THE SITE OF FERRICYANIDE PHOTOREDUCTION IN PEA CHLOROPLASTS PRETREATED BY SILICOMOLYBDIC ACID

Nili PORAT, Gosal BEN-HAYYIM and Ilan FRIEDBERG
Department of Microbiology, The George S. Wise Center for Life Sciences, Tel-Aviv University, Tel-Aviv, Israel

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1. Introduction

In the accepted scheme of photosynthetic electron transport in higher plant, the primary electron acceptor (Q) of photosystem II (PS II) is localized towards the outer surface of the thylakoid membrane [1]. However, fluorescence studies show that this acceptor is not exposed to impermeable reagents such as ferricyanide, as revealed by its inability to reduce ferricyanide in a 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) insensitive manner. Yet, chloroplasts, in which Q has been exposed by silicomolybdate treatment, can photoreduce ferricyanide in a DCMU insensitive reaction [2,3].

Ferrocyanide produced by photoreduction can be complexed with copper ions to form insoluble, electron-dense precipitates of Cu-ferrocyanide, easily detected by electron microscopy [4]. The specific formation of Cu-ferrocyanide precipitates, after silicomolybdate treatment, in the presence of DCMU, was used in this study to localize cytochemically the site of Q in the thylakoid membrane. The presence of such deposits on the outer surface of the membranes indicates that Q is located on or near to this surface.

2. Materials and methods

Pea chloroplasts were isolated as previously described [5] and chlorophyll was determined after Arnon [6]. Chloroplasts (40–50 μg chlorophyll/ml) were incubated in 33 mM 2-[*N*-morpholine] ethanesulfonic acid (MES) buffer, pH 6.0, 33 mM NaCl and 0.1 mM silicomolybdic acid, for 1 min at room temperature, centrifuged and resuspended in 0.2 M sucrose, 0.1 M NaCl and 50 mM *N*–Tris (hydroxymethyl)-methyl glycine (Tricine) buffer, pH 8.0.

Ferricyanide reduction was performed by two procedures: (a) Reaction mixture contained 17 mM MES buffer, pH 6.0, 0.1 M NaCl, 2 mM ferricyanide, 20 mM $CuSO_4$, 0.3 M Na–K-tartrate and 3 μM DCMU; (b) Chloroplasts were incubated for 15 min in 17 mM MES buffer, pH 6.0, 0.1 M NaCl, 20 mM $CuSO_4$, 0.3 M Na–K-tartrate in the dark. Ferricyanide and DCMU were added to a final concentration of 2 mM and 3 μM, respectively.

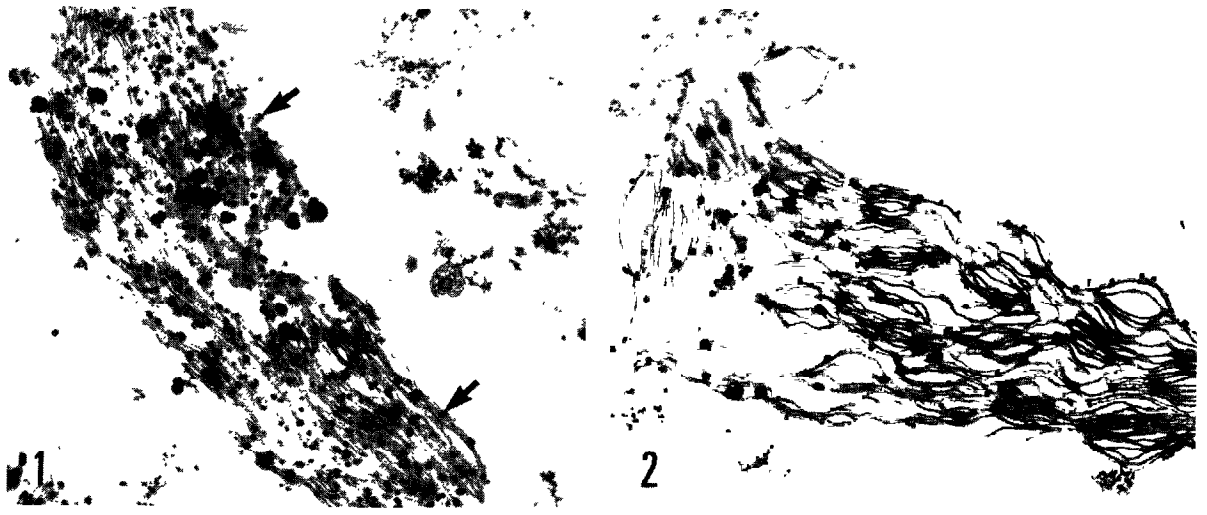
After illumination with a projector lamp (5.10^5 erg $\cdot$ sec^{-1} $\cdot$ cm^{-2}), the chloroplasts were centrifuged at 4°C, washed with 0.1 M phosphate buffer, pH 7.6, fixed for 45 min in 5% glutaraldehyde, and post-fixed for 1 h with 2% OsO_4 in the same buffer. The samples were dehydrated by a series of graded alcohols and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and were examined with Jeol 100-B electron microscope.

Control experiments were performed under the same conditions in the dark.

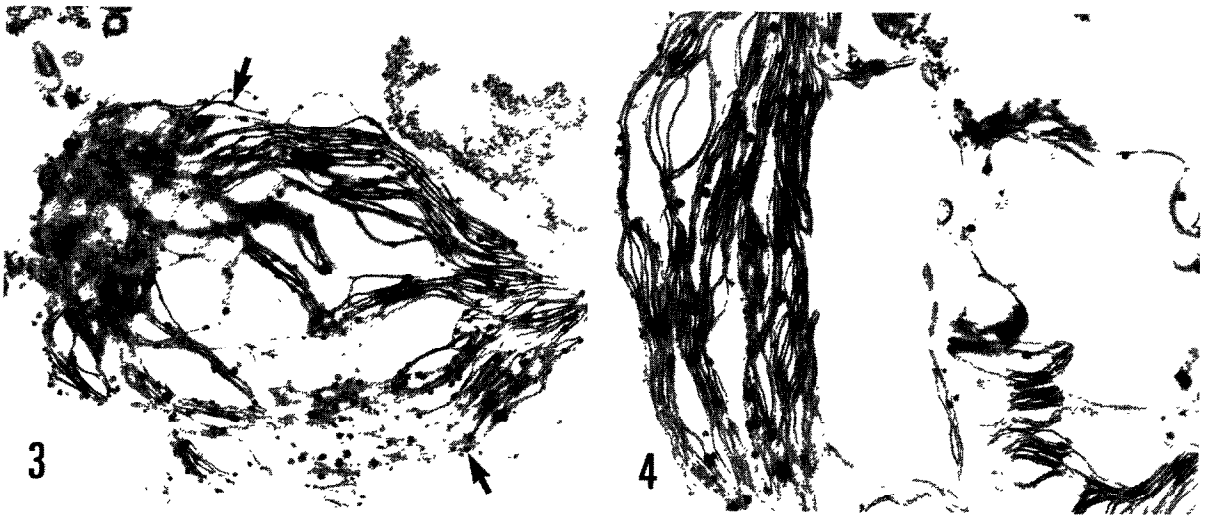
3. Results and discussion

Pea chloroplasts were treated with silicomolybdic acid to expose the primary electron acceptor, Q, of PS II [2,3]. The treated chloroplasts were illuminated in the presence of ferricyanide and Cu^{2+}, and the Cu-ferrocyanide deposits formed upon photoreduction were detected by electron microscopy.

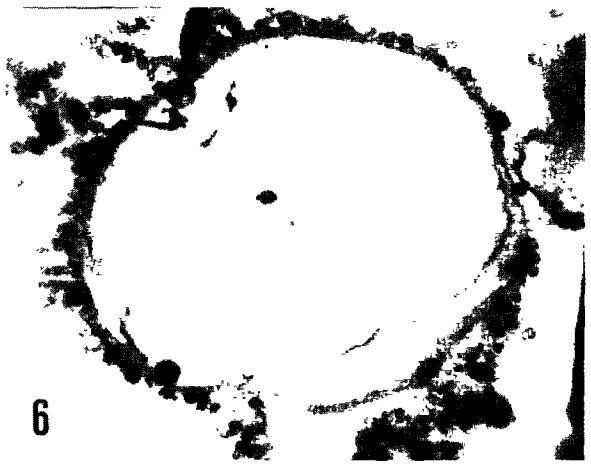
During 2-min illumination, the amount of deposits



Figs.1 and 2. Thin sections of pea chloroplasts. Chloroplasts, pretreated with silicomolybdate were incubated in the reaction mixture for 4 min either in the light (fig.1) or in the dark (fig.2), as described in section 2. Arrows indicate Cu-ferrocyanide deposits (× 16 800).



Figs.3 and 4. Thin sections of pea chloroplasts after preincubation in the dark. Chloroplasts, pretreated with silicomolybdate, were incubated for 15 min in the dark in the reaction mixture, omitting ferricyanide and DCMU as described in section 2. After addition of these reactants the chloroplasts were either illuminated (fig.3) or incubated in the dark (fig.4) for 2 min. Arrows indicate Cu-ferrocyanide deposits (× 16 800).



Figs.5 and 6. Thin sections of illuminated pea chloroplasts at high magnification. The chloroplasts were treated and incubated either as described in fig.1 (fig.5) or in fig.3 (fig.6). Cu-ferrocyanide deposits are localized on the outer surface of the thylakoid membrane (× 90 000).

was relatively small, but after 4 min numerous deposits were observed (fig.1). However, a certain amount of ferricyanide was reduced, as well, during 4-min incubation in the dark, as was shown by deposit formation in non-illuminated control systems (fig.2).

The relatively small precipitation of Cu-ferrocyanide complex appeared after 2-min illumination and might result from a limited penetration of the reactants into the chloroplasts [4]. The penetration was improved by 15-min preincubation of the chloroplasts with the reactants in the dark. Under these conditions, a considerable difference was observed between a 2-min illuminated preparation and a dark control. Numerous deposits were observed in the illuminated chloroplasts (fig.3) as compared to a relatively small amount of precipitates in the non-illuminated control system (fig.4).

Figure 5 and 6 show, at higher magnifications, that the deposits are located on the outer surface solely. Since the Cu-ferrocyanide complex was found to be immobile [7,8], its localization represents the location of Q which has been exposed by silicomolybdic acid. This location is in line with biochemical data, suggesting that Q is facing the outer surface of the thylakoid membrane [1].

References

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