Development of a multi-frequency ESR system with high sensitivity

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Abstract. We have developed a new Multi-Frequency (MF) ESR system for the frequencies between 35 to 130 GHz utilizing TE₀₁₁ single mode resonators. Their sensitivities (10¹⁰ spins/G at 1.5 K) are comparable to that of a conventional low frequency ESR resonator and an order of magnitude higher than that of a Fabry Perot resonator which was previously developed by us. Thanks to a newly developed precise and stable matching system, we observed for the first time MFESR spectra of a metalloprotein with an integer spin.

1. Introduction
Multi-Frequency Electron Spin (or Paramagnetic) Resonance (MFESR, MFEPFR) is a very powerful means to give us microscopic information, such as electronic states of magnetic materials. It has been widely and successfully used in condensed matter physics [1] but not much in chemistry and biochemistry, because high sensitivity in wide frequencies is required due to very few magnetic ions in the materials studied in their fields. We recently succeeded in observing and investigating MFEPFR spectra of a metalloprotein with a half-integer spin in the wide frequency range from 30 to 600 GHz and magnetic fields up to 14 T [2]. We, however, found that our system does not have enough sensitivity to research other metalloproteins with integer spins. Especially, in biochemistry, there has been no report on MFEPFR of metalloproteins with an integer spin as far as we know [3]. In order to develop a MFESR system with high sensitivity and wide frequency range (35-600 GHz) simultaneously, we selected two types of resonators depending on the frequency range. One of them is a frequency tunable Fabry-Perot Resonator (FPR) for the use of higher frequencies (50-600 GHz). The others are ordinary frequency fixed TE₀₁₁ cylindrical cavities for 35, 50, 70, 95 and 130 GHz, since they have theoretically an order of magnitude higher sensitivity than the FPR. In this paper, we report a MFESR system utilizing TE₀₁₁ cavities developed for the studies of such samples with dilute magnetic ions. We could achieve the sensitivity of 10¹⁰ spins/G at 1.5 K with highly stable matching system. These are an order of magnitude higher than that of a Fabry Perot resonator which was previously developed by us. By their higher sensitivity and stability, we successfully observed MFESR spectra of a metalloprotein with an integer spin system for the first time.
2. Results and Discussion

In Fig. 1, the block diagram of our MFESR spectrometer is presented. Our spectrometer design is based on a simple reflection method, since they have a potential to give a better dynamic range for small signals than a transmission type [4, 5]. It consists of a millimeter vector network analyzer MVNA (ABmm, France), a lock-in amplifier, a 16 T superconducting magnet (Oxford Instruments, UK) and cryostats including partially oversized wave guide and TE\textsubscript{011} single mode cavities. The MVNA was used as a millimeter source for excitation of the cavities (samples) and for detection of the reflected signals as well. The lock-in amplifier works as a part of the phase lock system to the TE\textsubscript{011} cavities in order to measure the pure imaginary part of high frequency magnetic susceptibility, namely ESR absorption ($\chi''$). Other details are written in the figure caption.

As an example of TE\textsubscript{011} cavities, some tuning curves of the amplitude and the phases of a 130 GHz cavity are shown in Fig. 2A and 2B, respectively. The matching between the fundamental-mode rectangular waveguide and the cavity is varied by changing the angle $\theta$ between the longitudinal axis of the wave guide and that of the TE\textsubscript{011} cavity [6, 7, 8]. This mechanism provides very stable matching under a large magnetic field change (e.g. 0-16 T) as well as a very precise matching control to produce the best performance from lossy to non-lossy samples due to widely variable matching from under to over coupling (see Fig. 2B).

We have estimated the performance of our MFESR system by measuring intrinsic quality factors ($Q_0$s) and absolute sensitivities which are listed in Table 1. The ratio of the $Q_0$ values with cryostats to the theoretical limits are in the range from 0.4 to 0.8. Then, absolute sensitivities estimated by using an ESR standard DPPH powder sample are in the range of 10\textsuperscript{10} spins/G at 1.5 K. At a glance, these values are much less sensitive than that (10\textsuperscript{7} spins/G) reported by Blok et al. [8] which is the highest sensitivity reported so far. Our system, however, keeps the same sensitivity for any broad signals, since our system measures the reflected MMW amplitude directly rather than the field modulated amplitude, whose sensitivity is inversely proportional to the linewidth, used by Blok and in many other high sensitive ESR systems. Therefore, if our MFESR system has very stable matching (no baseline fluctuation), our system may have a better sensitivity for much broader signals (>1 T) than the maximum field modulation amplitude which is practically less than 3 mT.
Figure 2. Tuning curves (A) and phases (B) of a 130 GHz TE$_{011}$ cavity versus the rotation angle $\theta$ between the longitudinal axis of the wave guide and that of the TE$_{011}$ cavity. Parallel horizontal gray lines in the right panel indicate the phase shift corresponding to the critical matching, that is $\pm 90$ degrees.

Table 1. Estimated sensitivities of our MFESR system utilizing TE$_{011}$ cavities by a DPPH powder sample. (a): Ratio to the theoretical limit, (b): At 260 K, (c): At 1.5 K.

| Frequency (GHz) | $Q_0$ at 300 K (ratio)$^a$ | Sensitivity (Spins/G) |
|----------------|-----------------------------|----------------------|
| 35             | 12250 (0.76)                | $4.9 \times 10^{10}$$^b$ |
| 50             | 10400 (0.78)                | $4.5 \times 10^{10}$$^c$ |
| 70             | 8850 (0.81)                 | $2.2 \times 10^{10}$$^c$ |
| 95             | 4020 (0.41)                 | $3.6 \times 10^{10}$$^c$ |
| 130            | 4900 (0.59)                 | $5.8 \times 10^{10}$$^c$ |

Figure 3 shows an example of MFESR spectra of a metalloprotein with an integer spin which is the powder of the sperm whale metmyoglobin (MnMb) substituted Fe$^{3+}$ by Mn$^{3+}$ ($S=2$). The concentration of MnMb was ca.$10^{-2}$ mol/dm$^3$. To the best of our knowledge, this is the first MFESR spectra of a metalloprotein with an integer spin. The detected signals are directly proportional to $\chi''$, since our system does not use the field modulation technique. In addition, signals contain only pure $\chi''$ because the MMW is phase locked to the cavities to observe only the changes the MMW reflections (i.e. magnetic loss of the sample) in stead of the resonance frequency shift of the cavities. By virtue of these features and very stable matching system, even such a broad line width (e.g. at 130 GHz, 6-11 T) can be clearly observed. The concentration sensitivity, which means the minimum concentration to observe ESR signals with the signal to noise ratio (SNR) larger than 1, is the order of $10^{-4}$ mol/dm$^3$ from these spectra (the sample concentration simply devided by the SNR). This corresponds to nearly the highest concentration sensitivity for metalloproteins measured with the ESR appratus including commercially available low frequency ESR machines which have usually better concentration sensitivity than many high frequency ESR systems [9].

The spin Hamiltonian of Mn$^{3+}$ ($S=2$) in MnMb is written as follows;

$$\mathcal{H} = \mu_B H \mathbf{g} S + D[S_z^2 - S(S + 1)/3] + E(S_x^2 - S_y^2)$$  \hspace{1cm} (1)

where $\mu_B$ the Bohr magneton, $H$ the external magnetic field, $\mathbf{g}$ the $g$-tensor of Mn$^{3+}$ ions, $D$ and
Figure 3. MFEPR spectra of the sperm whale metmyoglobin substituted Fe$^{3+}$ by Mn$^{3+}$ ($S=2$). The concentration of MnMb was ca. $10^{-2}$ mol/dm$^3$. All the spectral data were taken by a single field sweep. The solid and dashed curves are measured and simulated ones, respectively.

$E$ the axial and the rhombic anisotropy constants, respectively. From the frequency dependence as well as the temperature dependence of the ESR spectra, we could determine precisely the spin Hamiltonian parameters, $D$=-3.79 cm$^{-1}$ and $E$=0.077 cm$^{-1}$ with $g_x=g_y=g_z=2.0$. The experimental details which include the results of single crystal experiments and the temperature dependence of the ESR signals will be reported [10].

In conclusion, we have developed a new MFESR (35 to 130 GHz) system with high sensitivity ($10^{10}$ spins/G at 1.5 K). This system is especially powerful to survey the samples which have very broad ESR line width (>1 T), such as biomolecular intermediates as well as metalloproteins with integer spins which have been believed to be spin silent so far. In this work, we observed for the first time ESR spectra from a metalloprotein with an integer spin.

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