# Kinetic and spectral resolution of cytochrome *c*-553 and cytochrome *f* in the photosynthetic electron-transfer chain of heterocysts

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Oxidation of cytochrome *c*-553 and cytochrome *f* following single-turnover flashes was measured in isolated heterocysts. Half-times of oxidation for these two cytochromes were determined by measuring the rate of bleaching at 554 nm in heterocysts possessing cytochrome *c*-553, or in heterocysts in which cytochrome *c*-553 was replaced by plastocyanin. Flash-induced difference spectra demonstrate that a fast bleaching ($t_{0.5}$ = 20–35 $\mu$s) corresponds to the oxidation of cytochrome *c*-553, and a slower bleaching ($t_{0.5}$ = 87 $\mu$s) corresponds to the oxidation of cytochrome *f*.

*Heterocyst* *Cytochrome* f *Cytochrome* c-*553* *Plastocyanin* Anabaena
*Photosynthetic electron transfer*

## 1. INTRODUCTION

Energy transducing electron-transport chains from chloroplasts, mitochondria and bacteria share a number of common features. In each case a quinol donates two electrons to a membrane-bound complex containing a high potential Fe–S center, two cytochrome *b* hemes and a bound *c*-type cytochrome [1]. The oxidant for this complex in mitochondria and photosynthetic bacteria is a soluble *c*-type cytochrome which mediates electron flow to either cytochrome oxidase or a photosynthetic reaction center. In higher plants, this function has been replaced by a $Cu^{2+}$ protein, plastocyanin. Both proteins occur in some cyanobacteria [2] and function interchangeably in the electron-transport chains of these organisms [3]. Cytochrome *c*-553 and plastocyanin have similar $M_r$-values and midpoint potentials, and both are released into the soluble fraction upon disruption of thylakoids [4]. The relative abundances of these mediator proteins is determined by the level of $Cu^{2+}$ in the growth medium with cyt. *c*-553 substituting for plastocyanin under conditions of $Cu^{2+}$ deficiency [2].

*Anabaena* 7120 is a filamentous cyanobacterium in which both plastocyanin and cyt. *c*-553 occur. During growth under $N_2$-fixing conditions about 10% of the cells in the filament differentiate into heterocysts which are the site of $N_2$ fixation and have a modified photosynthetic apparatus designed to protect the $O_2$-labile nitrogenase complex from oxygen inactivation [5]. Photosystem II is eliminated during differentiation, but photosystem I and the associated electron-transfer chain are retained. Electrons from reductants such as $H_2$ may be donated to nitrogenase via the photosystem, with the necessary ATP being generated by cyclic electron transport [6]. The elimination of photosystem II results in low ratios of chlorophyll to P700 (90/1) [7] and makes heterocysts excellent subjects for the study of optical changes resulting from electron flow around photosystem I.

This report describes studies of flash-induced oxidations of *c*-type cytochromes in isolated heterocysts possessing either cyt. *c*-553 or plastocyanin.

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## 2. MATERIALS AND METHODS

Growth of *Anabaena* 7120 and isolation of heterocysts were done as in [6] except that culture bottles were rinsed with 12 N HCl before preparation of medium.

The plastocyanin content of isolated heterocysts was determined by EPR spectroscopy as in [2]. For determination of cyt. *c*-553, heterocysts suspended in 0.2 M KCl, 1 mM $MgCl_2$, 40 mM Hepes (pH 7.5) were broken by 3 passes through a French pressure cell at 140 MPa. The crude extract was frozen and thawed twice and centrifuged at 200000 × *g* for 1 h. Cyt. *c*-553 content of the supernatant was determined by measuring the ascorbate-minus-ferricyanide difference spectrum at 77 K and comparing the peak height at 553 nm to a standard curve prepared with purified *Anabaena* cyt. *c*-553. P700 was determined by measuring the reversible bleaching at 703 nm upon illumination in the presence of 1 mM ascorbate and 20 $\mu$M tetramethylphenylenediamine. An extinction coefficient of 70 $mM^{-1}.cm^{-1}$ was assumed [8].

Flash-induced absorbance changes and time-resolved difference spectra were measured under 90% $H_2$ plus 10% $O_2$ as in [9]. Cuvettes contained 16 $\mu$g chl./ml as isolated heterocysts in a reaction medium consisting of 50 mM KCl, 7% Ficoll, 1 mM $MgCl_2$, 25 $\mu$M KCN and 40 mM Hepes (pH 7.5). In all cases instrument response times were chosen which did not interfere with measurements of cyt. *c* oxidation kinetics.

## 3. RESULTS AND DISCUSSION

The levels of cyt. *c*-553 and plastocyanin in some cyanobacteria are determined by $[Cu^{2+}]$ in the growth medium [2]. The cyt. *c*-553 and plastocyanin content of cells of *Anabaena* 7120, grown at 3 different $Cu^{2+}$ levels, are shown in table 1. A small amount of cyt. *c*-553 is present even at 1.0 $\mu$M $Cu^{2+}$. The substitution of cyt. *c*-553 for plastocyanin is partial in cells grown at 0.04 $\mu$M $Cu^{2+}$ and is complete in $Cu^{2+}$-free cultures. Flash-induced absorbance changes were measured in heterocysts isolated from filaments grown at these different levels of $Cu^{2+}$, and the spectral and kinetic changes resulting from this substitution have been examined and are presented below.

Table 1

Cytochrome *c*-553 and plastocyanin content of heterocysts isolated from filaments grown at 3 $Cu^{2+}$ levels

| Culture $Cu^{2+}$ ($\mu$M) | Cyt. *c*-553/ P700 | Plastocyanin/ P700 | Plastocyanin/ Cyt. *c*-553 |
|---|---|---|---|
| 0.00 | 1.28 | 0.00 | 0.0 |
| 0.04 | 0.51 | 0.60 | 1.2 |
| 1.00 | 0.20 | 0.74 | 3.8 |

Fig.1 shows flash-induced spectra for heterocysts from cultures grown at 1 $\mu$M $Cu^{2+}$ (containing plastocyanin) or under $Cu^{2+}$-free conditions (containing cyt. *c*-553). These spectra are similar to those in [9]; however, large differences between the two are visible in the cytochrome $\alpha$-band and $\gamma$-band regions. The difference between

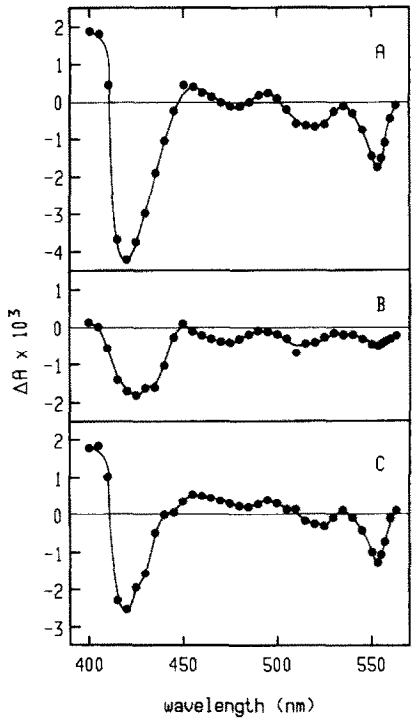


Fig.1. Flash-induced difference spectra of isolated heterocysts. Cuvettes contained 16 $\mu$ chl./ml as isolated heterocysts. The absorbance change at 400 $\mu$s after the flash was measured at each wavelength: (A) $Cu^{2+}$-free heterocysts; (B) Heterocysts from filaments cultured in 1 $\mu$M $Cu^{2+}$; (C) (A) – (B) difference.

the two spectra obtained 400 $\mu$s after a flash is plotted in fig.1c and corresponds to cyt. *c*-553. Light scattering and optical flattening effects probably account for the lower $\gamma$-band: $\alpha$-band ratio in this spectrum as compared to pure cyt. *c*-553.

The oxidation–reduction kinetics of *c*-type cytochromes in heterocysts grown at 3 different levels of $Cu^{2+}$ are shown in fig.2. Cells grown on 1 $\mu$M $Cu^{2+}$ have little cyt. *c*-553 and the absorbance change at 554 nm in these cells is primarily due to cyt. *f*. The observed bleaching at 554 nm is larger and appears more rapidly in heterocysts from $Cu^{2+}$-free cultures. In these cells the initial bleaching is attributable primarily to the oxidation of cyt. *c*-553 and only a slight absorbance change would be expected at this wavelength upon oxidation of cyt. *f* by cyt. *c*-553. In cells grown at intermediate levels of $Cu^{2+}$, cyt. *c*-553 and plastocyanin participate simultaneously in electron transfer from the cyt. *b*/*f* complex to P700. The extent of the turnover at 554 nm in these cells is intermediate between the two extremes and is comprised of contributions from the oxidation of cyt. *c*-553 by P700 and the oxidation of cyt. *f* by plastocyanin. The half-times for the relaxation of the cyt. *c* transient (fig.2) are similar regardless of whether the bleaching is due primarily to cyt. *f* or cyt. *c*-553. This suggests that rapid equilibration occurs between cyt. *f* and the cyt. *c*-553/plastocyanin pool.

Table 2 summarizes the kinetics of cytochrome oxidations and the magnitudes of the absorbance changes at the 3 $Cu^{2+}$ levels tested. An onset half-time of 35 $\mu$s was determined for the oxidation of cyt. *c*-553 in $Cu^{2+}$-free cultures. When cyt. *c*-553 was replaced by plastocyanin (1 $\mu$M $Cu^{2+}$), a slower onset time corresponding to the oxidation of cyt. *f* was observed. Both a fast and a slow component of cytochrome oxidation were apparent in cells grown at intermediate $Cu^{2+}$ levels corresponding to the oxidations of cyt. *c*-553 and cyt. *f*, respectively. Fig.3 shows time-resolved, flash-induced difference spectra obtained from heterocysts grown at the intermediate $Cu^{2+}$ level. The spectrum measured 40 $\mu$s after the flash has a peak at 552.5 nm and closely resembles the $\alpha$-band of cyt. *c*-553 [10]. The change occurring between 40 and 300 $\mu$s has a peak at 556 nm and a shoulder at 550 nm, as does the oxidized-minus-reduced difference spectrum of cyanobacterial cyt. *f* [11].

Table 2

Summary of cyt. *c*-type cytochrome oxidation rates and magnitudes of the corresponding absorbance changes

| Culture $Cu^{2+}$ ($\mu$M) | Fast phase | | Slow phase | |
|---|---|---|---|---|
| | $t_{0.5}$ ($\mu$s) | $A \times 10^3$ | $t_{0.5}$ ($\mu$s) | $A \times 10^3$ |
| 0.00 | 35 | 1.7 | – | – |
| 0.04 | 20 | 0.25 | 77 | 0.45 |
| 1.00 | – | – | 87 | 0.45 |

The rates of electron transfer reactions involving the soluble mediator proteins, cyt. *c* and plasto-

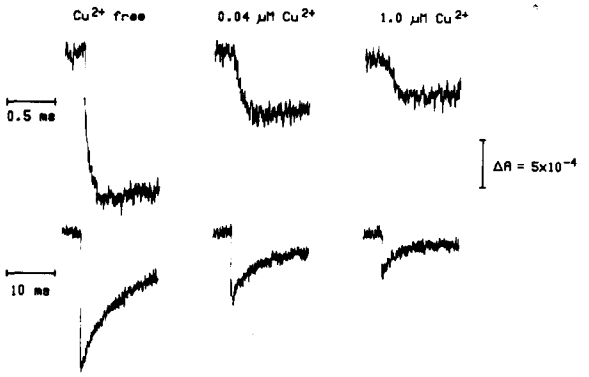


Fig.2. Oxidation and reduction kinetics of cyt. *f* and *c*-553, measured at 554 nm, in heterocysts grown at 3 $Cu^{2+}$ levels. Traces are averages of 512 events (1 ms sweeps) or 128 events (20 ms sweeps).

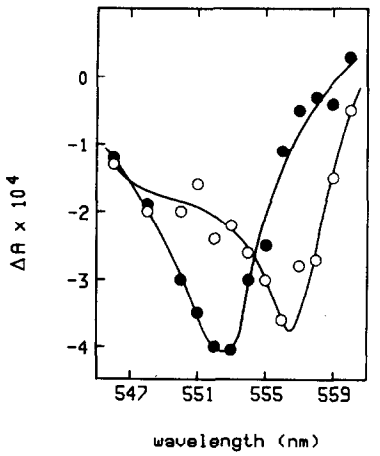


Fig.3. Time-resolved, flash-induced difference spectra of heterocysts grown in 0.04 $\mu$M $Cu^{2+}$: (●) 40 – 0 $\mu$s difference; (○) 300 – 40 $\mu$s difference.

cyanin, have been studied in chloroplasts, photosynthetic bacteria and cyanobacteria. A half-time of 15 $\mu$s for cyt. *c* oxidation and one phase of P700 reduction in filaments of *Plectonema boryanum* was measured in [12]. In *Rhodopseudomonas sphaeroides* a soluble cyt. $c_2$ is oxidized with a half-time of 5 $\mu$s following an actinic flash and this cytochrome accepts an electron from a membrane-bound cyt. $c_1$ with a half-time of 150 $\mu$s [13]. Similar kinetics have been reported for the analogous reactions in chloroplasts where P700 is reduced by plastocyanin with a half-time of 10–20 $\mu$s [14,15] and cyt. *f* is oxidized by plastocyanin following a flash with a half-time of about 300 $\mu$s [16].

The oxidation rate of cyt. *c*-553 (20–35 $\mu$s) is similar to that reported for other photosynthetic systems. The oxidation rate of cyt. *f* in heterocysts is somewhat faster than for the analogous reactions in chloroplasts and photosynthetic bacteria. If substantial reduction of cyt. *f* by the Rieske Fe–S center occurs before oxidation of cyt. *f* is complete, thereby diminishing the apparent extent of cyt. *f* oxidation, a shorter apparent half-time for cyt. *f* oxidation will be observed [17]. In chloroplasts, 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole (UHDBT) blocks this electron transfer and allows determination of the cyt. *f* oxidation rate without interference from the competing reduction reaction [16]; this inhibitor, however, is not effective in heterocysts [9]. The true half-time for cyt. *f* oxidation may thus be greater than the value of 87 $\mu$s reported here. On the other hand, the fastest phase of cyt. *c* reduction following a flash, presumably corresponding to the donation of an electron from the Rieske Fe–S center to cyt. *f*, occurs with a half-time of 0.4–0.5 ms [17]; hence, the error introduced by this competing reaction is probably small. The oxidation kinetics for cyt. *c*-553 and cyt. *f* in heterocysts are consistent with the sequential electron transfer:

cyt. *f* $\longrightarrow$ cyt. *c*-553 $\longrightarrow$ P700.

In *Anabaena* 7120 and some other cyanobacteria, cyt. *c*-553 and plastocyanin are produced interchangeably in response to the $Cu^{2+}$ status of the growth medium [2,18]. Both proteins can effectively reconstitute photosynthetic electron transfer in washed thylakoids [3]. Here, we demonstrate in vivo the effective substitution of cyt. *c*-553 for plastocyanin in flash-induced turnovers of the photosynthetic electron-transfer chain. Cells possessing either mediator protein have fully competent electron transfer chains with rapid turnover kinetics comparable to those seen in other photosynthetic systems.

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## REFERENCES

[1] Hauska, G., Hurt, E., Gabellini, N. and Lockau, W. (1983) Biochim. Biophys. Acta, in press.
[2] Sandmann, G. and Böger, P. (1980) Plant Sci. Lett. 17, 417–424.
[3] Lockau, W. (1981) Arch. Microbiol. 128, 336–340.
[4] Wood, P.M. (1978) Eur. J. Biochem. 87, 9–19.
[5] Stewart, W.D.P. (1980) Annu. Rev. Microbiol. 34, 497–536.
[6] Houchins, J.P. and Hind, G. (1982) Biochim. Biophys. Acta 682, 86–96.
[7] Almon, H. and Böhme, H. (1980) Biochim. Biophys. Acta 592, 113–120.
[8] Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160–171.
[9] Houchins, J.P. and Hind, G. (1983) Arch. Biochem. Biophys., in press.
[10] Susor, W.A. and Krogmann, D.W. (1966) Biochim. Biophys. Acta 120, 65–72.
[11] Böhme, H., Pelzer, B. and Böger, P. (1980) Biochim. Biophys. Acta 592, 528–535.
[12] Hiyama, T. and Ke, B. (1971) Biochim. Biophys. Acta 226, 320–327.
[13] Meinhardt, S.W. and Crofts, A.R. (1982) FEBS Lett. 149, 223–227.
[14] Haehnel, W., Döring, G. and Witt, H.T. (1971) Z. Naturforsch. 26b, 1171–1174.
[15] Bouges-Bocquet, B. (1975) Biochim. Biophys. Acta 396, 382–391.
[16] Whitmarsh, J., Bowyer, J.R. and Crofts, A.R. (1982) Biochim. Biophys. Acta 682, 404–412.
[17] Houchins, J.P. and Hind, G. (1983) submitted.
[18] Hawkesford, M.J., Houchins, J.P. and Hind, G. (1983) FEBS Lett. 159, 262–266.