REVERSIBLE UNCOUPLING OF THE COPPER AND COBALT SPIN SYSTEMS IN COBALT BOVINE SUPEROXIDE DISMUTASE AT LOW pH

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1. Introduction

A great deal of spectroscopic evidence, obtained with the Co(II) derivative of bovine superoxide dismutase [1,2], has indicated that the copper and the zinc sites are very close to each other. In particular, EPR studies on the Co(II)-Cu(II) protein have shown that the copper and cobalt spin systems are coupled antiferromagnetically at the temperature used to observe the EPR, and that the magnitude of the coupling is such as the two metal sites have a common ligand, probably an imidazole nucleus [3]. Recent X-ray work [4] has confirmed these suggestions, indicating the imidazole nucleus of His 61 as the bridging group. The present report will show that acid titration of Co(II)-Cu(II) bovine superoxide dismutase modifies the optical spectrum of the cobalt much more than that of the copper and brings about a reversible breaking of the magnetic coupling between the two metal sites. The results are interpreted in terms of protonation of the bridging imidazolate on the cobalt-facing nitrogen.

2. Materials and methods

Cobalt was substituted for zinc in bovine Superoxide dismutase, prepared with the procedure of McCord and Fridovich [5], as previously described [1]. Optical and EPR spectra and metal determinations were carried out as usually in this laboratory [1]. The protein was brought to different pH values below neutrality by addition of small amounts of concentrated HCl. The pH of the solutions was measured by a Radiometer PHM4c pH meter. Optical and EPR spectra were recorded only after a stable pH was achieved after each addition of acid (several minutes at room temperature).

3. Results

Fig.1 shows the optical spectra of the Co(II)-Cu (II) superoxide dismutase at various pH values between pH 5.6 and 3.0. As the d-d bands of the cobalt decrease with decreasing pH, the copper main absorption band around 700 nm is practically unchanged. The changes below 500 nm, that is the decrease of absorbancy around 450 nm and the modifications between 300 and 400 nm leading to a sharper shoulder at approx. 330 nm, are due to the copper chromophore, as evident by the control spectrum of the Zn-Cu protein at pH 3 (bottom curve). These minor optical changes of the copper chromophore are the optical counterpart of the axialization of the EPR spectrum in the same pH region which has already been described [6]. Raising the pH back to neutrality reversed the decrease of the cobalt absorption and the modifications of the copper spectrum.

Fig.2 shows the corresponding EPR spectra of the copper for the experiment described in fig.1. As the cobalt substitution is never 100%, the EPR spectrum of the protein near neutral pH (curve a) always shows the copper which is present in excess with respect to the cobalt. As pH is decreased (curves b–e) the typical rhombic shape of the copper EPR signal is evidently modified toward a more axial line shape, as already

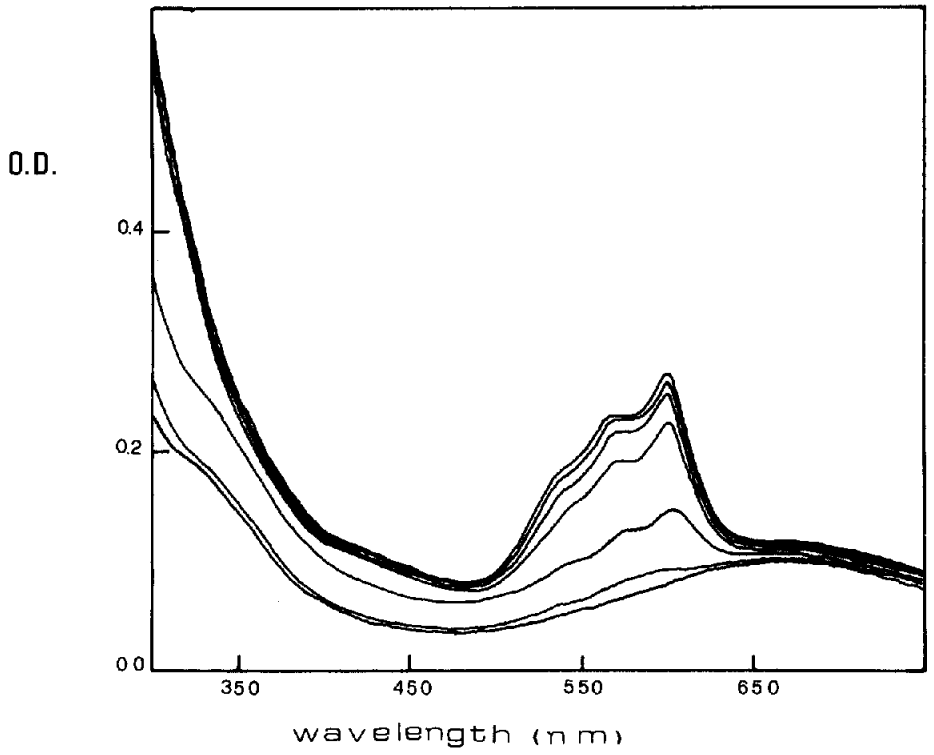
O.D.
0.4
0.2
0.0
350
450
550
650
wavelength (nm)

Fig.1

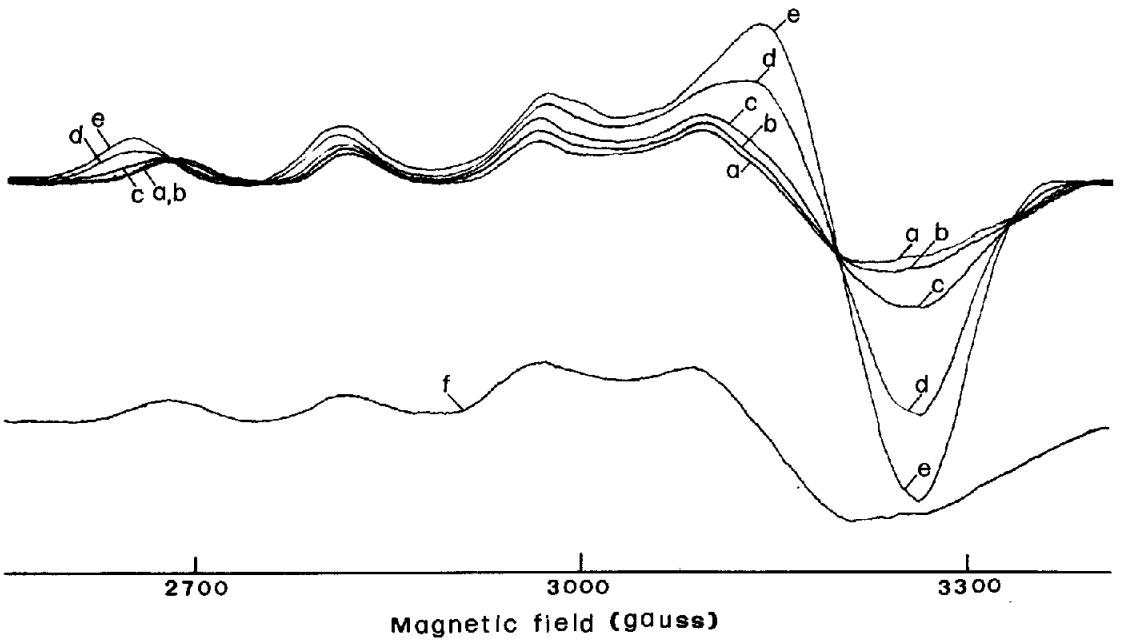
e
d
c
b
a
a b
c
d
e
f
2700
3000
3300
Magnetic field (gauss)

Fig.2

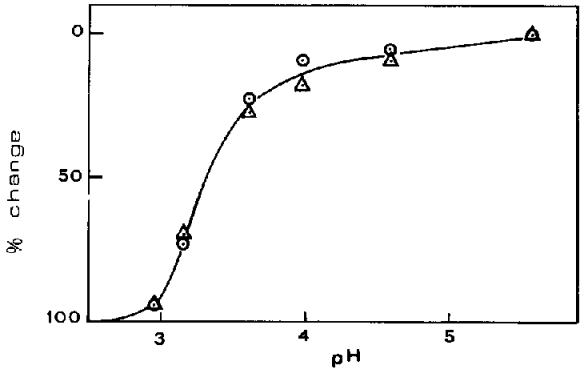

Fig.3. Percent changes of absorbancy of the cobalt cromophore and of the EPR-detectable copper in Co(II)-Cu(II) superoxide dismutase of acid pH values. Data from figs. 1 and 2. ●: optical changes; △: EPR changes. The line joining the experimental points is not derived from any theory.

reported for the zinc-copper protein [6]. Moreover, evaluation of signal intensities by double integration of the spectra showed a gradual increase of the EPR detectable copper with pH decreasing below pH 5. The original line shape and the initial amount of EPR-detectable copper were recovered after the solution was brought back to neutral pH and incubated for 10 min at room temperature (fig.2, bottom curve).

Fig.3 reports the phenomena described in the figs.1 and 2 in the form of a titration plot. Both types of change overlap satisfactorily and show the same apparent pK of pH 3.3.

4. Discussion

The results reported above indicate that uncoupling of the cobalt and copper spin system is brought about by a protonation in the coordination sphere of the two metals. Since they are bridged through an imidazolate group, it is reasonable to identify this group as that involved in the protonation equilibrium. It is likely that the zinc-facing nitrogen is undergoing the titration for the following reasons: a) the imidazole–copper bond is expected to be more stable than the imidazole–zinc or imidazole–cobalt ones; b) the copper optical spectrum is only slightly affected, while that of the cobalt disappears on titration.

These results also indicate that the copper–cobalt imidazolate bridge is very important in determining the physico-chemical properties of the two metal sites. As to the cobalt, the release of the bridging ligand dramatically changes its symmetry, as indicated by the disappearence of the optical spectrum due to the tetrahedral configuration of the site [7]. The changes at the copper site are obviously less striking, as it maintains the original ligands. However, its release from the contact to the zinc site produces also a loss of its typical native configuration, which is reflected by the axialization of the EPR spectrum and the optical changes below 500 nm. All these changes are reversible and therefore can not be due to protein denaturation at acid pH.

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Fig.1. Optical spectra of Co(II)-Cu(II) and Zn(II)-Cu(II) superoxide dismutase at acid pH values. The protein was 0.35 mM, with a cobalt content of 1.2 Co(II) (protein - Curves from top to bottom) are the Co(II)-Cu(II) protein at pH 5.56, 4.60, 4.01, 3.62, 3.15, 2.99, and the Zn(II)-Cu(II) protein at pH 2.96, respectively.

Fig.2. EPR spectra of Co(II)-Cu(II) superoxide dismutase at acid pH values. Spectra a–e correspond to the solutions at pH 5.56, 4.60, 3.62, 3.15 and 2.99 of fig.1. The bottom curve(f) is the sample at pH 2.99 brought to pH 6 by addition of NaOH and incubated 10 min at room temperature before freezing for EPR. Microwave frequency: 9.15 GHz. Microwave power: 10 mW. Modulation amplitude: 10 G. Temperature: −170°C.