TRANSMEMBRANE CHARGE SEPARATION WITHIN THE LARGE SUBUNIT OF PHOTOSYSTEM I-REACTION CENTERS FROM CHLOROPLASTS

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1. Introduction

Photosystem I-reaction centers, capable of ferredoxin photoreduction as well as of plastocyanin photooxidation, contain a minimum of six polypeptides, a large subunit of 65 kD and five smaller ones [1,2]. Removal of the smaller subunits by SDS-treatment results in the loss of ferredoxin-mediated $NADP^+$ photoreduction and plastocyanin photooxidation, but the light-induced bleaching of P700 is retained [1,2]. SDS-treatment also abolishes the light-induced EPR signals at cryogenic temperatures [3], which are characteristic for three electron accepting Fe-S-centers in photosystem I [3,4]. Recently yet a more primary electron acceptor was detected by fast flash spectroscopy [4–9], and by EPR measurements [4,7,10,11]. This function has been assigned to chlorophyll *a* [12], either in dimeric [4,9], or in monomeric form [7,8,10]. It is also observed in SDS-treated photosystem I-reaction center preparations [6,7], and therefore seems to be present in the large, 65 kD subunit, together with P700.

Recently we succeeded to incorporate the multisubunit photosystem I-reaction center into liposomes, and to observe light-induced proton movements across the membrane if reduced phenazine methosulfate was present as mediator [13]. In addition the system catalyzed photophosphorylation if purified chloroplast coupling factor complex, CF_1–CF_0, was added [14, 15]. In this communication we demonstrate that the large subunit of photosystem I-reaction centers alone is able to catalyze a transmembrane, proton translocating photoredox cycle, in the presence of reduced phenazine methosulfate.

Abbreviations: P700 stands for the electron donating chlorophyll *a* in the reaction center of photosystem I; phenazine methosulfate for *N*-methylphenazonium methosulfate; Tricine for *N*-(tris(hydroxymethyl)methyl)glycine; SDS for sodium dodecylsulfate

2. Materials and methods

Photosystem I-reaction centers were prepared from spinach chloroplasts after Bengis and Nelson [1]. From this multisubunit complex the largest subunit, which may be called P700-reaction center [2], was obtained by SDS-treatment followed by sucrose density gradient centrifugation [1]. Photosystem I-reaction centers were first concentrated to 0.5 mg chlorophyll per ml by ultrafiltration through an Amicon XM100A filter, then 10% SDS was added to a final concentration of 1%; the mixture was incubated for 1 h at room temperature and was then loaded on the sucrose gradient. The lower green band containing the P700-reaction centers was collected and was concentrated by ultrafiltration to about 0.5 mg chlorophyll per ml. It migrated as a single band in SDS-polyacrylamide electrophoresis [1], and had a chlorophyll/P700-ratio of 40. It was stored under liquid nitrogen before use. For control experiments the multisubunit reaction center was run through the procedure omitting SDS. This control preparation had a chlorophyll/P700-ratio of 60.

P700-reaction center liposomes were prepared with soybean phospholipids by sonication as described in detail elsewhere [13]; 1.5 nmol P700 were incorporated into 10 mg lipid. After sonication the external medium of the suspension was exchanged against 50 mM KCl by chromatography on Sephadex G-50 coarse, to reduce the buffer capacity. The measurement of light-induced changes of pH and of 9-aminoacridine fluorescence is described elsewhere [13], and in the legend to fig.1. P700 [1] and chlorophyll [16] were measured by routine methods.

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Nigericin was purchased from Eli Lilly, Indianapolis, USA; valinomycin and L-α-phosphatidyl choline, Type II-S (soybean phospholipids) from Sigma, USA; phenazine methosulfate, SDS and Triton X-100 (purified) from Serva, FRG.

3. Results and conclusion

Liposomes containing P700-reaction centers, consisting of the 65 kD-subunit only, exhibited light-induced quench of 9-aminoacridine, in the presence of reduced phenazine methosulfate, which was fully sensitive to nigericin (fig.1A). This phenomenon is also known for chloroplasts [17] and reflects acidification inside the lipid vesicles. From the extent of the quench a pH-difference of more than three units across the membrane can be estimated [13,17]. With the same liposome preparation net acidification of the external medium in the light was observed, which also was fully inhibited by nigericin (fig.1B). Control experiments with the multisubunit photosystem I-reaction centers gave the same results, and have been published in detail elsewhere [13]. Apparently the reaction centers are incorporated in two orientations into the membrane of the vesicles, one catalyzing the proton translocating redox cycle of phenazine methosulfate directed towards internal acidification, like in chloroplasts, and another, prevailing one, which leads to external acidification in the light [13]. In spite of this complication it may be concluded from the experiments reported here, that the primary charge separation in the light, from P700 to another chlorophyll *a* within the large subunit of the photosystem I-reaction center, which is known to occur in the picosecond range [18,19], already spans the membrane dielectric. In addition we can assume that phenazine methosulfate does not substitute for the primary electron acceptor in the reaction center [3], but is secondarily reduced by it.

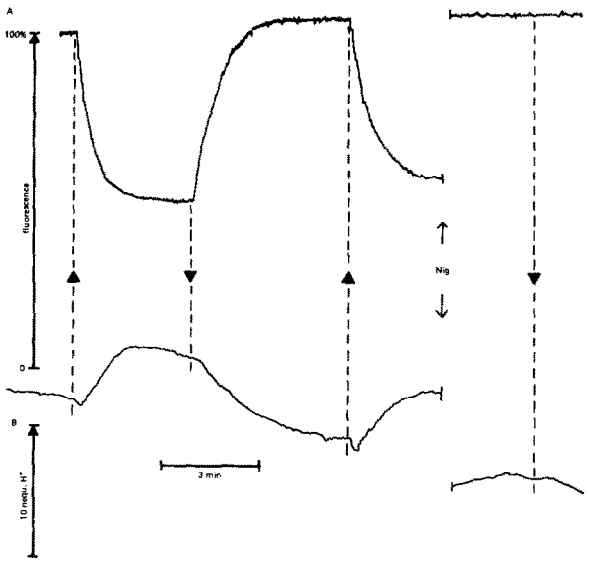

Fig.1. Light-induced proton movements in P700-reaction center liposomes. (A) Fluorescence of 9-aminoacridine was measured as described elsewhere [13]. The reaction mixture contained in 2.5 ml: 50 mM KCl, 20 mM Tricine-NaOH, pH 8.0, 5 mM ascorbate, 50 μM phenazine methosulfate, 5 μM 9-aminoacridine, 1 μg valinomycin and liposomes equivalent to 1 mg lipid containing 0.15 nmol P700. (B) pH-measurements with a glass electrode were carried out under anaerobic conditions [13]. The reaction mixture contained in 3 ml the same components as above, except that buffer was omitted, and the double amount of liposomes was added. The external pH was 7.0. Nigericin (Nig) was added to 1 μg/ml, where indicated. Upward and downward triangles denote light on and off, respectively.

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