Regulation of Ribulose Diphosphate Formation in Vivo by Light

W. KLOB, O. KANDLER, AND W. TANNER
Botanisches Institut der Universität München and Fachbereich Biologie der Universität Regensburg, 8400 Regensburg, Universitätsstraße 31-Postfach, Germany

ABSTRACT

Light-dependent formation of ribulose-1,5 diphosphate is completely inhibited by low concentrations of 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Also in Scenedesmus mutant number 11, capable of cyclic photophosphorylation, cellular ribulose-1,5 diphosphate-levels do not increase upon illumination. When mutant cells are H₂ adapted, however, a light-dependent formation of ribulose-1,5 diphosphate is observed in the presence of H₂. From these results it has been concluded that at least part of the Calvin cycle does not operate in the dark, since a reductant is lacking which is generated in the light.

The sequence of reactions in the Calvin cycle is known not to operate in the dark (1, 15). It has been suggested that changes in "energy charge," in the concentration of protons, ions like Mg⁻, and reducing equivalents, which occur when green cells are brought into the light, are positively affecting various Calvin cycle enzymes (1, 4–6, 8, 9, 11, 12, 15, 16). It is difficult, however, to assess from in vitro results what the actual effectors are in vivo. In this paper evidence will be presented to show that for the light-dependent formation of RuDP in vivo, reducing equivalents generated by light are required.

MATERIALS AND METHODS

Chlorella vulgaris (strain 211/11h, Göttingen) and Scenedesmus obliquus (mutant 11) of N. Bishop (2) were used. Ribulose diphosphate was determined according to Latzko and Gibbs (10); RuDP carboxylase for the test was prepared from spinach (13). Inorganic phosphate was determined following the procedure of Chen et al. (3). For Pi determination the algae were extracted in a 5% trichloroacetic acid solution at 0 °C for 1 hr; to determine RuDP the algae were boiled for 2 min in 70% ethanol.

RESULTS

The level of RuDP in green cells in the dark is low, but increases considerably when the light is turned on (17). This is shown for Chlorella vulgaris in Figure 1. In the presence of CO₂, this increase is reduced to about 50%. Since ATP is required for the formation of RuDP from pentose phosphates or possible other endogenous precursors, it seemed reasonable to assume that light generated ATP brings about this increase in RuDP. In addition it has been reported that AMP inhibits phosphoribulokinase of various photosynthetic organisms (4–6, 11, 16) and a change in energy charge by light has been considered important for regulation of this enzyme (4–6, 11, 15, 16).

It was surprising, therefore, to find that 1 μM DCMU completely inhibits the rise in RuDP (Fig. 1). This concentration of DCMU only slightly affects cyclic photophosphorylation in vivo (18), which was also seen in parallel ex-

![Figure 1. RuDP formation after a dark-light change in Chlorella and Scenedesmus mutant 11. Sixty milliliters of Chlorella or Scenedesmus cells (28 μl packed cells/ml culture medium) were brought into an Erlenmeyer flask, gassed in the dark for 15 min with pure N₂ and illuminated. Samples of 3 ml were taken at the times indicated and RuDP was determined. Five hundred cpm/ml algal suspension corresponds to a RuDP concentration in the cells of 0.1 mM (=0.2 μmole/10 mg chlorophyll).

In accordance with these data was the observation that Scenedesmus mutant 11, which possesses an intact photosystem I but not II (2), also is not able to raise its endogenous level of RuDP when light is turned on (Fig. 1), although this mutant is capable of cyclic photophosphorylation (14, 19, and Fig. 2b). When this mutant is H₂-adapted, it is able to photoreduce CO₂ directly with H₂ as in the wild type (2).

Under these conditions also in the absence of CO₂, part of a noncyclic electron flow must be operating, and it was thought possible that RuDP is formed. As seen in Figure 3, this is indeed the case; when light is turned on, RuDP does increase in an atmosphere of H₂ in hydrogen-adapted cells of mutant 11. For a control H₂ was replaced by N₂ and only a

Abbreviations: RuDP: ribulose diphosphate; Calvin cycle: reductive pentose phosphate cycle.
suspended ml) for illuminated.flushed with H2 gassing of RuDP from nous not see results with N2 and operating indicated of Scenedesmus Nr. 11. Sixty milliliters of algal cells, suspended in phosphate-free culture medium were gassed for 15 min with N2 and then illuminated. Samples of 3 ml were taken at the times indicated and Pi determined.

slight increase of RuDP was observed. It can be concluded from these experiments that noncyclic photosynthetic electron flow has to take place for RuDP to be formed from endogenous sources.

**DISCUSSION**

The results show that at least part of the Calvin cycle does not operate in the dark because a reductant produced only in the light is missing. Whether light-generated ATP (or a decrease in AMP) also is required for performance, as has been suggested before (4, 6, 11, 15, 16), cannot be clearly answered by the experiment described here. The light requirement of mutant 11 for RuDP formation in the presence of H2 could be interpreted in this way.

Latzko et al. (8, 9) have reported that the activity of phosphoribulokinase increased 4- to 5-fold when isolated chloroplasts of spinach were illuminated in an atmosphere of N2. This effect was inhibited by DCMU. Dithiols but not NADPH could activate the isolated enzyme. In photosynthetic bacteria and in *Hydrogenomonas*, however, NADH did act as a positive effector and, depending on its concentration, could cancel the inhibitory effect of AMP (11, 16). Recently it was reported by Ziegler (21) that in isolated chloroplasts NADPH or ATP activated the enzyme. From these results and the in vivo observations reported here, it seems clear that phosphoribulokinase is under strict control of a reductant produced in the light.

This had been suggested previously by Strotmann and Heldt (17) and recently Togasaki (20) has reached a similar conclusion from his studies of light-enhanced dark-fixation of CO2.

**LITERATURE CITED**


