ON THE PATHWAYS OF CYTOCHROME *b*-563 PHOTOREDUCTION IN SPINACH CHLOROPLASTS

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1. Introduction

The role of cytochrome *b*-563 in cyclic electron transport and phosphorylation is generally accepted for the following reasons:

(i) Cytochrome *b*-563 is reduced by photosystem I [1–7] and associated with phosphorylating photosystem I fragments [8,9];
(ii) In developing chloroplasts, it appears along with cyclic photophosphorylation [10,11];
(iii) In intact chloroplasts, the influence of inhibitors and uncouplers on cyclic phosphorylation could be correlated to cytochrome *b*-563 redox reactions [5,12,13].

Oxidation of cyt. *b*-563 by photosystem I involves plastoquinone and part of the non-cyclic electron-transport chain [5]; this also includes an energy conservation step [5,13]. Photoreduction of cyt. *b*-563 by system I depends on ferredoxin [9,14], but not on Fd-$NADP^+$ reductase. These results agree with the generally suggested role of Fd in cyclic phosphorylation [15].

Recent studies with intact, CO_2-fixing chloroplasts suggested the possibility of cyt. *b*-563 reduction not only by photosystem I but also by photosystem II. The evidence was as follows [16]: The red-light induced reduction of cyt. *b*-563 is more complete than that obtained with far-red light and this PS II-mediated cyt. *b*-563 reduction is DCMU-sensitive. Addition of PS I electron acceptors rather enhance than inhibit cyt. *b*-563 reduction. Additional evidence is provided by older data:

(i) Action spectra of cyt. *b*-563 reduction in the absence of DCMU suggested the participation of PS II [5];
(ii) Decrease of the plastocyanin donor pool by KCN-treatment of chloroplasts did not decrease the extent of cyt. *b*-563 photoreduction [12].

This report presents additional data for a direct reduction of cyt. *b*-563 by PS II. Moreover the data show that basically the same light-induced absorbance changes of cytochromes as observed in intact chloroplasts are obtained after mild osmotic shock of these chloroplasts.

Abbreviations: ΔA, absorbance changes; cyt., cytochrome; DCMU, 3-(3′,4′-dichlorophenyl)-1,2-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; Fd, Ferredoxin; PS, Photosystem; tricine, *N*-(tris (hydroxylmethyl)-methyl)-glycine

2. Materials and methods

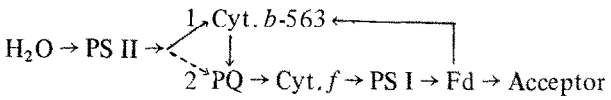
Intact chloroplasts were isolated from spinach according to [17]. The chloroplasts were 80–90% intact as judged by the ferricyanide assay [18]. Light-induced ΔA were measured at 20°C by the dual-wavelength method as in [9,14]. Reference wavelength in all experiments was 570 nm; spectral bandwidth 3 nm. Previous to each measurement the chloroplasts were shocked in a reaction mixture containing (in mM): 100 sorbitol; 50 tricine–NaOH; 10 NaCl; 5 $MgCl_2$, (pH 7.8); 0.1 methylviologen; as indicated. The properties of the Fd antiserum were in [14]. Chloroplasts were added to 2.5 ml reaction mixture at 60 μg chl/ml. Actinic light was defined by interference filters (Balzers, Filtraflex) with maximum transmission at 657 nm and 713 nm, light intensity 40 $J \cdot m^{-2} \cdot s^{-1}$.

3. Results and discussion

The light-induced redox reactions of cyt. *f* and cyt. *b*-563 in intact, CO_2-fixing chloroplasts revealed the following features in the presence of an electron acceptor [16]: After far-red illumination, or red light plus DCMU, the cytochromes assume a more oxidized state (state I). This state is characterized by a completely photooxidizable cyt. *f*, which stays more or less oxidized, after the light has been switched off. Under these conditions cyt. *b*-563 is only partially but reversibly photoreduced by far-red light.

Red light on the other hand reduces cyt. *b*-563 more completely; the cytochrome, however, is only incompletely reoxidized in the dark (state II). The same is true for cyt. *f*. The common pattern is that after red illumination both cytochromes react at a more reduced, after far-red at a more oxidized level.

The same pattern is also observed, if intact chloroplasts are subjected to mild osmotic shock. The tracings of fig.1 show the redox-reactions of cyt. *f* and cyt. *b*-563 under these conditions. Again a more oxidized (state I) and a more reduced (state II) level of reversible redox reactions of cyt. *b*-563 is observed after far-red or red illumination, respectively. Cytochrome *f* on the other hand stays more or less irreversibly reduced in the dark after red-light, whereas cyt. *b*-563 is partially oxidized in the dark. The following red light-induced ΔA are therefore mainly those of cyt. *b*-563 (fig.2). Fig.1 also shows that the rate and extent of cyt. *b*-563 reduction is enhanced by red light as compared to far-red excitation. Addition of the electron acceptor, methylviologen, has no significant influence on the light-induced ΔA measured. Two alternative explanations can be given: Photosystem II causes the additional reduction of cyt. *b*-563 either directly (pathway 1 below) or indirectly, by creation of a reduced plastoquinone pool; under these conditions cyt. *b*-563 could not feed electrons into the chain between both photosystems; hence the reduction of cyt. *b*-563 by system I should be more complete (pathway 2). This is schematically presented by the following sequence:



The distinguishing feature of pathway (1) as com-

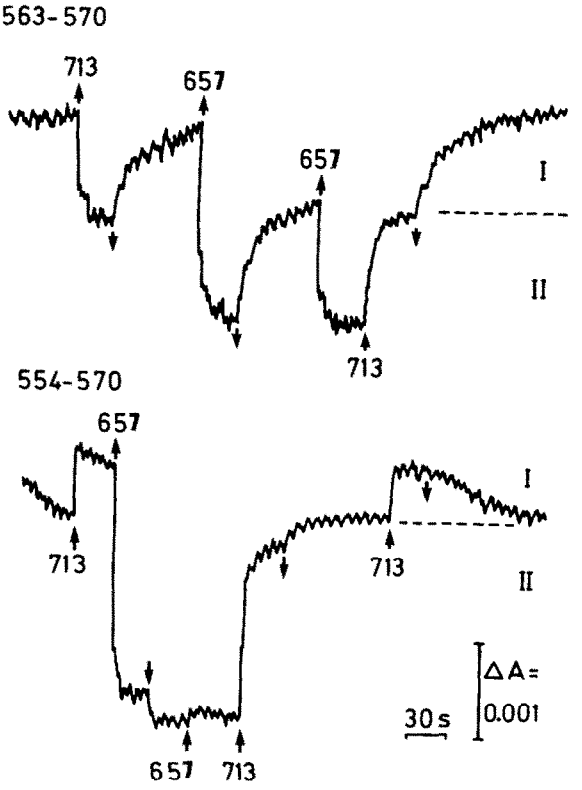


Fig.1. Light-induced ΔA of cyt. *b*-563 and cyt. *f* in the presence of methylviologen. Upward deflection indicates an absorbance decrease; upward arrows, light on; downward arrows, light off. The sample was preilluminated by far-red light. (I/II) System I/II state.

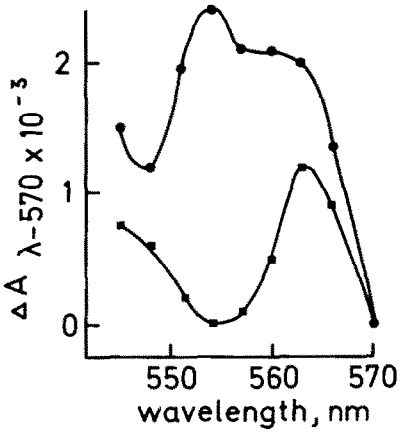


Fig.2. Spectra of the reversible, light-induced ΔA; (•—•) first; (▪—▪) second excitation by 657 nm light. Otherwise conditions as in fig.1.

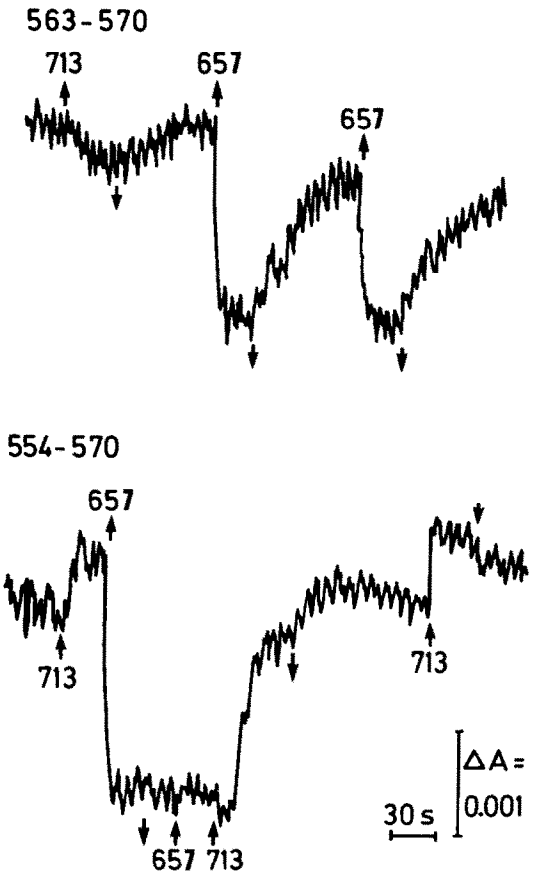


Fig.3. Effect of a ferredoxin antiserum on light-induced redox reactions of cyt. *b*-563 and cyt. *f*; Conditions as in fig.1. The higher noise level after addition of 150 μl of the antiserum is probably due to agglutination of the chloroplasts.

pared to the generally adopted scheme (2) with cyt. *b*-563 on a 'side path' of linear electron flow, is the obligatory participation of Fd in cyt. *b*-563 reduction by PS I.

Inhibiting Fd-dependent reactions by a specific antiserum should allow a distinction between the two possible pathways. The data in fig.3 show that cyt. *b*-563 reduction by PS I (713 nm light) is severely inhibited by the addition of Fd antiserum, whereas system II reduction is still observed. For comparison and control the effect of the same amount of Fd antiserum on cyt. *f* redox reactions is also shown in fig.3. Both red and far-red light-induced ΔA were not influenced. Similarily, addition of a null-serum was without effect. Occasionally it was observed that the antiserum inhibited the rate of both cyt. *f* oxidation and cyt. *b*-563 reduction, which could be restored by addition of methylviologen. Probably the antiserum prevented the reaction of ferredoxin with oxygen.

From these data it is concluded that cyt. *b*-563 is accessible to PS II electrons via a ferredoxin-independent pathway. In addition, it may receive electrons from PS I through a cyclic pathway including ferredoxin. Accepting pathway 1, it is conceivable why the photoreduction of cyt. *b*-563 is not in competition with the photoreduction of a system I electron acceptor [13,16]. Measurements of dark relaxation kinetics after a short flash of red light also indicated a DCMU-sensitive reduction of cyt. *b*-563 by PS II, however, via plastoquinone [19]. It was shown, on the other hand, in intact chloroplasts that system II reduction of cyt. *b*-563 is not inhibited by DBMIB, hence it should not involve plastoquinone [16]. From the data presented here, however, no statement can be made on the relative positions of cyt. *b*-563 and plastoquinone.

Acknowledgements

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