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ESR studies on iron-sulfur clusters of complex II in *Ascaris suum* mitochondria which exhibits strong fumarate reductase activity

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Complex II of *Ascaris suum* mitochondria, which functions as fumarate reductase in physiological conditions, contains three types of iron-sulfur clusters. These correspond to clusters S-1, S-2 and S-3 and are distinguishable by low-temperature ESR studies. Cluster S-1 is reduced by succinate, giving ESR signals with g_z, g_y and g_x values at 2.033, 1.939 and 1.920. The existence of cluster S-2 is suggested by an enhancement of the S-1 spin relaxation induced upon reduction of S-2 by dithionite. Cluster S-3 is ESR detectable under air-oxidized conditions and gives a strong signal at $g=2.025$. Cluster S-3 was only partially reduced even with an excess amount of sodium succinate, which is a common characteristic of fumarate reductase but this is not seen in the mitochondrial complex II.

Iron-sulfur cluster; Complex II; Fumarate reductase; ESR; (*Ascaris suum*, Nematoda)

1. INTRODUCTION

Adult *Ascaris suum* resides in the host's small intestine, and anaerobically respires with electron transfer from NADH to fumarate [2]. Reducing equivalents from NADH are accepted by the mitochondrial complex I, mediated by rhodoquinone, and oxidized by the complex II fumarate reductase [2–4]. Thus, the mitochondria are quite unique for its high ratio of fumarate reductase/succinate dehydrogenase activity (20.0), when compared with that of mammalian enzymes (0.05) [5]. The complex II was purified and was shown to be composed of four subunits with molecular masses of 68, 26, 15 and 13.5 kDa containing a flavin and a *b*-type cytochrome (cytochrome *b*-558) [5], as is well known for the mammalian complex II [6].

Succinate dehydrogenase in the mitochondrial complex II [6,7] and fumarate reductase in anaerobically growing bacteria [8] are two closely related enzyme systems. They have similar subunit compositions and active centers [FAD, iron-sulfur (Fe/S) clusters]. ESR studies have already suggested that both enzyme systems contain three corresponding types of Fe/S clusters, designated as clusters S-1, S-2 and S-3 [7,8]. Although compositions and ESR characteristics of these reaction centers are similar to each other, they play different roles in catalyzing the two directionally opposite enzyme reactions.

In this paper, Fe/S clusters from the *Ascaris suum* complex II were characterized by low-temperature ESR. The relation between the high fumarate reductase activity and the redox properties of these clusters was discussed by comparing them with the cases of bacterial fumarate reductase and mitochondrial succinate dehydrogenase.

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Abbreviations: E_m, oxidation-reduction midpoint potential; $P_{1/2}$, apparent half-saturation parameter of ESR signal [1]

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2. MATERIALS AND METHODS

2.1. *Preparation of* Ascaris *complex II*

Mitochondria of *Ascaris* were prepared by the method of Takamiya et al. [2]. Complex II was solubilized in a solution containing 1% deoxycholate and 1% Triton X-100, and purified by fractionation by ammonium sulfate precipitation and by Sephadex G-200 column chromatography [3]. The purified complex II, thus obtained, was free from rhodoquinone and other quinones [3]. Fumarate reductase activity (measured with methyl viologen as an electron donor [4]) of the purified complex II was 40 μmol/min per mg protein.

2.2. *ESR measurements*

The purified complex II was suspended in 40 μl of 1 M Hepes/NaOH buffer (pH 7.0) and adjusted to a total volume of 200 μl. The mixture was incubated with 4 μl of 1 M sodium succinate or saturated sodium dithionite solution for 20 min, transferred to an ESR sample tube under argon atmosphere at 20°C, and then frozen in liquid nitrogen. ESR signals were measured with an X-band Bruker ER 200D spectrometer (Bruker, FRG) equipped with a liquid helium model 900 cryostat (Oxford, England), as described previously [9,10].

3. RESULTS AND DISCUSSION

A strong ESR signal with positive and negative peaks at $g = 2.025$ and 2.014, respectively, was observed from the air-oxidized complex II at 13 K (fig.1a). Fig.1b shows a spectrum after reduction with 20 mM sodium succinate. It gives a positive peak at $g = 2.033$ in addition to the one seen in fig.1a and weaker signals at $g = 1.939$ and 1.920. Fig.1c shows a spectrum of the complex II reduced by sodium dithionite. In this condition, the peaks at $g = 2.025$ and 2.014 disappeared and the signals at $g = 2.033$, 1.939 and 1.920 were detected.

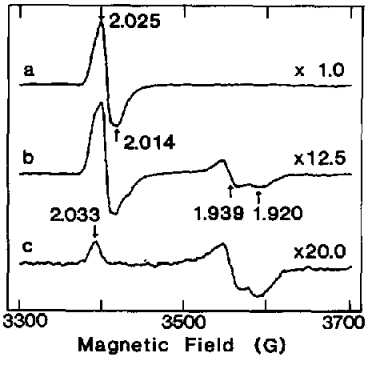

Fig.1. ESR signals of the *Ascaris* complex II. (a) Air-oxidized sample (5.3 mg protein/ml); (b) succinate-reduced sample (2.1 mg protein/ml); (c) dithionite-reduced sample (1.3 mg protein/ml). Samples contained the *Ascaris* complex II and 200 mM Hepes/NaOH buffer (pH 7.0). ESR measurement conditions were: modulation amplitude, 10 G; microwave power, 100 μW; sample temperature, 13 K.

The dependence on the microwave power of intensities of the negative peak at $g = 1.920$ was examined to study the spin relaxation rate of the signal at 13 K (fig.2). The apparent $P_{1/2}$ value of the signal at $g = 1.920$ of the succinate-reduced complex II was about 0.3 mW, while that from the dithionite-reduced sample was 30 mW. The difference of the $P_{1/2}$ value of the same signal under these conditions suggests that the complex has two interacting Fe/S clusters; one is reduced with succinate and shows microwave power saturation at 0.3 mW, and the other is reduced by dithionite only and enhances the spin relaxation rate of the former signals by spin-spin interactions which results in the higher $P_{1/2}$ value. These clusters can be assigned as S-1 and S-2, respectively [11]. The E_m values of clusters S-1 and S-2 are reported to be 0 and −260 mV, respectively, in bovine complex II [12], and −20 and −330 mV, respectively, in *E. coli* fumarate reductase [8]. In both cases, spin-spin interactions between clusters S-1 and S-2 were reported; for example, the $P_{1/2}$ values of 0.07 mW (succinate-reduced, noninteracting condition) and 6.0 mW (dithionite-reduced, interacting condition) were reported in a mammalian mitochondrial succinate dehydrogenase preparation at 12 K [11,13]. The g values of the signals of the *Ascaris* cluster S-1 closely resemble those of the bovine mitochondrial centers ($g = 2.03$, 1.93 and 1.91) [6,12] and the *E. coli* fumarate reductase ($g = 2.03$, 1.93 and 1.91) [8]. The broad ESR signals of cluster S-2 showing strong rhombicity ($g = 2.06$, 1.99 and 1.85 for bovine [13] and $g = 2.17$, 1.90 and 1.68 for *E. coli* fumarate reductase [14]) could not be detected

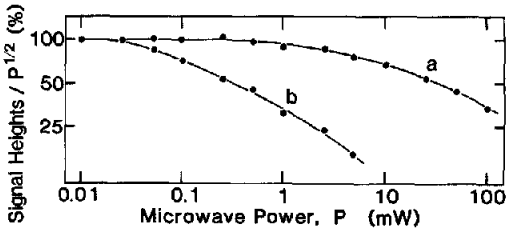

Fig.2. Microwave power saturation behavior of the ESR signal at $g = 1.920$ (a) under the dithionite-reduced condition; (b) under the succinate-reduced condition. Measurement conditions and sample preparations were the same as in fig.1.

in the *Ascaris* sample of this spin concentration (fig.1c).

The strong signal near $g = 2.03$ with positive and negative peaks observed in fig.1a is due to cluster S-3 [15]. The $P_{1/2}$ value of the signal was 30 mW at 13 K and 0.4 mW at 7 K (not shown). The distinct dependence on temperature of the power saturation behavior reflects its short relaxation time. The signal of bovine S-3 also shows a short relaxation time (high $P_{1/2}$ value), and is diminished at temperatures higher than 15 K at a microwave power setting of 1 mW [15]. Thus, we measured the temperature dependence of the broad signal near $g = 2.03$ seen in fig.1b. At 50 K, the broad signal near $g = 2.03$ became small (fig.3), and typical peaks of cluster S-1 at $g = 2.033$, 1.939 and 1.920 were observed. Therefore, the higher field positive peak at $g = 2.025$ in fig.1b, which disappears at higher temperatures, could be attributed to the oxidized form of cluster S-3.

These results indicate that more than 15% of the $g = 2.025$ signal remains after reduction with sodium succinate. This strongly suggests that S-3 in *Ascaris* is not fully reduced even by excess sodium succinate. However, S-3 in the bovine complex II was reported to be completely reduced under similar conditions (E_m value is 65 mV) [15]. The succinate-ubiquinone reductase activity of the *Ascaris* complex II used in this experiment is as high as that in the bovine complex II (2.0 μg mol/min per mg protein) [16], suggesting its high reactivity with sodium succinate. Therefore, the E_m value of *Ascaris* S-3 may be lower than that of the sodium succinate/fumarate couple (E_m = 30 mV). The lower E_m value of cluster S-3 is a common feature of fumarate reductase systems in *Wolinella succinogenes* (E_m = −24 mV) [17] and *E. coli* (−70 mV) [8]. We also reported that TTFA (2-thenoyltrifluoroacetone) shows no effect on the succinate dehydrogenase activity of the *Ascaris* complex II [16], although it is a potent inhibitor of the bovine complex II [18]. In the case of the bovine complex II, TTFA inhibits reoxidation of the cluster S-3 [19,20]. Thus, it indicates a difference in reactivity of the *Ascaris* cluster S-3 from the bovine mitochondrial cluster S-3. This may be related to the different behavior of the ESR signal of cluster S-3 observed here. These results indicate the unique characteristics of cluster S-3 in *Ascaris*, and help us to understand its high fumarate reductase activity.

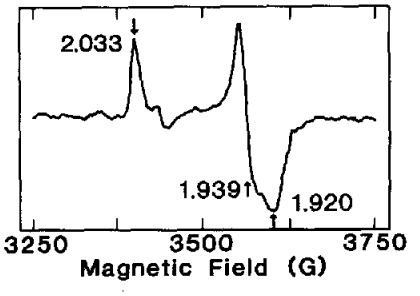

Fig.3. ESR signals from succinate-reduced *Ascaris* complex II at 50 K. ESR measurements conditions were: modulation amplitude, 10 G; microwave power, 1.0 mW; protein concentration, 3.1 mg protein/ml.

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