Electron Spin Resonance of Ferritin

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Abstract

Recently various biological substances have being studied by electron spin resonance. Ferritin is a sort of biological substance and contains large amount of iron compound in its protein shell. In this paper ESR spectra of ferritin at room and liquid air temperatures are reported. According to our experimental results most part of iron ions in ferritin may be in a state $S=3/2$ and a little part of them in a state of $S=5/2$. An ESR spectrum of $\gamma-$FeOOH at room temperature is also reported and compared with that of ferritin.

1. Introduction

Ferritin is a biological substance containing iron ions as nonhem iron. It exists in a spleen, a liver, intestines and many other organs of an animal. Ferritin of a horse spleen has been most investigted [1-5].

According to an experiment with radioactive iron, it has become clear that ferritin is used as a source of iron for hemoglobin formation and stores the liberated iron by the destruction of hemoglobin in a body of an animal. Ferritin contains as much as 23% of ferric iron and the iron can be removed from ferritin, leaving colourless iron free protein called appoferritin.

Ferritin has a molecular weight of $(9 \sim 10) \times 10^5$ and a diameter of 120Å, while iron free ferritin (appoferritin) has a molecular weight of 48×10^4.

Ferritin is made of a protein shell and an iron oxide core in it (Fig. 1). The shell is made of 20 subunits, molecular weight of a subunit being about 24000, and they are arranged at the vertices of a pentagonal dodecahedron. The composition of the iron core is supposed to be $(FeOOH)_8$ (FeO, OPO_3H_2) and cotains about 5000 iron ions. The diameter of the core is about $50 \sim 70$Å and it is made of several subunits, but the numbers and the shapes are not identical. The investigations are in a step of

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dispute about the shape or number
of micells (subunits) of the core.
No knowledge about the structure
of them has been obtained. It is
said in the reference (1) that
though the core has composition
of FeOOH, a little common featu-
res of α-FeO$_3$, β-FeO$_3$, α-FeOOH,
and β-FeOOH were obtained in
an electron diffraction experiment.

55 Å   120 Å
iron core

Fig. 1   Ferritin molecule

To study the states of iron ions in the core, ESR experiments were attempted.

2. Experimental Procedures

Usually ferritin of horse spleen is used as a sample for it offers larger amount of
ferritin than other animals because of high concentration of ferritin in it. So we used
also the horse spleen ferritin. It may be prepared as follows [2]. Mashed spleen is
extracted with same amount of water a day, filtered, the filtrate is heated to 80°C
(not higher) and then filtered. To 100cc of the red-brownish filtrate is added 30g of
ammonium sulfate, the precipitate is collected by a centrifuge and dissolved in as
small an amount of water as possible. By adding cadmium sulfate to the solution so
that the final concentration of cadmium sulfate becomes 5%, crystallization begins.
After 24 hours the brownish crystals are separated by centrifugation from mother
liquid. The crystals, when free from ammonium sulfate, are not readily soluble in
water, but readily dissolved in 2% ammonium sulfate, yielding a deep red-brownish
solution.

The samples used in our experiments were prepared in the laboratory of Dr.
Sugita of Faculty of Medicine of Kanazawa University, till the step of crystallization
by adding solution of cadmium sulfate. After several repeats of centrifugation (about
8000 rpm) for ten minutes and dissolution in 2% ammonium sulfate, it was dialyzed
to water to remove ammonium and cadmium sulfates.

As water absorbs the microwave power in a sample cavity of ESR apparatus,
samples in a state of solution are not suitable for ESR investigations. Also, if the
sample contains an appreciable amount of water, there exists a possibility of breaking
the sample tube when cooled to liquid air temperature, so we must dry the sample.
Fortunately it is said that in a dried state even if the protein shell may be changed
noticeably, the iron core perhaps may not be disturbed, as there would not be enough
vacancy for existing water molecules in the core, considering from the volume of the
core and the amount of substance making the core. However, from our experiments it
has become clear that the drying methods are very important problems.

We prepared two smaples, one was dried under vacuum and the other in a usual
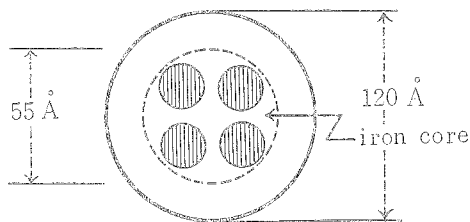desiccator with conc. sulfuric acid for ten days. Two samples showed different behav-

iours, so they were distinguished as "sample 1" and "sample 2" for the one dried by the former and the other by the latter method respectively. The "sample 1" showed a remarkable behaviour. When it was ceased to be dried in a state of half-dried, it did no more come back to the original solution which was divided into precipitate and colourless liquid, meaning that the iron core might be removed out from the ferritin. The "sample 2" was thought not to be in such a situation and its colour was red-brown as same as the original mother liquid, while the colour of the "sample 1" was yellow-brown.

Both two samples could be dissolved neither in water nor in ammonium sulfate rapidly, and the water was slightly coloured in brown in two days, but they were never dissolved completely, leaving a doubt that they were changed in the process of drying.

γ-FeOOH which was also prepared in Dr. Sugita's laboratory was investigated to compare its spectrum with that of ferritin.

3. Experimental Results

The "sample 2" offered characteristic spectra at room and liquid air temperatures.

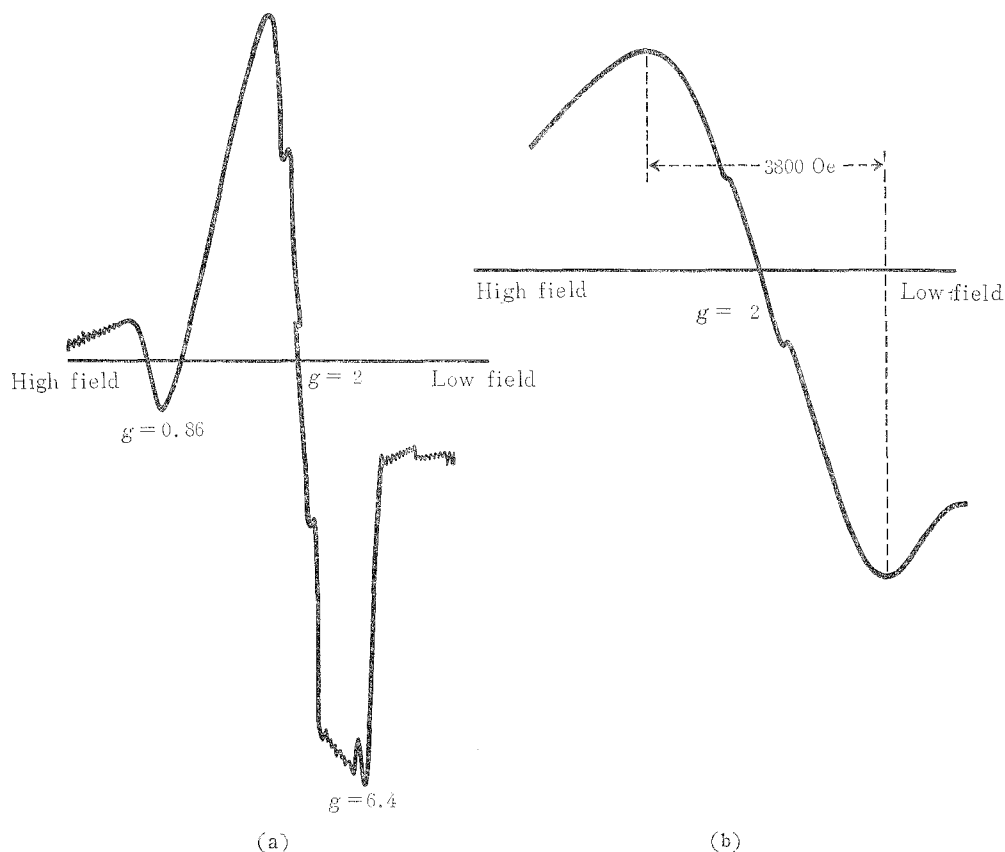(a)   (b)

Fig. 2   The spectra of the "sample 2"; (a) at room temp. (b) at liquid air temp.

The spectra are shown in Fig. 2. The experimental results are listed in Table 1 in which the indices of paramagnetic centers represent the resonance peaks indicated in Fig. 3.

The "sample 1" showed only a strong signal at $g=2$ similar to that represented as center 4 in Fig. 3. γ-FeOOH also showed a strong signal at $g=2$ and a weak signal at $g=4$ which could not be seen in any other spectra. The spectrum of γ-FeOOH is shown in Fig. 4.

It was found that when the "sample 2" was reexamined after two months from the period of sampling the lines at $g=0.86$ and 6.4 had vanished. This will be discussed later.
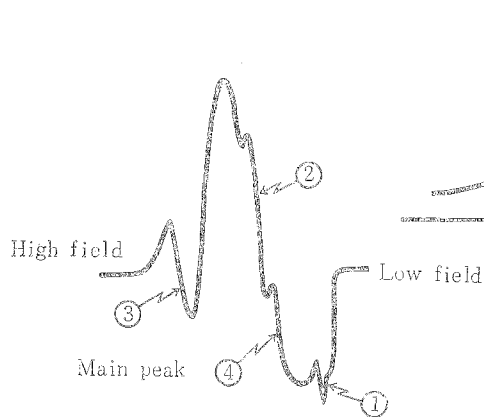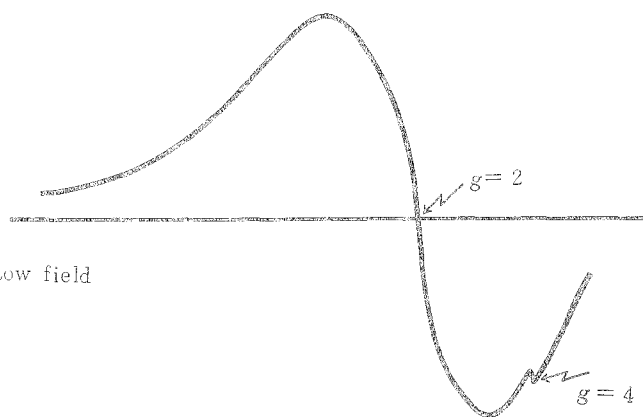

Fig. 3 Indices of spectra

Fig. 4 The spectrum of γ-FeOOH at room temp.

Table 1 Experimental Results of the "sample 2"
g: g-value, w: line width, $*$: calibrated by DPPH

paramagnetic center		room temp.	liquid air temp.
1	g w	6.4 63.4 Oe.	vanished
2	g	2.06*	2.03*
3	g w	0.86 100 Oe.	nearly vanished
4	g w	2.0 1900 Oe.	2.0 3800 Oe.

4. Discussion

The spectrum of the "sample 2" (Fig.2-a) shows a complexity of theoretical analysis. It can be noted that there are two different paramagnetic centers giving

g-values of 2 and they do not vanish at liquid air temperature (Fig. 2-b), while other two centers giving g-values of 6.4 and 0.86 exist at room temperature only and vanish at liquid air temperature. The relative absorbed powers by centers 1, 3, and 4 are in ratio of 500:70:7. But these figures do not represent directly the numbers of ions because the transition probabilities and the relaxation times are different from each other.

To obtain a knowledge about the iron ions, we have to calculate the energy states and the expected resonance lines on relatively simple assumptions according to Pryce's effective spin Hamiltonian method, and compare them with the experimental results. Even in an absence of a static magnetic field, the spin may prefer to align itself to a certain crystalline direction. Such an effect may arise from spin-orbit or dipole-dipole couplings. If the spin prefers some crystalline direction it no longer precesses freely in a static magnetic field, resulting g-value different from 2.

As in the case of iron ions in glass [6], we assume the spin Hamiltonian takes the form

$$\mathcal{H} = g_0 \beta H S_{z'} + D S_z^2 + E(S_x^2 - S_y^2), \qquad (1)$$

where D and E are constants of crystalline field, S_x, S_y and S_z are the components of spin along three mutually perpendicular crystalline axes x, y and z, and z' is the direction of the applied magnetic field H, g_0 is the g-value of a free spin. We consider the following four cases for simplicity (A) $S=5/2$, $E=0$ (B) $S=5/2$, $D=0$ (C) $S=3/2$, $E=0$ (D) $S=3/2$, $D=0$.

Case (A) $S=5/2$, $E=0$

In this case the effective spin Hamiltonian becomes

$$\mathcal{H} = g_0 \beta H S_{z'} + D S_z^2 \qquad (2)$$

and it is an easy problem to solve (2), assuming $D \gg H$ as in the case of iron in glass. When the applied magnetic field is parallel to x, y or z axes we can expect resonance lines with effective g-values

$$g_x = g_y = 6, \quad g_z = 2$$

respectively.

Case (B) $S=5/2$, $D=0$

In this case as the effective spin Hamiltonian we have

$$\mathcal{H} = g_0 \beta H S_{z'} + E(S_x^2 - S_y^2) \qquad (3)$$

and (3) has been solved in the reference [6], assuming $E \gg H$. When the applied field is parallel to x, y or z axes, we can expect several resonance lines with effective g-values

$$g_x = g_y = g_z = 4.29,$$

$$g_x = 9.7, \ g_y = 0.61, \ g_z = 0.86$$
$$g_x = 0.61, \ g_y = 9.7, \ g_z = 0.86.$$

Case (C) $S = 3/2, \ E = 0$

In this case the spin Hamiltonian is (2), solving it on the assumption $D \gg H$ as before, we may expect resonance lines with

$$g_x = g_y = 4, \ g_z = 2$$

Case (D) $S = 3/2, \ D = 0$

In this case the spin Hamiltonian is (3), solving it on the assumption $E \gg H$ we may expect

$$g_x = 5.47, \ g_y = 1.46, \ g_z = 2$$
$$g_x = 1.46, \ g_y = 5.47, \ g_z = 2$$

The observed g-values of the "sample 2" are 0.86, 2.0 and 6.4, comparing this fact with the simple analysis mentioned above, we can deduce the following several conclusions.

(1) As the observed g-value of 0.86 is just coincident with the calculated value of g_z in case (B), some part of the iron ions may be in a state of $S = 5/2$, but some doubts are remained why lines corresponding to $g = 9.7$, 0.61 or 4.29 are not observed.

(2) The fact there exists a line with g-value of 6.4 can not be explained satisfactorily, suggesting a necessity of a more complex model.

(3) As it is said that the total spin of iron ion in ferritin is 3/2 from the measurements of static susceptibility [2], it is reasonable to guess the main peak at $g = 2$ may be due to the ions in a state of $S = 3/2$. But, as no signal was found at $g = 4$, 5.47, 1.46 the ions in this state may not be under the influence of crystalline field.

(4) As the lines at $g = 6.4$ and 0.86 disappear at liquid air temperature, the ions showing these lines may have an intimate contact with the protein shell and be dominated by the modification of the shell. The fact in the spectrum of the "sample 2" (at room temperature) taken after two months these lines were not found may support this reasoning.

(5) FeOOH in the iron core may be imagined not to be in a state of γ-FeOOH because of the existence of a line at $g = 4$ in latter's spectrum (Fig.4).

(6) Weak line at $g = 2$ may come from free radicals in the protein shell or some paramagnetic impurities.

In the conclusions mentioned above some ambiguous points may be pointed out and to understand the states of iron ions in ferritin more elaborate studies are desired.

5. Acknowledgement

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