Reconstitution of photosynthetic electron transfer in cyanobacterial heterocyst membranes

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Photosynthetic membranes have been isolated from heterocysts of the cyanobacterium *Anabaena* 7120. Electron transfer reactions associated with photosystem I have been investigated using single turnover flashes. Turnover rates, which were dependent on reconstitution with cytochrome *c*-553 or plastocyanin, were maximal when a suitable electron donor system was provided. Hydrogen (via an endogenous uptake hydrogenase), NADH (via a dehydrogenase) or the ascorbate/dichlorophenolindophenol couple were all effective donors. NADPH could also serve as a donor but this sytem was dependent on addition of ferredoxin and ferredoxin–NADP reductase. Turnovers of P700, soluble cytochrome *c*-553 and cytochrome *b*-563 could be demonstrated.

Anabaena *Heterocyst* *Photosystem I* *Reconstitution* *Cytochrome* *Plastocyanin*

1. INTRODUCTION

Cyanobacterial heterocysts provide a unique system in which to study cyclic electron transfer around a higher plant type of PS I. The heterocyst is a distinct cell type, occurring at a frequency of 5–10% of the total cells in some filamentous cyanobacteria, and it is the site of N_2-fixation under aerobic conditions [1].

The photosynthetic organization of cyanobacteria resembles that of higher plants; however, during differentiation of heterocysts, the O_2-evolving PS II is lost [2]. This is thought to facilitate N_2-fixation by the O_2-sensitive nitrogenase enzyme. The absence of the PS II reaction centres and the diminished levels of accessory pigments in heterocysts [3] give the cells excellent optical properties for spectroscopic investigation.

Abbreviations: chl, chlorophyll; cyt., cytochrome; DCIP, dichlorophenol-indophenol; FNR, ferredoxin:NADP oxidoreductase; HEPES, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid; HQNO, 2-heptyl-4-hydroxyquinoline *N*-oxide; PS I and II, photosystems I and II; PC, plastocyanin

The electron transport components remaining in the heterocysts, following differentiation, include the plastoquinone pool, cyt. *b–f* complex, soluble cyt. *c*-553 and plastocyanin [4,5]. The PS I complex is similar to that of higher plants, consisting of P700 and the membrane-bound Fe–S centres A, B and X [6]. The plastoquinone pool and electron transfer chain can be reduced by an endogenous uptake hydrogenase [7,8].

Flash spectroscopic characterization of photosynthetic electron transfer in isolated intact heterocysts has been reported in [9]. This paper describes photosynthetic oxidation–reduction turnovers in isolated heterocyst membrane preparations that are dependent upon reconstitution with soluble mediators, and upon a suitable supply of reductant.

2. MATERIALS AND METHODS

Anabaena sp. strain 7120 (ATCC 27893) was grown in batch cultures from which heterocysts were isolated under H_2 as in [10].

Photosynthetic membranes were prepared under H_2 from isolated intact heterocysts suspended in

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50 mM KCl, 1 mM $MgCl_2$, 40 mM HEPES (pH 7.5) by passage twice through a French pressure cell at 110 MPa. The broken cell preparation was then centrifuged at 3000 × *g* for 5 min to remove unbroken cells and cell wall fragments. The resulting supernatant was centrifuged at 40000 × *g* for 90 min; the pellet was resuspended in a small volume of buffer to a chlorophyll concentration of 0.8 mg/ml and stored under Ar.

Absorbance changes elicited by brief (4 μs) flashes of saturating red light were measured as in [10,11] under an atmosphere of H_2, except where indicated. Samples in 1.5 ml buffer contained 20 μg chl. *a*. The flash frequency was 1 Hz and traces were the average of 64 (P700 turnovers) or 128 (cytochrome α-band region) records. Traces in the cytochrome α-band region were corrected for contributions of P700, which was measured at 430 nm, using a published spectrum of *Anabaena* P700 [12]. The 430 nm signal was first corrected for the contribution of P430, estimated at 410 nm (a P700 isosbest) and normalized to 430 nm using a P430 spectrum [13].

Cytochrome *c*-553 and plastocyanin were purified from extracts of *Anabaena* 7120 as in [14,15]. Spinach ferredoxin and FNR substituted effectively for the *Anabaena* enzymes.

3. RESULTS AND DISCUSSION

In preparations of photosynthetic membranes from heterocysts there is little apparent turnover of electron-transfer components in response to repetitive flash excitation under Ar. This is shown (fig.1) by monitoring absorbance at 554 nm and 430 nm. Absorbance transients seen at 554 nm may include contributions from P700, cyt. *f*, and cyt. *c*-553 or plastocyanin; at this wavelength, P700 [12] and plastocyanin oxidation produce an increase, and cytochrome oxidation produces a decrease in absorbance. Absorbance changes observed at 430 nm were primarily due to P700 turnover. In the absence of added reductant, plastocyanin and cyt. *c*-553, only a small, rapidly appearing and slowly decaying transient was apparent at 430 nm, indicating that little rereduction of $P700^+$ occurred between flashes. This situation could arise because repetitive flash illumination depletes endogenous reductant or because soluble carriers mediating between cyt. *f* and P700 are deficient in the isolated membrane system.

H_2, in conjunction with the membrane-bound hydrogenase, effectively reduced the electron transfer systems in the membrane and a large, flash-induced, P700 signal was observed, both at

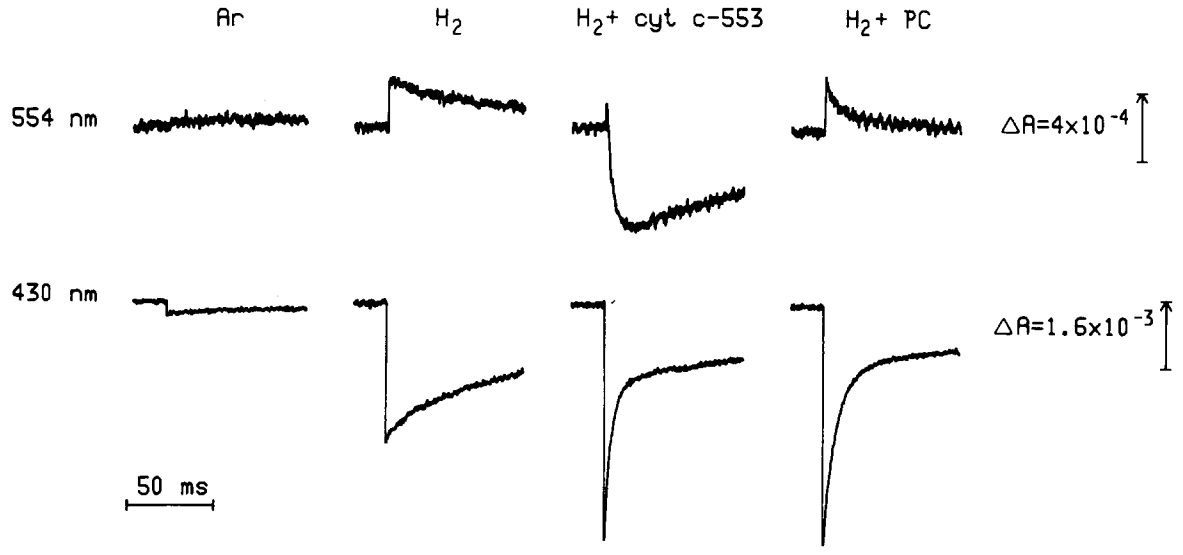

Fig.1. Flash-induced absorbance changes at 554 nm and 430 nm in isolated heterocyst membranes. Cytochrome *c*-553 or plastocyanin (2.2 μM) were added where indicated.

430 nm and 554 nm. Although P700-oxidation was rapid, relaxation of the signal was slow. With the addition of a soluble carrier (cyt. *c*-553 or plastocyanin) the relaxation of the signal at 430 nm became clearly biphasic, with a rapidly decaying component ($t_{0.5}$ = 2.5 ms) and a slow component ($t_{0.5}$ = 150 ms). The carriers facilitated electron transfer between the *b–f* complex and P700, giving rise to the fast decaying component. The slow component may be attributable to membrane components orientated in membrane vesicles such that their active sites were inaccessible to the externally added carrier. P430, the optical signal of the Fe–S centres A and B, which act as electron acceptors for PS I, also contributed at this wavelength [13]. Following a flash, this component would be rapidly reduced, eliciting an absorbance decrease at 430 nm, and would relax only slowly in the absence of O_2 or other PS I acceptors.

Fig.2 shows flash-induced absorbance changes at 554 nm and 563 nm which, after correction for the P700 contribution as in section 2, reflect turnovers of cyt. *c*-553 and cyt. *b*-563, respectively. In the system reconstituted with cyt. *c*-553, oxidation of this cytochrome occurred concomitantly with the fast phase of $P700^+$ reduction (fig.1). Slow relaxation of the signal at 554 nm ($t_{0.5}$ = 93 ms) may result from diffusion of the soluble cytochrome away from the membrane before reduction by the *b–f* complex. Turnover of cyt. *b*-563 was apparent only in the presence of the inhibitor HQNO, which prevented its rapid reoxidation [9].

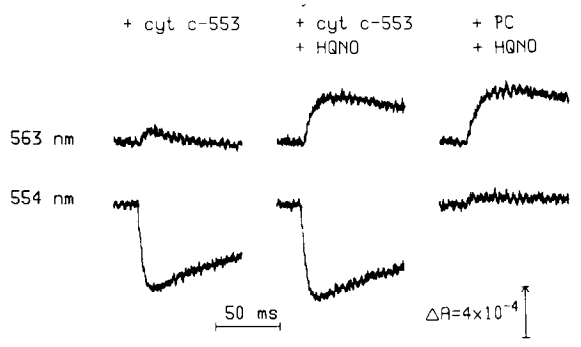

Fig.2. Effect of HQNO (5 μM) on flash-induced turnovers of cyt. *c*-553 and cyt. *b*-563, as measured at 554 nm and 563 nm, respectively. Data have been corrected for contributions of P700.

Flash-induced difference spectra of heterocyst membranes, in the cytochrome α-band region, are shown in fig.3. The bleaching around 554 nm in the system reconstituted with cyt. *c*-553 originated in the oxidation of added cyt. *c*-553 rather than of endogenous cyt. *f*, as this bleaching was absent when electron flow was reconstituted with plastocyanin. Absorbance transients attributable to cyt. *f* would not be expected in response to single-turnover flash excitation, because electron transfer from plastoquinol to cyt. *f* ($t_{0.5}$ = 2–3 ms) [11] is considerably faster than the transfer from cyt. *f* to cyt. *c*-553 in reconstituted systems ($t_{0.5}$ = 93 ms, see fig.2). This kinetic feature prevents significant accumulation of oxidized cyt. *f*.

The flash-induced spectrum of cyt. *b*-563 is apparent (fig.3) in membranes reconstituted with either cyt. *c*-553 or plastocyanin, as an increase in absorbance around 563 nm. No well-defined spectral signal attributable to plastocyanin was discerned owing to its small extinction coefficient.

The effectiveness of alternative electron donor systems was examined by monitoring P700 turnover at 430 nm, in the presence of cyt. *c*-553, as shown in fig.4. P700 relaxed slowly in the absence of an added donor ($t_{0.5}$ = 35 ms) indicating the

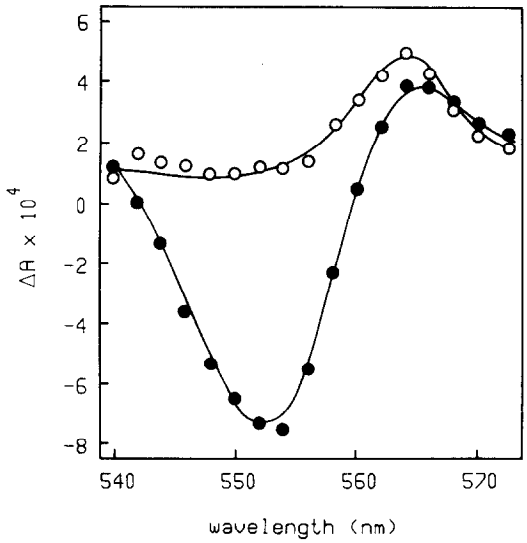

Fig.3. Flash-induced difference spectrum of membranes reconstituted with 2.2 μM cyt. *c*-553 (●—●) and 2.2 μM plastocyanin (○—○), in the presence of HQNO (5 μM). Absorbance changes were measured 50 ms after the flash, when the contribution of P700 was minimal.

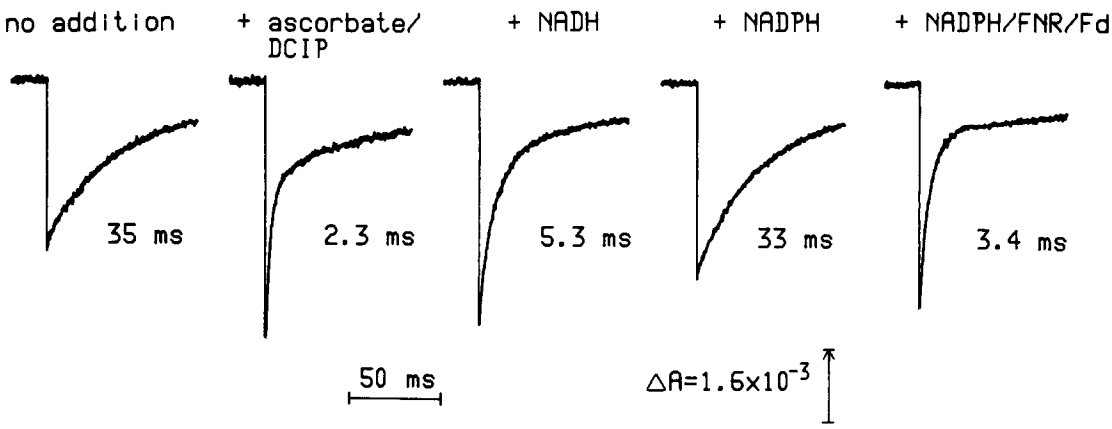

Fig.4. Effect of various electron donor systems on the turnover of P700, as measured at 430 nm, under an Ar atmosphere and in the presence of 2.2 μM cyt. *c*-553. Sodium ascorbate (0.5 mM), DCIP (10 μM), NADH or NADPH (100 μM), ferredoxin (6.6 μM) and FNR (0.5 units) were added as indicated. The half-time for the fast phase of relaxation is given in ms.

presence of some endogenous reductant which, however, was less efficient than H_2 (fig.1) in reducing the cyt. *c*-553 pool, as evidenced by the $P700^+$ rereduction rates. In the presence of an electron donor such as ascorbate, and the mediator DCIP, P700 turnover occurred; this was extensive and relaxed with a rapid ($t_{0.5}$ = 2.3 ms) and a slow phase. Reduced DCIP probably donates electrons to both cyt. *c*-553 and P700 since omission of cyt. *c*-553 resulted in a slower ($t_{0.5}$ = 45 ms) rereduction.

NADH was able to reduce the electron transfer chain as in [10]. NADPH was ineffective as a donor unless ferredoxin and FNR were also included and this donor system probably reduced the cyt. *c*-553 pool directly. These findings confirm that a membrane-bound NADH dehydrogenase is able to reduce the plastoquinone pool, whereas electron input from NADPH depends on restoration of ferredoxin and FNR [10]. Turnover of components of the electron transport pathway requires addition of an exogenous donor because pools of endogenous reductants are lost during isolation of heterocysts. In vivo, reductant is normally provided as fixed carbon compounds, which are transported into the heterocyst from adjacent vegetative cells [16].

During membrane isolation, several functional components are lost, including plastocyanin, cyt. *c*-553, ferredoxin and FNR. The rates of electron transfer between the *b–f* complex and P700 were enhanced considerably by addition of plastocyanin or cyt. *c*-553 and both carriers were equally effective in this function. These components occur interchangeably in some cyanobacteria [17], with cyt. *c*-553 replacing plastocyanin in copper-deficient cultures. Selective reconstitution with one or other of these components is a useful tool in distinguishing and assigning absorbance transients observed with single turnover flashes. This system should prove useful, in further studies, for investigating the acceptor side of photosystem I and mechanisms involved in cyclic electron transfer.

ACKNOWLEDGEMENTS

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