Studies of heme-proteins by high-field multi-frequency ESR with high sensitivity

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To elucidate the electronic structures of transition metal ions in a transition metal complex is of great importance in chemistry and biology. Electron spin resonance (ESR) spectroscopy has long been used extensively to investigate structural and electronic environments of the paramagnetic metal centers. For many years, the conventional low frequencies (< 35 GHz) ESR apparatus have been used for metal complexes with odd number of unpaired electrons, i.e. half-integer spin systems (Kramers system), whereas paramagnetic transition metal complexes with an integer spin (non-Kramers system) were hardly investigated by this method. Thus, the paramagnetic ions with an integer spin and a large zero-field splitting (ZFS) have been regarded as ESR-inactive or ESR-silent species under conventional ESR techniques. The necessity for observing the ESR spectra in these paramagnetic species is to extend the frequency and magnetic field range of ESR. In recent years, high-field multi-frequency ESR (HFMF ESR) apparatus have been developed at some places including KYOKUGEN in Osaka University to investigate a variety of spin systems in the transition metal complexes [1].

Iron ions are ubiquitous in biological systems, namely metalloproteins. Among iron containing metalloproteins, we have a particular class called hemoproteins, which have iron-porphyrin, i.e. heme, as the prosthetic group in the active center. The electronic states of the ferric (Fe^{3+}) hemoproteins with a half-integer spin have been investigated in detail by conventional ESR techniques. Several years ago, the ferric high-spin myoglobin (Mb) was measured with our HFMF ESR apparatus and the ZFS parameters were determined precisely [2]. On the other hand, the electronic structures of the ferrous (Fe^{2+}) high-spin hemoproteins with an integer spin have not been definitely analyzed. In spite of many efforts to detect the ESR absorption of deoxy Mb and deoxy hemoglobin (Hb) by means of HFMF ESR techniques, no meaningful results have yet been reported. It should be noted that the paramagnetic ions are buried in the biological macromolecule and thus the concentration of the paramagnetic ions is very dilute and hard to detect the ESR signals as compared with the condensed chemical substances.

The main aim of our study is to detect the ESR signals of hemeproteins with an integer spin such as the deoxy Mb and the deoxy Hb, and to analyze their electronic structures that

correlate with their biological functions, but it must be meaningful to establish analytical methods for an S = 2 integer spin system by using Mn(III)-containing protein molecules, namely, Mn(III)-protoporphyrin reconstituted Mb, Mn(III)Mb. The aquomet Mb used in this study was extracted from native sperm whale muscle and the Mn(III)Mb was prepared by reconstitution of apo Mb with the manganese (III) protoporphyrin IX. For HFMF ESR measurements, we used a frozen solution of the Mn(III)Mb in potassium phosphate at pH 7.0. The HFMF ESR measurements were performed with a millimeter wave vector network analyzer and a 16 T superconducting magnet. In order to obtain a high sensitive ESR spectra from biological substances with dilute paramagnetic ions, we have developed home made TE_{011} single-mode cylindrical cavities for 33, 50, 70, 90, and 130 GHz with high stable matching mechanism and the sensitivity of 10^{10} spins/G at T = 1.5 K [3].

Figure 1 shows the HFMF ESR spectra of the Mn(III)Mb in a frozen solution at 10 K for several frequencies between 33.3 GHz and 122.1 GHz and the magnetic fields up to 14 T [3]. To analyze the EPR spectra, we adopted the standard spin Hamiltonian for S = 2 spin state comprised of the Zeeman and the ZFS terms given by,

$$\boldsymbol{H} = \mu_B \boldsymbol{B} \cdot \tilde{\boldsymbol{g}} \cdot \boldsymbol{S} + D\,(S_z^2 - 2) + E\,(S_x^2 - S_y^2) \qquad (1),$$

where μ_B is the electron Bohr magneton, $\boldsymbol{B}$ the external magnetic field, $\tilde{\boldsymbol{g}}$ the g tensor of Mn^{3+} spins, $\boldsymbol{S}$ the S = 2 spin operator, D and E the axial and rhombic ZFS parameters, respectively. The observed MFEPR spectra (blue lines) were analyzed with the spectral simulation software, and the red lines in Fig. 1 are the simulation results. The agreement between the experimental and the simulated spectra is satisfactory. Assuming isotropic g-values, $g_x = g_y = g_z = 2.00$, the ZFS parameters were determined by this comparison to be $D = -3.79 \pm 0.03$ cm^{-1} and $E = 0.08 \pm 0.01$ cm^{-1}. In the presentation, these ZFS parameters will be compared with those in Mn(III) porphyrin complexes and other Mn(III) complexes.

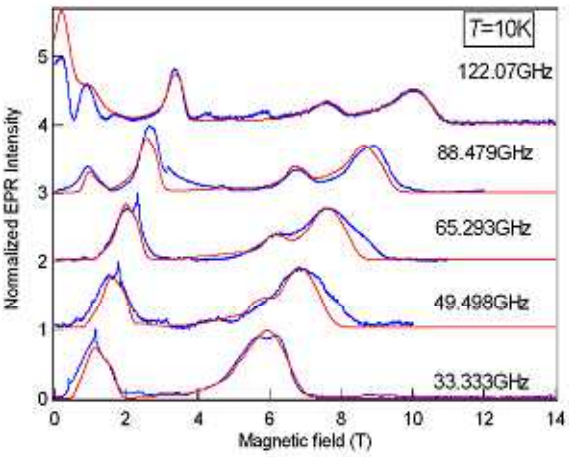

Fig. 1. HFMF ESR spectra of the Mn(III)Mb

Finally, if we have the time, we will briefly talk about ESR studies on the deoxy Hb (Fe^{2+}: S = 2), which is now in progress.

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