THE PHOTOOXIDATION OF CHLOROPLAST CYTOCHROME b_6 BY PHOTOSYSTEM I

David B. KNAFF
Department of Cell Physiology, University of California, Berkeley, Calif. 94720, USA

Received 10 March 1972

1. Introduction

The existence of a *b*-type cytochrome with an α-band absorbance maximum at 563 nm in chloroplasts of green plants was first demonstrated by Hill [1] who designated it cytochrome b_6. Hill and Bendall [2] determined a value of 0.0 V for the midpoint potential of cytochrome b_6 in etiolated barley chloroplasts. However, Fan and Cramer [3] have recently reported a value of −180 mV for the midpoint potential of cytochrome b_6 in spinach chloroplasts.

Although there is some disagreement regarding the midpoint potential of cytochrome b_6, there is general agreement that cytochrome b_6 is associated with Photosystem I. It has been shown in several laboratories [4–6] that cytochrome b_6 can be photoreduced by the long-wavelength light ($\lambda > 680$ nm) characteristic of Photosystem I. In addition, Photosystem I particles prepared by digitonin treatment contain cytochrome b_6 [7, 8].

Arnon and co-workers [9] have proposed that cytochrome b_6 functions as an electron carrier in a phosphorylating Photosystem I cyclic electron transport pathway:

$$\mathrm{Chl_I} \xrightarrow{h\nu} \mathrm{Fd} \rightarrow \mathrm{cyt.}\, b_6 \rightarrow \mathrm{cyt.}\, f \rightarrow \mathrm{Chl_I}$$

where $\mathrm{Chl_I}$ is the photoactive chlorophyll of Photosystem I and Fd is the iron–sulfur protein, ferredoxin.

If cytochrome b_6 actually does function in such a cyclic pathway, it should be both photoreduced [4–6] and photooxidized by Photosystem I. The photooxidation of cytochrome b_6 by Photosystem I light in chloroplasts poised at an ambient potential low enough to reduce cyrochrome b_6 has been investigated and the midpoint potential of the cytochrome b_6 that is photooxidized has been estimated to be near 0.0 V.

2. Methods

Washed, "broken" spinach chloroplasts (P_{1S1}) were prepared by the method of Whatley and Arnon [10] and Tris-treated spinach chloroplasts were prepared by a modification [11] of the method of Yamashita and Butler [12]. Chlorophyll was determined by the method of Arnon [13].

Absorbance changes were measured with a dual wavelength spectrophotometer (Phoenix Precision Instrument Co.) as described previously [14]. The half-band width of the measuring beam was 2.0 nm.

All of the absorbance measurements were made under anaerobic conditions using a cell similar to that described by Cramer and Butler [15, 16] and by Dutton [17]. Sufficiently anaerobic conditions were obtained by this technique to reductively titrate benzyl viologen ($E'_0 = -332$ mV, $n = 0.97$; in good agreement with reported values [18]). A chloroplast sample poised for 5 min at a potential of −150 mV and then returned to its original potential retained the ability to reduce NADP from water ($Q_{2e} = 215$ μmole/mg chlorophyll per hr) and showed a 2.8-fold increase in the rate of NADP reduction on addition of ADP, indicating that the chloroplasts retained photophosphorylation activity.

To facilitate equilibration between the chloroplast electron carriers and the platinum electrode, the following mediators were used: 30 μM 2,5-dimethylbenzoquinone ($E'_0 = +180$ mV); 10 μM 1,4-naphthoquinone ($E'_0 = +60$ mV); 10 μM 5-hydroxy-1,4-naph-

North-Holland Publishing Company – Amsterdam

thoquinone (E'_0 = +30 mV); 10 μM 2-hydroxy-1,4-naphthoquinone (E'_0 = −145 mV); and 10 μM anthraquinone-1,5-disulfonate (E'_0 = −170 mV). These mediators gave no detectable absorbance changes on reduction in the spectral region from 540 to 580 nm at the indicated concentration.

After the desired potential became stable, the sample was allowed to remain at this potential for several minutes before illumination to insure thorough equilibration. The ambient potential after illumination was always within 5 mV of the potential before illumination.

3. Results and discussion

Fig. 1 shows that illuminating chloroplasts poised at either −75 mV or −205 mV with Photosystem I light (715 nm) results in a rapid decrease in absorbance at 564 nm. The spectrum of this light-induced absorbance decrease at these two potentials, shown in fig. 2, exhibits a minimum at 564 nm, consistent with a photooxidation of cytochrome b_6 [1–8, 19]. As would be expected for a Photosystem I reaction, neither addition of the Photosystem II inhibitor 3-(3′,4′-dichlorophenyl)-1,1-dimethyl urea (DCMU) [9] nor Tris-treatment (a treatment which inhibits oxygen evolution without affecting Photosystem I

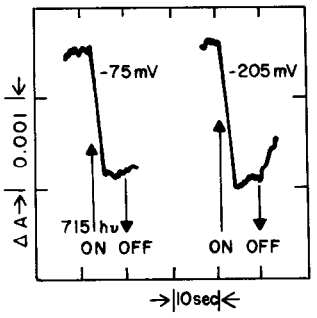

Fig. 1. Photooxidation of cytochrome b_6 by Photosystem I light (564 nm *minus* 570 nm). The reaction mixture contained (per 1.0 ml) washed, broken spinach chloroplasts (P_1S_1) equivalent to 75 μg chlorophyll and the following, in μmoles: Tricine [*N*-Tris(hydroxymethyl)methyl glycine] buffer (pH 7.9), 50; 5-hydroxy-1,4-naphthoquinone, 0.01; 2-hydroxy-1,4-naphthoquinone, 0.01; and anthraquinone-1,5-disulfonate, 0.01. The 715-nm actinic light had an intensity of 1 × 10^4 ergs/cm^2 per sec.

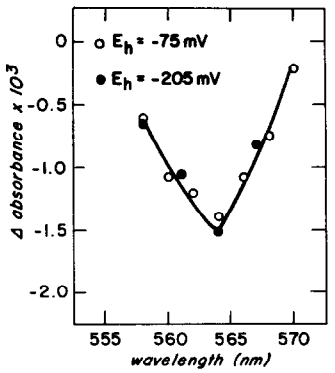

Fig. 2. Spectrum of cytochrome b_6 photooxidation by Photosystem I light (715 nm). Experimental conditions were as described in fig. 1. Reference wavelength, 570 nm.

activity [12]) had any effect on the cytochrome b_6 photooxidation.

The fact that one can observe a photooxidation of cytochrome b_6 at a potential of −75 mV and that the extent of the photooxidation is the same at −75 mV and −205 mV indicates that cytochrome b_6 is fully reduced at −75 mV. This would be true if the potential of the cytochrome were 0.0 V, as reported by Hill and Bendall [2]. However, if the potential were −180 mV, as reported by Fan and Cramer [3], the cytochrome would be completely oxidized at −75 mV and no photooxidation would be expected to occur.

More recent evidence for a potential near 0.0 V for cytochrome b_6 comes from the work of Erixon and Butler [20] who showed (see fig. 1 in [20]) that cytochrome b_6 was completely oxidized at +55 mV and completely reduced at −67 mV. This result has been confirmed in our own laboratory. An absorbance increase at 563 nm *minus* 572 nm caused by the reduction of cytochrome b_6 may be observed in a transition from +60 mV to −60 mV, indicating that the potential of cytochrome b_6 is near 0.0 V. No absorbance changes at 563 nm *minus* 572 nm were observed at other potentials in the range from +100 mV to −300 mV. In particular, there was no increase in absorbance at 563 nm *minus* 572 nm observed in a transition from −100 mV to −250 mV, as would be expected from the reduction of a *b*-type cytochrome with the potential of −180 mV reported by Fan and Cramer [3] for cytochrome b_6. Addition of uncoupler (5 mM NH_4Cl) or ATP (5 mM) had no effect on this pattern.

4. Concluding remarks

Cytochrome b_6 can be photooxidized by the long-wavelength light characteristic of Photosystem I. This observation, along with the previously demonstrated Photosystem I reduction of cytochrome b_6 [4–6], is consistent with the idea that cytochrome b_6 functions as an electron carrier in a cyclic electron transport chain [9]. The oxidation–reduction potential of the cytochrome b_6 that can be oxidized by Photosystem I appears to be 0.0 V.

Acknowledgements

The author would like to thank Mr. William Ufert for excellent technical assistance. This investigation was aided by a grant from the National Science Foundation to Daniel I. Arnon (GB-30494X).

References

[1] R. Hill, Nature 174 (1954) 501.
[2] R. Hill and D.S. Bendall, in: Biochemistry of Chloroplasts, Vol. 2, ed. T.W. Goodwin (Academic Press, London, 1967) p. 559.
[3] H. Fan and W.A. Cramer, Biochim. Biophys. Acta 216 (1970) 200.
[4] G. Hind and J.M. Olson, Brookhaven Symp. Biol. 19 (1966) 189.
[5] W.A. Cramer and W.L. Butler, Biochim. Biophys. Acta 143 (1967) 332.
[6] J. Weikard, Z. Naturforschung. 23b (1968) 245.
[7] N.K. Boardman and J. Anderson, Biochim. Biophys. Acta 143 (1967) 187.
[8] D.I. Arnon, H.Y. Tsujimoto, B.D. McSwain and R.K. Chain, in: Comparative Biochemistry and Biophysics of Photosynthesis, eds. K. Shibata, A. Takamiya, A.T. Jagendorf and R.C. Fuller (Univ. Park Press, State College, Pa., USA, 1968) p. 113.
[9] D.I. Arnon, H.Y. Tsujimoto and B.D. McSwain, Nature 214 (1967) 562.
[10] F.R. Whatley and D.I. Arnon, in: Methods in Enzymology, Vol. 6, eds. S.P. Colowick and N.O. Kaplan (Academic Press, New York, 1963) p. 308.
[11] D.B. Knaff and D.I. Arnon, Biochim. Biophys. Acta 223 (1970) 201.
[12] T. Yamashita and W.L. Butler, Plant Physiol. 43 (1968) 1978.
[13] D.I. Arnon, Plant Physiol. 24 (1949) 1.
[14] D.B. Knaff and D.I. Arnon, Proc. Natl. Acad. Sci. U.S. 63 (1969) 956.
[15] W.A. Cramer and W.L. Butler, Biochim. Biophys. Acta 172 (1969) 503.
[16] W.A. Cramer, Biochim. Biophys. Acta 189 (1969) 54.
[17] P.L. Dutton, Biochim. Biophys. Acta 226 (1971) 63.
[18] W.M. Clark, Oxidation-Reduction Potentials of Organic Systems (Williams and Wilkins, Baltimore, Md., 1960).
[19] K. Wada and D.I. Arnon, Proc. Natl. Acad. Sci. U.S. 68 (1971) 3064.
[20] K. Erixon and W.L. Butler, Biochim. Biophys. Acta 234 (1971) 381.