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EPR studies of a 9 kDa polypeptide with an iron-sulfur cluster(s) isolated from photosystem I complex by *n*-butanol extraction

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A 9 kDa polypeptide was isolated with an iron-sulfur cluster(s) from spinach photosystem I complex [(1988) J. Biochem., in press]. Its EPR spectrum indicated that this protein was an iron-sulfur protein similar to bacterial-type ferredoxins. The reduction profiles and temperature dependence of its EPR signals suggested the existence of at least two iron-sulfur clusters: the one with a lower redox potential shows similar characteristics to those of center B, while the other seems to be a degradation product of center A (or B).

Photosystem I complex; Iron-sulfur center; Iron-sulfur protein; EPR

1. INTRODUCTION

The PS I complex consists of at least five electron acceptors serving on the reducing side; a primary electron acceptor A_0, an intermediate electron acceptor A_1, and three iron-sulfur centers X, A and B [1,2]. A_0 is now assumed to be the immediate electron acceptor from P700 and to be a monomeric chlorophyll [3–7]. A_1 is suggested to be vitamin K_1 functioning between A_0 and center X [2–5,8,9]. Centers X, A and B are considered to be iron-sulfur clusters with a series of redox potentials [10]. As centers A and B are present in equal amounts and show a magnetic interaction in the reduced states, they are supposed to be in a close arrangement, possibly on the same polypeptide [11–13].

An alternative approach has come from the analysis of zero-valence sulfur atoms covalently bound to the denatured polypeptides in the PS I complex, showing that the iron-sulfur centers were present together with high molecular mass polypeptides (59 and/or 63 kDa) as well as an approx. 8 kDa polypeptide [14,15]. Recent studies on the amino-terminal sequence analysis of 9 kDa polypeptide carried out by three groups have independently proved it to be an apoprotein of one of the iron-sulfur center proteins, probably carrying center(s) A and/or B [16–18].

In the previous paper, we demonstrated that the 9 kDa polypeptide was isolated with iron-sulfur cluster(s) from the PS I complex by *n*-butanol extraction [19], a method that is convenient for the dissociation of this unstable protein from the complex. We subsequently succeeded in showing some details of the EPR spectroscopic characterization of this protein and present them in this report.

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Abbreviations: PS I, photosystem I; ISP, iron-sulfur protein

2. MATERIALS AND METHODS

The 9 kDa polypeptide from spinach PS I complex, which was prepared by the method of Sakurai et al. [14], was isolated with iron-sulfur cluster(s) by an *n*-butanol extraction procedure under anaerobic conditions as previously reported [19].

EPR spectra were recorded with an X-band EPR spectrometer (model EPR-200, Bruker, FRG), equipped with a liquid-helium cryostat (model ESR-900, Oxford Instruments,

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England) in the Center of Analytical Instruments of the National Institute for Basic Biology [20]. Samples were incubated with dithionite (final concentration of 2 mM) in a medium containing 50 mM Tris-HCl (pH 8.1), 300 mM NaCl, 5 mM dithiothreitol, for varied times at 0°C and then frozen in liquid nitrogen.

3. RESULTS AND DISCUSSION

In the previous paper [19], we reported the isolation method of the 9 kDa polypeptide with iron-sulfur cluster(s) from the PS I complex of spinach, spectroscopic properties and contents of iron and acid labile sulfur of this protein. It contained 4.1 atoms of non-heme iron and 3.2 atoms of inorganic sulfide per molecule, only half of those expected from the primary sequence with a capacity of 8Fe and 8S [21]. Although our present preparation still needs further improvements to decrease the loss of iron and sulfur atoms, we studied EPR properties of iron-sulfur cluster(s) remaining in this protein preparation to obtain information on its character.

Fig.1A shows the reduction time course of the ISP after addition of dithionite studied by low temperature EPR spectra. When the sample was frozen after a long incubation time, the spectrum of the fully reduced ISP showed the typical signals for iron-sulfur cluster(s) and was relatively similar to those of the bacterial-type ferredoxins [22]. The present spectrum markedly resembled that of the crude ISP which was isolated from spinach thylakoid membranes by Malkin et al. [23]. The difference spectrum between spectra of the samples frozen after 2 and 7 min incubation with dithionite was different from the spectrum at 30 s (fig.1B). Therefore, there are at least two species of iron-sulfur cluster with different reduction rates. This may reflect the difference in redox potentials between them. In this paper, we call the one with the faster and the one with the slower reduction rate high potential (H)- and low potential (L)-type components, respectively. The broad EPR spectrum of the H-type component suggests its nature to be that of the partially degraded iron-sulfur cluster(s).

EPR spectra of the ISP were measured at various temperatures as shown in fig.2. With increasing the temperature, the $g = 2.05$ signal as well as fine structures at $g = 1.92$ and 1.90 of the spectrum of fully reduced ISP disappeared

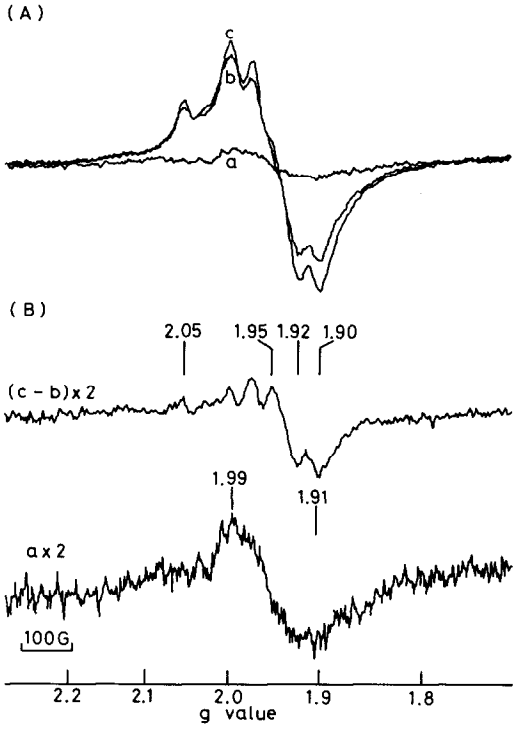

Fig.1. EPR spectra of the isolated ISP. (A) Reduction profiles of EPR spectra after incubation with dithionite as mentioned in section 2: reduction times; a, 30 s; b, 2 min; c, 7 min. (B) Difference spectrum of (c − b) × 2 (upper) and the spectrum of a magnified 2-fold (lower). Concentration of the sample was about 0.6 mg/ml. Conditions of EPR spectroscopy: temperature, 10 K; microwave frequency, 9.65 GHz; microwave power, 20 mW; receiver gain, 1×10^5; modulation amplitude, 10 G; scan width, 3000–4000 G; scan time, 50 s; time constant, 80 ms.

gradually, and that of the broad H-type component remained (fig.2A). On the other hand, in the sample with the shorter reduction time (30 s), only the broad (H-type) signal was observed between 4 and 70 K (figs 2B and 3). The former fully reduced spectrum is, thus, understood to be the sum of the spectra of H- and L-type components.

Signal intensities of the H- and L-type components were plotted against temperature (fig.3). Those of center A and B in the PS I complex (frozen in light, i.e., interacting forms) were superimposed on them (broken lines). The L-type component and center B showed similar temperature dependence, but the behavior of H-type did not resemble that of either center A or B. Since the present ISP contained about half the

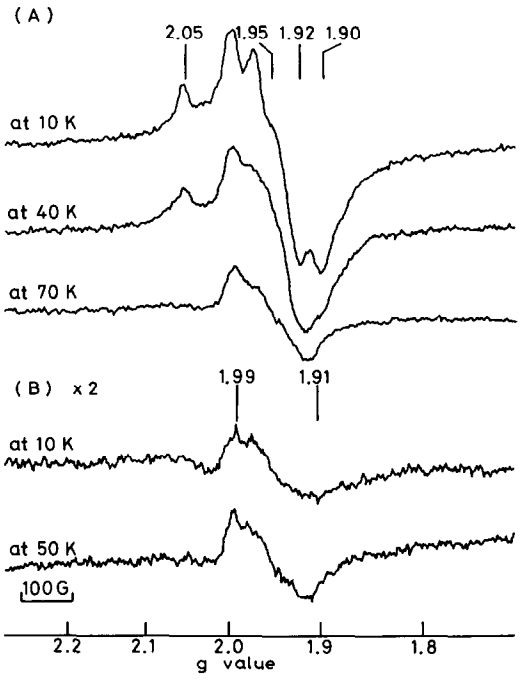

Fig.2. Effect of temperature on EPR spectra. (A) Frozen after reduction for 7 min, measured at 10, 40 and 70 K. (B) Frozen after reduction for 30 s, measured at 10 and 50 K. Other conditions of EPR spectroscopy were the same as in fig.1.

number of iron and sulfur atoms per molecule expected, it is reasonable to consider that the H-type component was the derivative(s) from center(s) A and/or B, and that L-type remained relatively in situ in the PS I complex. Although there is no exact correspondence between H- and/or L-type component(s) and center(s) A and/or B, the L-type spectrum seems to resemble that of center B, which shows g values of 2.06, 1.94 and 1.89 [24]. Center A in the PS I complex, which has a higher redox potential [25] and was suggested to be less stable to treatment with organic solvent [9], might have partially been destroyed during extraction.

During preparation of this manuscript, Wynn and Malkin [26] described isolation of the 9 kDa polypeptide from thylakoid membranes by the method using methanol and acetone as the extraction solvent, as previously reported [23]. Their EPR spectrum of the protein with 7.9 iron and 6.4 sulfur atoms per molecule was slightly different from that of their previous preparation and ours in the region of $g = 2.00$. These differences may partially be interpreted by the different amounts of H- and L-type components reported here.

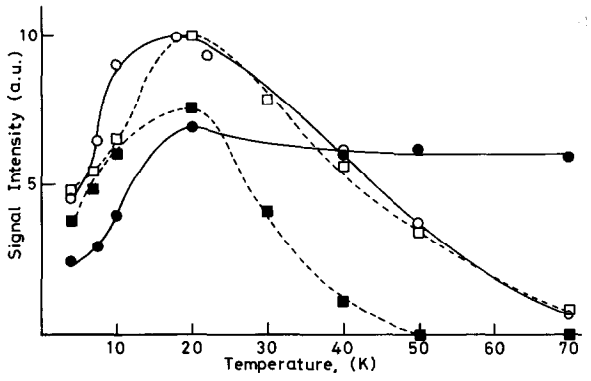

Fig.3. Comparison of temperature dependence of signal intensities. Signal intensities (arbitrary units) of H-(●) and L-(○)type components were measured at $g = 1.91$ and 2.05, respectively. Those of centers A (■---■) and B (□---□) were obtained from the dithionite-reduced PS I complex (3.0 mg chlorophyll/ml) frozen during illumination. Other conditions of EPR spectroscopy were the same as in fig.1.

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