Abstract

We report a novel force-detected high-frequency electron spin resonance (HFESR) technique using a microcantilever in the terahertz region. In this technique, we attach a tiny sample on the microcantilever end and the ESR signal is detected as the cantilever bending. The bending is sensitively detected by fiber-optic Fabry-Perot interferometry. We applied this technique to a tiny amount (~16 ng) of metalloporphyrin, a model substance of hemoproteins, and successfully observed ESR signals at multiple frequencies up to 0.5 THz. This result indicates that the sample volume needed in multi-frequency HFESR can be greatly reduced by several orders of magnitude, and our novel technique would be a promising tool for HFESR studies of metalloproteins in the future.

Keywords: Electron spin resonance, Terahertz (THz), Microcantilever, Metalloporphyrin

Introduction

Electron spin resonance (ESR) is a powerful method for understanding the microscopic properties of a sample [1]. Conventional ESR techniques are usually performed in the microwave region (GHz). On the other hand, the high-frequency ESR (HFESR) technique in the terahertz region has several advantages over conventional techniques [2]. For example, high g-value resolution can be obtained. In addition, broad ESR spectra, which we often observe in magnetic samples, can be measured in the extended frequency and magnetic field range. The HFESR technique also enables us to observe an ESR transition across large zero-field splitting beyond 100 GHz. This feature becomes particularly important for metalloproteins such hemoglobin or myoglobin, since they exhibit large zero-field splitting ranging from $D$$\sim$$5$–$10 \text{ cm}^{-1}$ [3–7]. Moreover, multi-frequency ESR measurements provide more detailed information about the electronic structure of a sample from a spectroscopic point of view. HFESR in the wide frequency range is thus very useful for studying samples like those described above [8–10].

However, there have been few reports on metalloprotein samples in the high-frequency range [6, 11, 12]. One of the reasons is that the output power of a terahertz source is less than approximately 10 mW, which is too low for performing transmission-type experiments, whose spin sensitivity is lower than that of a commercial ESR system using a cavity. Thus, in the case of low spin-concentration samples such as protein or diluted solution samples, a sample weight on the order of 10 mg is needed. In this sense, a reduction of the sample volume is strongly desired for the HFESR measurements of proteins, which are often difficult to prepare in large quantities.
To solve this problem, we have developed a force-detected highly sensitive HFESR technique using a microcantilever [13–16]. In this technique, a tiny sample is mounted on the end of a microcantilever and irradiated with electromagnetic waves. When ESR absorption occurs, the corresponding magnetization change is detected as a force change applied on the microcantilever. Using this technique, HFESR of a tiny sample has been demonstrated in the millimeter-wave region.

In this paper, we first extend the frequency region of our force-detected ESR technique to the terahertz range. We then apply our technique to metalloporphyrin, which is a model substance of hemoproteins such as hemoglobin. In the following sections, we will explain the principle of force-detected ESR, the details of our apparatus, and the results of HFESR analysis of the metalloporphyrin sample.

Principle of Force-detected ESR

The Faraday method is used for ESR detection in this study. When a magnetized sample is placed in the magnetic field gradient along the z-axis, a gradient force along the z-axis, \( F_z \), written as

\[
F_z = \frac{\mathrm{d}B_z}{\mathrm{d}z} \cdot M_z,
\]

(1)
is induced where \( \frac{\mathrm{d}B_z}{\mathrm{d}z} \) is the magnetic field gradient along the z-axis and \( M_z \) is the z-component of sample magnetization. This equation shows that the gradient force is proportional to the field gradient and the sample magnetization. Here, we consider a setup where an external static magnetic field is externally applied along the z-axis, while the sample is irradiated with an electromagnetic wave traveling along the z-direction. The z-component of sample magnetization \( M_z \) is given by the following equation:

\[
M_z = M_z^0 \left\{ 1 - \frac{\gamma^2 B_1^2 T_1 T_2}{1 + (\gamma B_z - \omega)^2 T_1 T_2 + \gamma^2 B_1^2 T_1 T_2} \right\},
\]

(2)

where \( B_1 \) is the transverse magnetic field of the electromagnetic wave, \( M_z^0 \) is the z-component of sample magnetization without irradiation, \( \gamma \) is the gyromagnetic ratio, \( T_1 \) is the spin-lattice relaxation time, \( T_2 \) is the spin-spin relaxation time, and \( \omega \) is the frequency of the electromagnetic wave. Therefore, when the ESR condition, \( \gamma \omega = B_z \), is satisfied, \( M_z \) is sharply changed. The difference of magnetization between the on-resonance and off-resonance conditions, \( \Delta M_{z, \text{res}} \), is written as below:

\[
\Delta M_{z, \text{res}} = -M_z^0 \frac{\gamma^2 B_1^2 T_1 T_2}{1 + \gamma^2 B_1^2 T_1 T_2}.
\]

(3)

So, the expected change of gradient force \( \Delta F_{z, \text{res}} \) at the resonance is given by

\[
\Delta F_{z, \text{res}} = \frac{\mathrm{d}B_z}{\mathrm{d}z} \cdot \Delta M_{z, \text{res}}.
\]

(4)

In our setup, the output power of the electromagnetic wave is modulated by a synthesizer to increase the sensitivity. To detect cantilever bending sensitively, we used a fiber-optic interferometry technique. A Fabry–Perot cavity is formed between the cantilever surface and the end of the optical fiber. The interference intensity \( I \) is written as

\[
I = I_0 \left\{ 1 - A \cos \frac{4 \pi d}{\lambda} \right\},
\]

(5)

where \( I_0 \) is the average optical intensity, \( A \) is the fringe visibility, \( \lambda \) is the wavelength, and \( d \) is the cavity length. Thus, the interference intensity \( I \) exhibits sinusoidal dependence on the cavity length \( d \). Around the middle point of the interference intensity, \( I \) is proportional to \( d \), and cantilever displacement is detected most sensitively. This interferometric technique has several advantages, such as its high sensitivity and compact setup, but it also has the drawback of a limited dynamic range originating from the sinusoidal dependence. This property sometimes makes ESR detection difficult, particularly under high-field conditions, since the cavity exhibits several fringes due to the background magnetization change. As a result, the sensitivity of the cavity \( \frac{\mathrm{d}I}{\mathrm{d}d} \) is field-dependent. To avoid this problem, a wavelength tunable laser was used to control the
The low-frequency data (105, 130, 160 GHz) could be fitted by a straight line, as shown by the black dashed line in Figure 1, which indicates that an oscillating magnetic field B\_1 is spatially uniform inside the sample and the ESR signal was detected as a change in the oscillation amplitude of the cantilever driven at the modulation frequency.

**Experimental**

A schematic of our force-detected ESR system is shown in Figure 1. In this setup, a 15-T superconducting magnet is used to produce an external static magnetic field. All experiments were performed at 4.2 K. As an electromagnetic wave source, we used a Gunn oscillator in the frequency range up to 160 GHz and a backward travelling wave oscillator (BWO) beyond 160 GHz. The output power of BWO was generally on the order of 1 – 10 mW depending on the frequency. The electromagnetic wave from the light source was introduced through a polished stainless-steel pipe, whose inner diameter was 12 mm and was irradiated to a sample-holding cantilever through a brass horn. Because the diameter of the light pipe was larger than the wavelength of the electromagnetic wave, multiple frequencies could be transmitted to the sample position with low dissipation. The diameter of the horn end was 4 mm, and the corresponding cut-off frequency was about 80 GHz. A dysprosium rod of 1.7 mm length and 0.5 mm diameter was held at the exit of the horn end by a Macor ceramic piece and used to produce a magnetic field gradient at the sample position. Macor is a kind of ceramics that transmits microwaves or terahertz waves. Figure 2 shows our handmade cantilever holder. A sample-holding cantilever was set on the commercial alignment disk for quick assembly and was fixed by a thin brass clamping plate. An optical fiber was held beneath the cantilever surface to form a Fabry-Perot interferometer. The cavity length was typically kept at about 100 μm. The output power of the electromagnetic wave was modulated by a synthesizer which was also used as a reference for the lock-in amplifier. Then, the ESR signal was detected as a change in the oscillation amplitude of the cantilever driven at the modulation frequency.

We used a commercially available cantilever (PPP-CONTSCR) supplied from Nanosensors. The nominal cantilever dimensions were \( (225 \times 48 \times 1) \) μm\(^3\), and the spring constant and eigenfrequency of the cantilever were \( 0.1 - 1 \) N/m and \( (\sim 23 - 23) \) kHz, respectively.

Figure 3 shows a photo of the cantilever mounted with a hemin-chloride sample from bovine, \( \{\text{C}_{34}\text{H}_{32}\text{ClFeN}_{4}\text{O}_{4}\} \), which was obtained from Sigma-Aldrich and used as is. The molecular chemical structure of hemin is shown in the inset of Figure 3. The molecular mass is 651.94 g/mol, and the magnetic ion \( \{\text{Fe}^{2+}\} \) is located at the center of the porphyrin ring, and ring under the high-spin state (\( S = 5/2 \)). Four nitrogen atoms and a chlorine atom are coordinated within the basal plane and normal to the basal plane, respectively. The porphyrin structure including metal ion in the center is called metalloporphyrin, and the porphyrin structure containing Fe is called heme. Hemoproteins contain heme in their molecular structure as a functionally active center, and hemin is one of the model substances of hemoprotein [19, 20]. Numerous ESR studies of hemin have been reported, and it has been well established that hemin shows ESR spectra similar to those of hemoproteins [21–23]. In this study, microcrystals of hemin were glued together with a small amount of epoxy and mounted on the cantilever. The sample mass was typically about 16 ng, as estimated by the eigenfrequency shift of the cantilever after sample mounting. The sample size was about \( (100 \times 50 \times 10) \) μm\(^3\), which was always shorter than the wavelengths of the electromagnetic wave used in this study. This fact indicates that an oscillating magnetic field \( B_{\perp} \) is spatially uniform inside the sample and the electromagnetic wave couples with each spin moment in the same manner.

**Results and Discussion**

Figure 4 shows the high-frequency ESR data of hemin obtained using a microcantilever. In this figure, we subtracted the background and vertically shifted the ESR spectra for clarity. We were able to observe a resonance signal at each measurement frequency range up to 0.5 THz. These signals were considered to correspond to the case in which a magnetic field was applied perpendicular to the heme normal \( \langle g \rangle_{\perp} \langle \text{eff} \rangle \mathrm{sim} \langle 6 \rangle \), in X-band. With the change in measurement frequency, the peak field was shifted to a higher magnetic field, as expected. Figure 5 shows the relation between the frequency and peak field. The low-frequency data (105, 130, 160 GHz) could be fitted by a straight line, as shown by the black dashed line in Figure 1.
line in the figure. According to the slope of the curve derived from the equation \( \langle h|nu = g^*\text{magneton}\rangle_{\text{magnet}}|\mu \text{u}_\text{magneton}(B)\rangle\times(\langle B|\text{magneton}()\rangle+|\delta B\rangle), \) where \( h \) is the Planck constant, \( |\text{magneton}\rangle \) is the Bohr magneton, \( \mu \text{u}_\text{magneton}(B) \) is the horizontal shift, \( g^*\text{magneton} \) and \( |\delta B\rangle \) is the horizontal shift of \( g^*\text{magneton} \) determined in the microwave range. From the X-band ESR measurement \([21]\), it is well known that the signals \( \langle g_{\perp}\text{magneton}\rangle\times\text{magneton}(eff)|\mu \text{u}_\text{magneton}(B)\rangle\times(\langle B|\text{magneton}()\rangle+|\delta B\rangle) \) are observed when a static magnetic field is applied perpendicular and parallel to the heme normal, respectively. This is because the \( \langle |\text{magneton}\text{Fe}^+|3+\rangle\) ion in hemin \( \langle S=5/2\rangle \) possesses an axial zero-field splitting parameter arising from the axial symmetry of the crystal field, and exhibits prominent anisotropy due to strong hybridization between the iron and porphyrin orbitals. Thus, in the low-frequency limit, the system can be approximated as a Kramers doublet with a different \( g \) value, but we have to take into account the higher spin levels in the high-frequency region, whose energy scale is comparable to the splitting. In particular, for the \( \langle g_{\perp}\text{magneton}\rangle\times\text{magneton}(eff)|\mu \text{u}_\text{magneton}(B)\rangle\times(\langle B|\text{magneton}()\rangle+|\delta B\rangle) \) case, mixing between the energy levels occurs, and a linear relationship is no longer expected in the high-frequency region. By analyzing this behavior, more precise determination of the splitting parameter becomes possible. This is of particular benefit for elucidating the local electronic structure of spin systems, which cannot be investigated by conventional ESR techniques.

Here, we consider the spin-Hamiltonian model, including the axial zero-field splitting parameters in the second order, as follows: \( \langle H=D S_{z}^2+E(S_{x}^2-S_{y}^2) \rangle \), where \( D \) and \( E \) represent the axial and in-plane anisotropy.

The energy diagram of hemin was calculated according to this spin-Hamiltonian model for the cases where the magnetic field was applied perpendicular to the heme normal in Figure 6. It is clearly seen that the level mixing occurs in the high-field region. The \( \langle g_{\perp}\text{magneton}\rangle\times\text{magneton}(eff)|\mu \text{u}_\text{magneton}(B)\rangle\times(\langle B|\text{magneton}()\rangle+|\delta B\rangle) \) signal corresponds to the transition between lowest energy level and second lowest level, as indicated by an arrow.

The data were fitted with this model, and can be reproduced with fitting parameters \( \langle g_{\text{m}}, g_{\text{y}}, g_{\text{z}}\rangle \). The horizontal axis of Figure 5 is plotted as a function of the field strength \( B = B_{\text{ext}} + B_{\text{local}} \) generated by the superconducting magnet, and the local field strength at the sample site differed from this magnet-generated field strength due to the local magnetic field produced by the dysprosium rod \( |B_{\text{magneton}}(local)|\rangle \). Thus, the total magnetic field \( B \) is written as \( B = B_{\text{magneton}}(ext) + B_{\text{magneton}}(local) \). The horizontal shift of the \( \langle H=D S_{z}^2+E(S_{x}^2-S_{y}^2) \rangle \) is equal to \( \langle B_{\text{magneton}}(local)|\rangle \), which we estimated as \( B_{\text{magneton}}(local)|\rangle = 0.48 \text{T} \) in this experiment. In addition, we were able to calculate the rod-sample distance for a cylindrical ferromagnetic piece, and the field gradient value along the \( z \)-axis at the sample position was estimated by \( \langle \text{frac}\text{magneton}(d)B\text{z}^2\rangle\times\text{magneton}(d)\text{z}\rangle \) as 1000 T/m.

As observed in Figure 5, the linewidth was about 100 mT, which was ten times broader than the intrinsic linewidth \( \langle |\text{magneton}|\rangle \). This was due to the field distribution inside the sample arising from the local field of the dysprosium rod. Indeed, the field gradient of 1000 T/m corresponded to a field distribution of \( \langle \text{Delta B} \rangle = 100 \text{mT} \) for typical sample dimensions on the order of 100 \( \text{u}m \). The total number of spins in the hemin sample was about \( 10^{13} \) spins. With a signal-to-noise ratio \( S/N \) of about 100 for 130 GHz, the minimum detectable spin number \( \langle N_{\text{magneton}}(min)\rangle \) was \( 10^{10} \) spins. However, the \( S/N \) value was \( 5 \) for 500 GHz, and \( \langle N_{\text{magneton}}(min)\rangle \) was 20 times worse than that of 130 GHz. This is because the output power of the electromagnetic wave at 500 GHz was weaker by several tens than that at 130 GHz. This sensitivity was not sufficient for practical application to a metalloprotein sample in the terahertz range, and will need to be further improved. For this purpose, fabrication of a customized microcantilever with a softer spring constant would be useful \([25]\).

**Summary**

We have developed an HFESR technique using a microcantilever. We used the Faraday method and detected ESR signals as changes in magnetization. We applied our technique to \( \langle |\text{magneton}|\rangle \) ng of hemin,
which is one of the metalloporphyrin complexes and also one of the model substances of hemoprotein, and we observed \( g_{\perp}^{\text{eff}} \) signals of hemin in the terahertz range up to 0.5 THz and estimated the \( g \) tensor and axial zero-field splitting parameters. Using this HFESR method, we succeeded in reducing the sample volume required for metalloporphyrin measurements by \( 10^5 \) times compared to the volume required for the conventional ESR. Further improvements will be needed to observe the ESR signals from samples with even lower spin concentration, such as hemoprotein. Our technique will be applicable not only as a general ESR technique but also as an alternative magnetic resonance technique for metalloproteins such as ENDOR.

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**Fig. 1** Schematic of our force-detected ESR system.

**Fig. 2** Photo of our handmade cantilever holder. The optical fiber used for detection is seen through the hole under the cantilever.

**Fig. 3** Photo of hemin sample mounted on the cantilever.

**Fig. 4** ESR spectra of hemin in the frequency range up to 0.5 THz. Arrows indicate the \( g_{\perp} \) signal.

**Fig. 5** Frequency-field plot of observed ESR signal of hemin. An arrow indicates the position of the intercept between the fitting curve and the horizontal axis. This intercept corresponds to \( B_{\text{local}} \) generated by a dysprosium rod.

**Fig. 6** Energy diagram of the \( S = \frac{5}{2} \) system for a magnetic field applied within the heme plain. The curves are drawn using the following parameters: \( g_x = g_y = 1.98, g_z = 2.00, D = 7.2 \text{ cm}^{-1}, \text{ and } E = 0.0 \text{ cm}^{-1} \). The observed ESR transitions correspond to the transition between the lowest and second lowest energy, as indicated by an arrow.