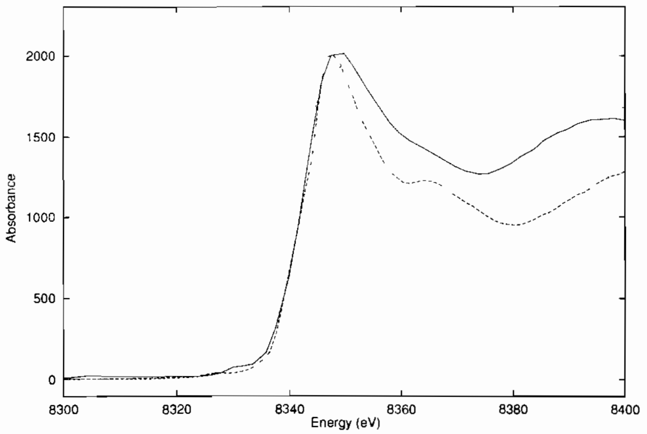

Fig. 3 Pre-edge and edge structure of Cu_2Ni_2SOD, nickel edge (——), Niprop (· ·) and $Ni(H_2O)_6^{2+}$ (– – –).

The analysis of the EXAFS spectrum, especially from these quite noisy and limited data, does not allow the question about the coordination number of the Ni(II) ion to be definitively answered. However, inspection of the pre-edge region shows the presence of a small peak at 8315 eV. This feature is assigned to a 1s–3d transition [15], which is forbidden according to spectral selection rules. Its presence can be interpreted in terms of quadrupole and symmetry breaking effects. Furthermore, its intensity is correlated with the geometry of the absorber coordination sphere. Figure 3 reports the pre-edge region of Cu_2Ni_2SOD compared with that of the pentacoordinated Niprop model compound and with that of the $Ni(H_2O)_6^{2+}$ ion. It can be seen that the intensity of the pre-edge peak is smaller than that of Niprop, strongly supporting the coordination number of five obtained from the EXAFS analysis for the Ni(II) ion in Cu_2Ni_2SOD.

These results are in agreement with a spectral investigation of Bertini *et al.* [8], where the high energy transition and the low molecular absorptivity of the absorption bands in the electronic spectrum of Cu_2Ni_2SOD have been interpreted in terms of a five-coordinated stereochemistry for the Ni^{2+} ion, Asp 81 acting as a bidentate ligand.

Also substitution of the zinc atom by nickel in carboxypeptidase A results in a five-coordinated stereochemistry around the metal atom. In that case it is Glu-72 which acts as bidentate. Although Oε2 of Glu-

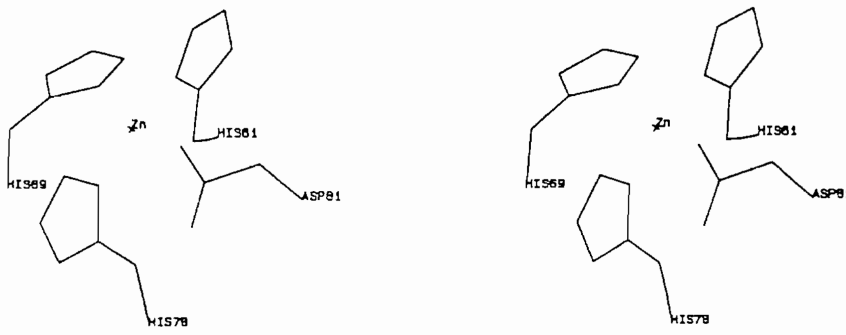

Fig. 4. Stereoview of the zinc coordination in native bovine Cu_2Zn_2SOD [5]. Coordinates are taken from entry 2SOD of the Protein Data Bank [16].

72 has moved a little further from the metal ion, the overall distribution of the ligands around nickel has clearly changed to a five-coordinated square pyramidal geometry [7].

Figure 4 shows the coordination of zinc in native SOD. We see that, by a slight shift of Oδ2 the aspartate ion can become bidentate completing a five-coordinate stereochemistry around the zinc atom.

It is well known that the EXAFS spectroscopy cannot distinguish between nitrogen and oxygen ligands and between oxygen atoms coming from a water molecule or from a carboxylate group of an aspartate residue [17]. Nevertheless, the most reasonable interpretation of our EXAFS results is that in Cu_2Ni_2SOD the nickel ion is five-coordinated, Asp 81 acting as a bidentate ligand, because the alternative hypothesis of the addition of a solvent molecule to the nickel coordination sphere seems less probable on the basis of spectroscopic data [8] and on the fact that the Zn site in SOD is completely buried in the protein and thus not accessible to the solvent itself [5].

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