Poster Session: Metalloproteins

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Environment of Mn(III) and its Function in a Red-Violet Colored Acid Phosphatase

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Purple colored metal-containing acid phosphatases have been purified from beef spleen, uterine fluid, sweet potato, soybean, and spinach leaves [1–3]. These acid phosphatases are divided into subgroups of Mn-containing acid phosphatases from some plants and Fe-containing acid phosphatases from mammalian organs. We have isolated and crystallized novel Mn-containing acid phosphatase from *Kintoki* sweet potato tubers [4]. Atomic absorption analyses indicated the presence of one Mn ion per one mol of enzyme molecule (MW 110,000). This red-violet Mn-containing acid phosphatase gave an intense absorption maximum at 515 nm ($\epsilon = 2460$) and a CD extreme at 555 nm ($\Delta\epsilon = -0.53$), respectively. The ratio of $|\Delta\epsilon/\epsilon|$ (Kuhn's anisotropic factor) was 2×10^{-4} for the characteristic visible band. The value is typical of an electrically allowed charge-transfer transition. No ESR signals were detected in the native enzyme at 293 and 77 K. In contrast, the acid- and heat-treated colorless enzyme showed characteristic six-line ESR patterns, around g = 2, based on the aquated Mn(II) ion (I = 5/2). The result is reasonable if it is assumed that the violet chromophore represents as ESR-silent form of the metal, Mn(III). In addition this enzyme showed the absorption band at 1160 nm, which can be assigned to a transition $^5A-^5B$ of high-spin Mn(III). The intense 515 nm band was also assigned to an electrically allowed charge-transfer band from the ligand to the metal, which was expected in Mn(III)(d^4) rather than Mn(II)(d^5). The resonance Raman spectrum of the native enzyme, excited by the 5145 Å line of argon ion laser, exhibited prominent Raman lines at 1230, 1298, 1508, and 1620 cm^{-1}. Similar resonance Raman lines have been reported in Mn(III)-ovotransferrin (1173, 1264, 1501, and 1603 cm^{-1}), Fe(III)-transferrin (1174, 1264, 1508, and 1613 cm^{-1}) and uteroferrin (1177, 1265, 1505, and 1607 cm^{-1}). In these Mn(III)- and Fe(III)-proteins the four characteristic Raman lines have been assigned to the vibration of the coordinated phenolate anion. Accordingly, the resonance Raman spectrum of the Mn(III)-containing acid phosphatase is interpreted in terms of the internal vibrations of a coordinated phenolate anion [5]. In the shorter wave number region (<1000 cm^{-1}), the resonance Raman spectra of the native enzyme were obscured by fluorescent background, which is due to the tryptophan residues. Tryptophan modification of the enzyme by N-bromosuccinimide showed a marked decrease of fluorescence and the N-bromosuccinimide-treated enzyme exhibited a positive resonance Raman band at 370 cm^{-1}. Such a Mn(III)-S stretching mode at approximately 370 cm^{-1} was reported in the infrared spectrum of the tris(N,N-diethyl dithiocarbamato) Mn(III) complex. Symmetric stretching vibrations of sulfhydryl sulfur to Fe(III) bonds have been assigned at 315–365 cm^{-1} in iron sulfur proteins and synthetic iron–sulfur clusters. Therefore, the resonance Raman line at 370 cm^{-1} is assigned to Mn(III)–S(cysteine) stretching mode [6].

In the Mn(III)-containing acid phosphatase the enzyme activity was reduced in parallel with a decrease in the 515 nm absorption attributed to the Mn ion directly coordinated with some amino acid residues. Mn(III)-chelating reagents strongly inhibited the phosphatase activity and decreased PRR (proton relaxation rate of water) enhancement of enzyme solution. ^{31}P NMR spectra of the enzyme in the presence of phosphate as substrate showed the pronounced broadening (full width at half-height: 37.8 ppm) ^{31}P phosphate resonance line. The broadening effect corresponded well to ^{31}P NMR signal of model pyrophosphate–Mn(III) complex (full width at half-height: 36.2 ppm). Addition of phosphate to this enzyme solution decreased PRR enhancement by 25%. These evidences indicated strongly that Mn(III) is located on the active center in the enzyme, binds to phosphate esters, and plays an essential role in the catalytic reaction of hydrolysis of phosphomonoesters.

In conclusion, (1) only single Mn ion per one enzyme molecule is contained in the enzyme, (2) the trivalent Mn is coordinated by tyrosine and cysteine residues of the protein molecule, and (3) phosphate binds to the Mn(III) active center of this enzyme. Acid phosphatase uses Mn(III) ion to induce effective binding of phosphate substrate in an acidic environment. In our latest experimental

results we note the similarities and differences between plant's Mn(III)- and mammalian Fe(III)-containing acid phosphatases.

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Temperature Dependence in the MCD Spectrum of Horseradish Peroxidase Compound I

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Ater considerable experimental study, the analysis of ENDOR [1, 2], and magnetic circular dichroism (MCD) spectra of horseradish peroxidase (HRP) compound I [3], as well as the MCD spectra of a series of porphyrin π-cation radical species [4] has led to the confirmation that the electronic configuration of the heme in the compound I species was that of an Fe(IV) porphyrin π-cation complex. While the MCD data of the enzyme were measured at 273 K, and these spectra can be obtained at low temperatures, the ENDOR [1] and EPR [5] signals can only be detected below 30 K. The MCD experiment is very sensitive to ground state degeneracy and in this paper spectra of HRP compound I are discussed that were obtained between liquid helium temperatures and 100 K.

Figure 1 shows how the reduction in temperature has a far more pronounced effect on the MCD spectrum of HRP compound I than on the comparable absorption spectrum. While there are only slight changes in the MCD spectrum between 273 K and

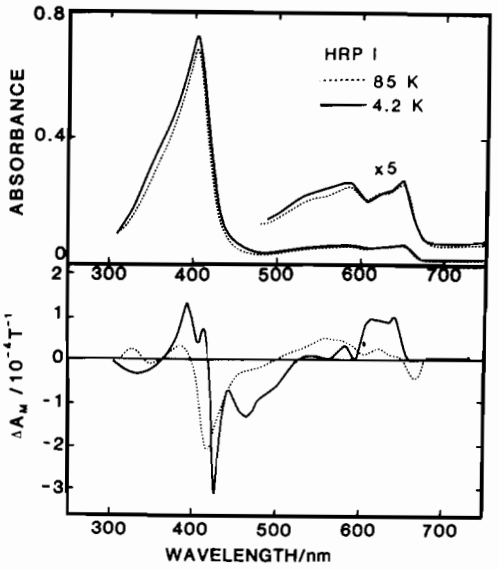

Fig. 1. The optical absorption (upper) and the MCD (lower) spectra of HRP compound I at 4.2 K and 85 K. The spectra were obtained in a 1/1 v/v glycerol/water solution. The HRP concentration was 7.3×10^{-5} mol l^{-1} and 4.4 mol l^{-1} in the absorption and MCD experiments, respectively. The sample path length was 0.11 cm, and the magnetic field used was 4.58 T.

30 K, there are very dramatic changes between 30 K and 4 K. Below 30 K, there is a loss in intensity at 660 nm with a corresponding increase in the 640 nm band and an increase in the dominance of the 300 nm to 500 nm region by the 420 nm and 460 nm bands. The temperature dependent band at 420 nm appears to be derived from a small impurity of the photochemical product of HRP compound I [6] which is formed during preparation of the sample and as a consequence of the visible and UV light used to measure the MCD spectra.

The most striking feature of the spectrum below 30 K is the apparent relationship between the 640 nm and 660 nm bands, and the simple intensity increase with inverse temperature that is observed in the 460 nm and 640 nm bands. The latter effect is characteristic of a **C** term and thus indicates the presence of an orbitally degenerate ground state. Coupling between the S = 1 iron and the S = ½ porphyrin to form the degenerate ground state as a set of three Kramers doublets has been used to explain the observations of the temperature dependence of the EPR spectrum [5]; this hypothesis can also be used to describe the appearance of the MCD C term [7]. The lack of temperature dependence above 30 K suggests that the iron and the porphyrin radical are not now strongly coupled together. These data also suggest that a structural change has occurred at the low temperatures which results in the tempera-

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