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Heme-heme magnetic interaction of cytochrome c_3 studied by Mössbauer effect

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Résumé. — Nous avons étudié le cytochrome c_3 par effet Mössbauer afin de déterminer l'interaction heme-heme magnétique. Nous concluons des résultats du spectre obtenu à 4,2 K sur le cytochrome c_3 partiellement réduit qu'il existe une interaction magnétique heme-heme fortement augmentée.

Abstract. — A Mössbauer effect study was carried out on cytochrome c_3 to investigate the heme-heme magnetic interaction. Results observed in the spectrum of partially reduced cytochrome c_3 at 4.2 K shows that a strongly enhanced heme-heme magnetic interaction exists between cytochrome c_3 molecules.

1. Introduction. — The heme-heme magnetic interaction has been rarely observed in biological molecules because of small contents of magnetic ions in them. Cytochrome c_3 , a bacterial electron transfer protein, has four iron ions per molecule with a molecular weight of 14 000 and has a rather high iron density compared with other biological molecules [1]. The heme-heme magnetic interaction has been observed at 4.2 K in ferricytochrome c_3 through the spin-spin relaxation effect observed in Mössbauer spectra, and the effective field was strongly enhanced and estimated to be about 50 Oe by Ôno *et al.* [2].

Cytochrome c_3 is reversibly reducible with molecular hydrogen by the catalytic action of Desulfovibrio hydrogenase. The reduction kinetics has been studied by us recently [3]. The electronic states of iron ions both ferri- and ferrocytochrome c_3 are found to be the low spin states and the ferric ions are magnetic with an effective spin of 1/2, while the ferrous ions are nonmagnetic. Therefore, a system of partially reduced cytochrome c_3 is expected to be a magnetically diluted system.

In the present experiment, a Mössbauer effect study was carried out to estimate the intermolecular spin-

spin interaction using an absorber of partially reduced cytochrome c_3 .

2. Experiment and results. — A preparation of lyophilized ferricytochrome c_3 absorber will be reported elsewhere [3]. A partially reduced cytochrome c_3 was prepared by the following procedure. Wet and deposited ferricytochrome c_3 with a small amount of hydrogenase (cytochrome c_3 : hydrogenase = $1:10^{-4}$), packed in a Teflon sample cell, was set in a cryostat. After a rough evacuation of the cryostat, pure hydrogen gas at atmospheric pressure was introduced in it. A progress of the reaction was monitored by taking the Mössbauer spectra. When the spectrum area ratio of the ferrous to the ferric ions became about 3:1, the absorber was cooled down by liquid nitrogen to stop the reaction and the cryostat was evacuated for several hours. After the experiment at 4.2 K, the Mössbauer spectrum at 300 K was observed again in the evacuated cryostat to confirm the ratio of the ions.

The Mössbauer source consisted of 10 mCi of ^{57}Co diffused into a rhodium foil. A metallic iron absorber was used for a velocity calibration.

Because of very small content of iron in the absorber, magnetic impurities may have serious effect on the heme-heme interaction. To examine the effect of magnetic impurities, the static magnetic susceptibility

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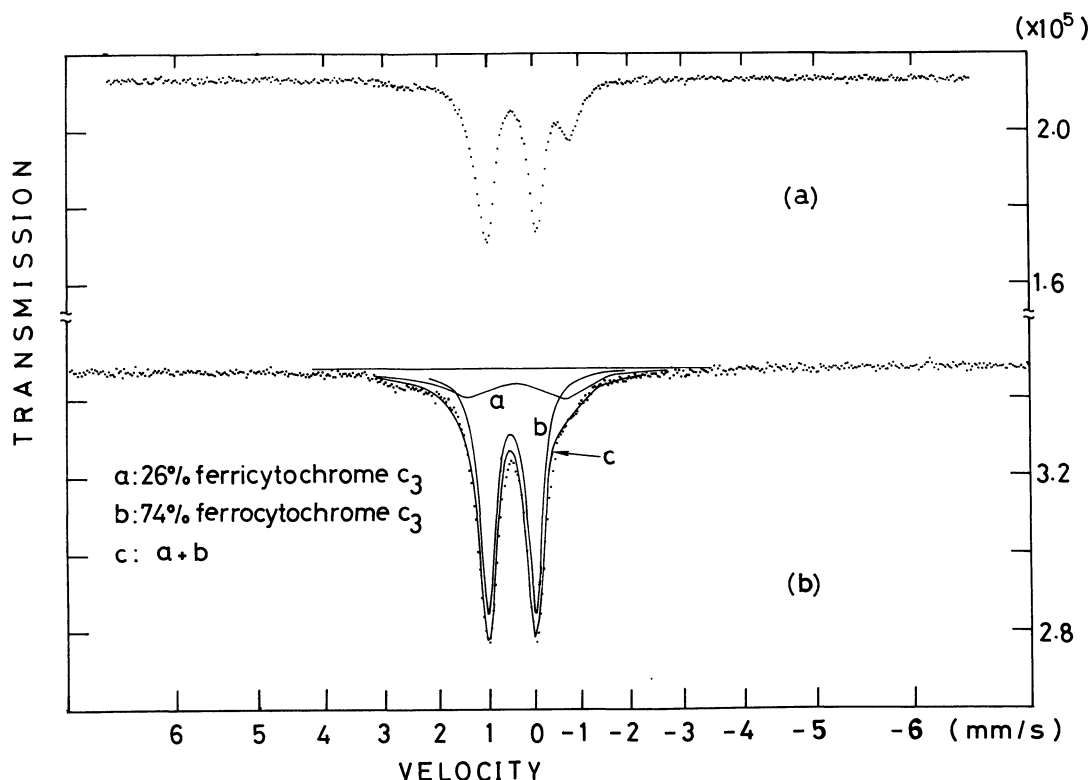


Fig. 1. — Mössbauer spectra of partially reduced cytochrome c_3 observed at (a) 300 K and (b) 4.2 K. Solid lines, a and b, are spectra of ferri- and ferrocytochrome c_3 with the ratio 26 : 74. $c = a + b$.

of cytochrome c_3 in ferric form between 4.2 and 1.5 K was measured by rf-SQUID magnetometer, with an external magnetic field of 176 Oe.

The temperature dependence of the magnetic susceptibility was well expressed by the Curie-Weiss law : $\chi = C/(T - \theta)$, with

$$C = 2.16 \pm 0.18 \text{ erg.K.G}^{-2}/\text{mol.}$$

and

$$\theta = 0.00 \pm 0.04 \text{ K.}$$

g -value calculated from Curie constant, C , using $s = 1/2$, was 2.39 ± 0.10 , agreed well with those obtained by other methods, namely 2.36 by Mössbauer effect [2] and 2.32 by EPR [4]. θ and g obtained in this experiment indicate that the specimen was free from magnetic impurities.

The Mössbauer spectra of partially reduced cytochrome c_3 obtained at 300 and 4.2 K are shown in figure 1. From the area ratio analysis of the spectrum at 300 K, the fraction of ferric state ions was estimated to be $28 \pm 5\%$. The Mössbauer parameters of ferri- and ferrocytochrome c_3 are listed elsewhere [3]. Hyperfine spectra with a long electron spin relaxation time could not be observed at 4.2 K in this diluted spin system as shown in figure 1.

3. Discussion. — As cytochrome c_3 has four iron ions per molecule, there will be several kinds of molecules such as fully reduced, fully oxidized and partially

oxidized (one ferric ion, two ferric ions or three ferric ions per molecule) in a partially reduced absorber. A polarography measurement revealed that redox potentials of four hemes in a cytochrome c_3 molecule were not equal, and that the spacings between them were estimated to be 52.5, 19.5 and 40.5 mV [6]. By electrochemical calculation, in the absorber with 28 % ferric ions, about 50 % of ferric ions are isolated in a molecule. If there is no intermolecular spin-spin interaction and the slow spin-lattice relaxation situation is achieved at 4.2 K, magnetically split Mössbauer pattern of these isolated ferric ions should be observed.

Many heme proteins such as ferricytochrome c , hemoglobin cyanoide and hemoglobin azide, which have local environment of the Mössbauer nucleus similar to that in ferricytochrome c_3 , show the slow relaxation limit at 4.2 K. The values of their hyperfine fields were observed to be about 400 kOe [7]. In the Mössbauer spectrum of cytochrome c_3 of 28 % ferri-form at 4.2 K, no hyperfine splitting could be found between 3.0 and 6.0 mm/s, -3.0 and -6.0 mm/s. The spectrum consisted of two doublets and is well fitted as is shown in figure 1b, by assuming that 26 % of the ions are in the ferri-form and 74 % in the ferro-form. The ratio of ferri- and ferro-form is in good agreement with that obtained from the 300 K spectrum.

The result shows that the spin relaxation time of the ferric ions is less than $\tau = \hbar/S = 30$ ns, the charac-

ristic time of the nuclear spin precession, where S is the over-all magnetic hyperfine splitting in the Mössbauer spectrum for ferricytochrome c_3 obtained in an applied magnetic field of 900 Oe [2]. This relaxation time is about the same as that of ferricytochrome c_3 and is more than ten times shorter than that expected in the partially reduced cytochrome c_3 (magnetically diluted system).

Recently, the authors found that there exists both intra- and intermolecular transfer of electrons in a redox reaction of cytochrome c_3 catalyzed by hydrogenase even in the solid phase [3]. Therefore, the spin relaxation may be attributed to the intermolecular transfer of electrons, which may be very important in the bacterial electron transfer protein, cytochrome c_3 .

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