LIGHT INDUCED REDOX REACTIONS OF CYTOCHROME C BY CELL-FREE PREPARATION OF ANABAENA CYLINDRICA

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In our work on hydrogenase system of Anabaena cylindrica (4), we found that the subcellular fraction which retained a hydrogenase system also had activities for Hill reactions (DCPIP and ferricyanide) and for NADP photoreduction similarly to green plant chloroplasts. This preparation, however, was found to carry out the light induced redox reaction of cytochrome c in a different way from that observed in spinach chloroplasts by Nieman et al (9,10) and Kok et al (7,8). In this communication we report the essential features of the reactions observed.

Cell-free preparations were obtained with use of polyethylene glycol medium following previous work on Anacystis by Van Baalen (11) and Fredricks et al (3). Composition of the medium per liter was as follows: Tris buffer (pH 7.0), 0.1 moles; $MgCl_2$, 0.001 moles; $CaCl_2$, 0.001 moles; EDTA, 0.001 moles; polyethylene glycol, 400 g. Cells suspended in this medium were disrupted by a Virtis homogenizer with glass beads for 5 minutes, and the homogenates were fractionated by

differential centrifugation. The subcellular fraction obtained as precipitate by 21,000 X g, 3 hour centrifugation was used. Cytochrome redox reaction was followed by a Cary Recording Spectrophotometer Model 14 which had an added illumination system for the sample cuvette. Each measurement during illumination required interruption of the actinic light which generally took about 1 second.

In Fig. 1 are shown time courses of cytochrome redox reaction by the present preparation. With highly oxidized cytochrome c, initial illumination with 630 mμ

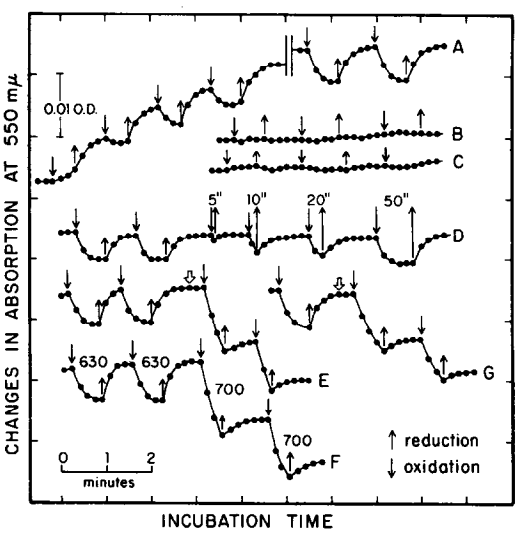

Fig. 1. Cytochrome c redox reactions by Anabaena cell-free preparation under various conditions. Reaction mixture contained cell-free preparation with 12 μg of chlorophyll a, 6 mμ moles of horse heart cytochrome c type II (for Curve A, 15 percent reduced state; for others, 38 percent reduced state); total volume, 1 ml. The open arrows indicate addition of DCMU to give 2 X 10^{-4} M concentration (Curve E) and benzyl viologen to give 2 X 10^{-5} M (Curve G). For Curve F illumination was 385 μ watts at 700 mμ (half band 10 mμ); for all other curves illumination was 310 μ watts at 630 mμ (half band 9.5 mμ). Upward arrows mean light-on; downward arrows mean light-off. All reactions were carried out at room temperature (≈25°C).

gave a slow reduction followed by more rapid reduction in a subsequent dark period (Curve A). With repetition of light and dark periods and progressive increase in level of reduction, the light reduction changed to an oxidation which became more and more pronounced until it finally balanced the dark reduction (Curve A). With proper choice of initial redox level of cytochrome c (about 40 percent reduced) a light oxidation balanced by an equivalent dark reduction was observed from the first illumination. Observed absorption changes are attributable to the redox reaction of cytochrome c, since at 500 mμ (Curve B) or without cytochrome c (Curve C) no changes were observed.

The amount of cytochrome c reduced in the dark depended on the preceding illumination (Curve D), indicating that the dark reduction was caused by a reducing substance(s) which had been formed in the preceding light period. We shall call this the cytochrome reducing substance (CRS).

DCMU addition slightly stimulated the initial rate of light oxidation, prolonged extent of light oxidation, and strongly inhibited the dark reduction (Curve E). Similar effects on the dark reduction were observed with 700 mμ illumination (Curve F), or by addition of benzyl viologen (Curve G). FMN, vitamin K_3 and substrate amount of NADP gave similar effects.

An important difference was observed when the cytochrome c was presented in larger amount (17 mμ moles) and almost completely reduced (ferrocytochrome c). Then photooxidation was rapid and prolonged, as observed in

detergent-treated spinach chloroplasts (7,8), but always followed by a dark reduction of the same magnitude observed as in Fig. 1. No effect of DCMU was then observable but the dark reduction was still suppressed by benzyl viologen.

We interpret the results as follows. With ferrocytochrome c the rapid and continued photooxidation is taken as evidence that electron transfer couples to molecular oxygen at the level of system 1 (longer wavelength photochemical system). Insensitivity to DCMU requires that CRS reduction is caused by system 1. Benzyl viologen, which stimulates electron flow from system 1 to O_2 (8), competitively inhibits reduction of CRS. The contribution of system 1 to both cytochrome oxidation in light and following dark reduction suggests that cytochrome c enters into a cyclic electron flow with system 1 and CRS. The cycle has an electron input to system 1 from cytochrome c, competing with system 2, and an output to CRS competing with oxygen. With ferrocytochrome c electron supply to system 1 is sufficient so that action of system 2 (shorter wavelength photochemical system) is obscured.

With partially reduced cytochrome c the rate of oxidation in light by system 1 becomes balanced by cyclic reduction via CRS (Fig. 1, Curve A). And a continued electron supply from system 2 is required to compensate for electron loss to O_2. A decreased rate of system 2, as with DCMU or 700 mμ (Fig. 1, Curves E, F), or increased electron loss to O_2 via benzyl viologen (Fig. 1, Curve G) then lower the electron reservoir in CRS and suppress dark reduction.

The amount of reduced CRS present after prolonged illumination, calculated as equivalents of cytochrome c reduced in the dark, was 1/40 to 1/60 of chlorophyll a content. This may be compared to cytochrome f in chloroplasts, 1/400 (2); Pigment 700 in Anacystis cells, 1/400 (6); ferredoxin in Anacystis cells, 1/200 (5) and total content of plastoquinone in chloroplasts (1). Evidently the present preparation contains a rather large amount of redox substance(s) which is reduced by the action of system 1.

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