Note

Glycolate-stimulated Photoreduction of Ferricytochrome *c* in Lamellar Fragments of *Anabaena variabilis*

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In *Anabaena cylindrica*, cytochrome *c* reducing substance (CRS) was found to be reduced by photosystem I.¹⁾ In spinach, CRS was found to have a role of the primary acceptor of photosystem I.²⁾ In *A. cylindrica*, CRS stimulated the photoreduction of cytochrome *c*.³⁾ These facts indicate that the photoreduction of cytochrome *c* is a probe for the reduction of the primary acceptor of photosystem I. A high photoreduction activity of cytochrome *c* was observed in sonicated preparations of *A. cylindrica*.⁴⁾

In *Anabaena variabilis* glycolate induces a light-dependent oxygen uptake coupled to the autooxidation of photoreduced methyl viologen in the presence of 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU), and reduces the photooxidized P700 in the dark.⁵⁾ These facts suggest that glycolate donates an electron to photosystem I. In the previous report, the reduction of the electron acceptor of photosystem I was monitored by an indirect method measuring oxygen uptake coupled to the reduction of methyl viologen. This communication presents the effects of glycolate on the photoreduction of cytochrome *c* in lamellar fragments of *A. variabilis* and the results of experiments on whether glycolate-stimulated photoreduction of cytochrome *c* is mediated by oxygen.

A. variabilis was grown in the culture medium of Kratz and Myers⁶⁾ containing 0.1% sodium glycolate, with continuous aeration of air containing 1% CO₂ under continuous illumination by fluorescent lamps (5,000 lux). At the late logarithmic growth phase, the cells were harvested and were washed three times with distilled water. The washed cells were suspended in 3 volumes (v/w) of 0.4 M sucrose containing 50 mM Tricine-KOH (pH 7.8) and 10 mM NaCl, and were sonicated for 3 min at 20 KHz with a Ohtake ultrasonicator at 0°C. The sonicate was centrifuged for 10 min at 10,000 × *g* and the supernatant was further centrifuged for 30 min at 40,000 × *g*. The pellet was suspended in the same medium and was used as lamellar fragments. For inactivation of photosystem II, the lamellar fragments were treated in a water bath at 50°C for 5 min.⁷⁾ The concentration of chlorophyll was measured

spectrophotometrically.⁸⁾

The photoreduction of ferricytochrome *c* was measured by the procedure of Asada *et al.*,⁹⁾ by measuring the absorbance increase at 550 nm. The standard reaction mixture contained in a final volume of 2 ml: 50 mM potassium phosphate (pH 7.8), 10 mM NaCl, 20 μM ferricytochrome *c* and lamellar fragments containing 5.2~25.9 μg of chlorophyll. After incubation in the dark at 25°C for 5 min, the reaction was started by the turning on of an actinic light at the point indicated. Superoxide dismutase from spinach leaves purified to crystals¹⁰⁾ was donated by K. Asada (Kyoto University). Horse heart ferricytochrome *c*, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), and Tricine were purchased from Sigma. Other chemicals were of analytical grade. DCMU was dissolved in ethanol.

The effects of the inhibitor of photosystem II (DCMU) and glycolate on cytochrome *c* photoreduction were examined using lamellar fragments of *A. variabilis* (Fig. 1). The activity was inhibited to 20% by DCMU, but restored to 41% by glycolate. This was also the case when glycolate was added during illumination of the lamellae in the presence of DCMU (Fig. 2). To completely inhibit photosystem II, DCMU was added at a high concentration. The course of cytochrome *c* photoreduction was not linear. This seems to be due to an increase of oxidation rate of photoreduced cytochrome *c*, elicited by the increase of the reduced cytochrome *c*.

Fig. 1. Effects of DCMU and Glycolate on Photoreduction of Cytochrome *c* by Lamellar Fragments of *Anabaena variabilis*.

The reaction conditions were the same as those described in the text, except that the reaction mixtures contained lamellar fragments (25.9 μg chlorophyll). Illumination was started at the arrow. A, control, no addition; B, addition of 25 μM DCMU to the reaction mixture; C, addition of 25 μM DCMU and 10 mM sodium glycolate to the reaction mixture.

Fig. 2. Effects of Glycolate Addition during Illumination on Photoreduction of Cytochrome *c* in the Presence of DCMU by Lamellar Fragments of *Anabaena variabilis*. The reaction mixture was the same as in Fig. 1, except that it contained 25 μ M DCMU. The reaction procedures were the same as in Fig. 1, except that, after the preincubation and the illumination for 5 min, the absorbance change of reaction mixture during illumination was recorded. At the arrow, sodium glycolate was added to give a final concentration of 10 mM.

The effects of glycolate on cytochrome *c* photoreduction were examined using heat-treated lamellar fragments of *A. variabilis* (Fig. 3). When photosystem II of lamellar fragments was inactivated by heat-treatment, the activity of the photoreduction was stimulated to 250% by 10 mM glycolate.

These results indicated that, in the lamellar fragments of *A. variabilis*, glycolate stimulated the photoreduction of cytochrome *c* under the conditions where photosystem II was inactivated.

The effects of superoxide dismutase (SOD) on the cytochrome *c* photoreduction in the presence of glycolate and DCMU were examined using lamellar fragments of *A. variabilis*. SOD (10 μ g/ml) did not inhibit the glycolate-stimulated photoreduction of cytochrome *c*, indicating that the activity of photoreduction was not mediated by oxygen.

These lines of evidence confirm that glycolate donates an electron to the donor side of photosystem I in *Anabaena variabilis*. It is a problem for future study to identify the site at which glycolate is oxidized in the photosynthetic electron transport system of *A. variabilis*.

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Fig. 3. Effects of Glycolate on Photoreduction of Cytochrome *c* by Heat-treated Lamellar Fragments of *Anabaena variabilis*.

The reaction conditions were the same as in Fig. 1, except that the reaction mixtures contained heat-treated lamellar fragments (5.2 μ g chlorophyll). Illumination was started at the arrow. A, control, no addition; B, addition of 10 mM sodium glycolate to the reaction mixture.

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