ELECTRON SPIN RESONANCE STUDIES OF BOUND FERREDOXIN IN CHLOROPLAST PHOTOSYSTEM I REACTION CENTER

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1. Introduction

The primary photochemical reaction in chloroplast photosystem I has been identified as the photooxidation of a reaction center chlorophyll complex P_{700} [1]. Bound ferredoxin was proposed as the primary electron acceptor for this reaction [2,3]. However, recently an alternative primary electron acceptor was suggested based on the observation of a light induced EPR signal at g = 1.76 under conditions in which the bound ferredoxin was fully reduced [4–6]. We have recently isolated a photosystem I reaction center which was active in NADP photoreduction and contained 5–6 polypeptides and P_{700} reaction center associated with a single polypeptide [7,8]. It is the purpose of this work to follow the bound ferredoxin content during the purification steps of P_{700} reaction center.

2. Materials and methods

Reaction centers were prepared as described previously [8] except that the sucrose gradient centrifugations were carried out using a SW27 rotor. Samples were illuminated for 2 min at room temperature in the presence of dithionite, then frozen with liquid nitrogen and transferred to the EPR spectrometer (Varian E 4), the cavity of which was cooled by a helium flow system to about 18°K. Occasionally the samples were illuminated in the cavity by a 500 W slide projector providing light intensity of about 10^4 ergs/cm^2/sec at the level of the sample. Photoreduction of NADP was measured spectrophotometrically in a Cary 118 C spectrometer by recording the light induced absorbance changes at 350 nm. The actinic beam was provided by a 150 W slide projector and passed through a red filter (Corning 2403). The phototube was projected from the acetinic beam by a blue filter (Corning 9782). The light intensity was 5.10^5 ergs/cm^2/sec at the level of the cuvette.

Abbreviations: SDS, sodium dodecyl sulfate; Tricine, *N*-Tris (hydroxymethyl) methylglycine

3. Results and discussion

The single polypeptide P_{700} reaction center is derived from the active photosystem I reaction center by SDS treatment [8]. Therefore it was of interest to test the effect of SDS treatment of the NADP photoreduction activity of this preparation. Treatment with 0.1% SDS decreased the NADP photoreduction activity by about 80% (fig.1). Treatment with 0.5% SDS abolished the NADP photoreduction activity. However, the light induced P_{700} signal at 430 nm in the presence of *N*-methylphenazonium methosulfate was not altered by a similar SDS treatment.

Fig.2a shows EPR spectrum at 18°K of the photosystem I reaction center which is active in NADP photo-

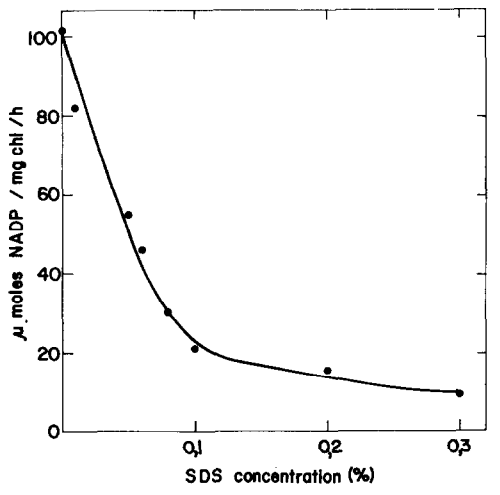

Fig. 1. Effect of SDS treatment of NADP photoreduction activity of photosystem I reaction center. Photosystem I reaction center containing 0.1 mg chlorophyll per ml was incubated at 0°C for 10 min with the specified concentrations of SDS. Aliquots of 50 μl were assayed for NADP photoreduction activity in a reaction mixture containing in a final volume of 1 ml; 20 μmol of 2-(*N*-morpholino) ethanesulfonic acid-Tricine (pH 7), 40 μmol of NaCl, 2 μmol of ascorbic acid, 2.5 nmol of plastocyanin, 5 nmol of ferredoxin, 0.05 nmol of ferredoxin-NADP-reductase, 1 μmol of NADP and 0.1% Triton X-100.

reduction. The spectrum is typical of that observed for bound feredoxin [2–4]. In order to obtain a similar spectrum with the active fractions from the DEAE cellulose column, double the amount of chlorophyll was required. A chlorophyll concentration of 3 mg/ml was needed for comparable peak intersity using digitonin photosystem I particles. Hence, the bound ferredoxin was enriched by about fivefold during the purification of the active reaction centers from the digitonin photosystem I particles. This is in line with the increase in the specific activity of NADP photoreduction during the purification of the reaction centers [8]. Treatment with rising concentrations of SDS gradually diminished the bound ferredoxin in the preparation (fig.2b and c). No bound ferredoxin could be detected in the single polypeptide preparation of P_{700} reaction center (fig.2d). Treatment of the active reaction center of the active fractions of the DEAE cellulose column with 0.5% SDS and without further separation also completely abolished the EPR signal of bound ferredoxin. Moreover, we could not detect the light EPR signal at g = 1.76 and reversible photooxidation of P_{700} at 8°K.

These data suggest that the P_{700} reaction center is lacking both the primary electron acceptor and the bound ferredoxin. At room temperature either oxygen

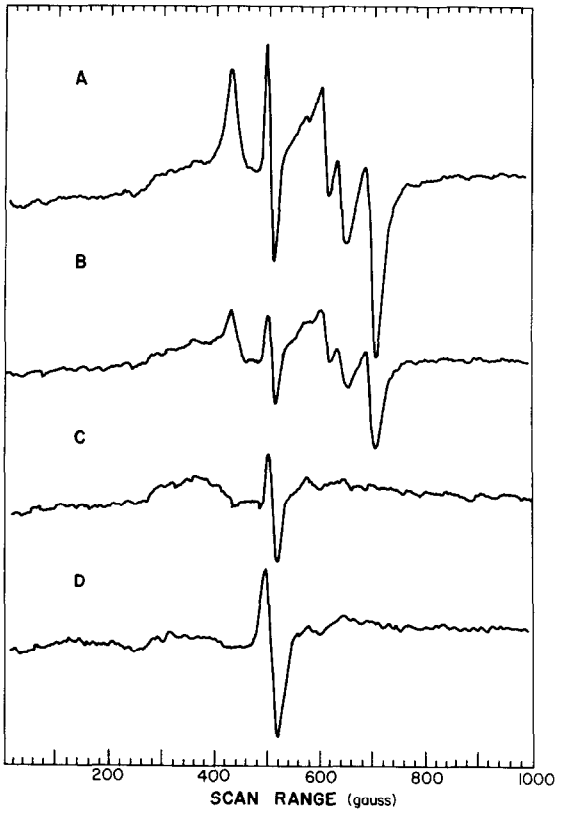

Fig. 2. Effect of SDS treatment on the EPR signal of bound ferredoxin. Active fractions after DEAE-cellulose column containing about 0.3 mg chlorophyll per ml were treated with various concentrations of SDS and applied on sucrose gradients 5–25% in 50 mM Tris–Cl (pH 8) and 0.2% Triton X-100. After centrifugation for 15 h at 3°C the lower green fractions were collected, dialysed for 2 h against 2 liters of 2 mM Tris–Cl (pH 8) and concentrated either by lyophilization or by pre-evaporation at 4°C a) Control without SDS treatment (Active photosystem I reaction center). Chlorophyll concentration 0.65 mg/ml. (B) After 0.1% SDS treatment. Chlorophyll concentration 0.61 mg/ml. (C) After 0.3% SDS treatment. Chlorophyll concentration 0.88 mg/ml. (D) After 0.5% SDS treatment (P_{700} reaction center). Chlorophyll concentration 0.6 mg/ml. Instrument settings; frequency 9.25 GHz, power 20 mW, modulation amplitude 10G, scan rate 500 G/min and gain 1250.

or *N*-methylphenazonium methosulfate can serve as artificial electron acceptor for the P_{700} reaction center. This implies that artificial electron acceptors can extract electrons directly from P_{700} prior to the natural primary electron acceptor. Kinetic studies are required to decide whether this reaction might be significant in isolated chloroplasts.

Apparently the sensitivity of the NADP photoreduction activity to SDS treatment is similar to that of the EPR signal of the bound ferredoxin. Since SDS treatment destroys the EPR signals we cannot conclude whether the primary electron acceptor is a constituent of the 70 000 mol. wt polypeptide.

Further studies are required to establish the involvement of the primary electron acceptor and bound ferredoxin in the various activities of the chloroplast photosystem I.

Acknowledgements

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