Source of Glycolate and Cyclic Changes in Photosynthetic and Photorespiratory Activity during the Development of Barley Leaves

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Abstract. ¹⁴CO₂ assimilation, RuBP carboxylase and PEP carboxylase activities show cyclic changes during the development of barley leaves. Cyclic changes, but in phase opposition with respect to carboxylating enzymes, are shown by RuBP oxygenase, phosphoglycolate phosphatase, glycolate oxidase and nitrate reductase activities. The oxygenase function of RuBP carboxylase appears to be the primary source of glycolate in young leaves, whereas in old ones glycolate could be supplied from some source in addition to RuBP oxygenase activity.

Leaves of many higher plants evolve CO₂ and take up oxygen in response to light stimulation. This apparent reversal of the photosynthetic process is called photorespiration (Jackson and Volk 1970). The substrate for photorespiration is considered to be glycolate, formed during the photosynthesis. The most recent hypothesis of photorespiratory synthesis of glycolate is based on the discovery by Bowes et al. (1971) that RuBP oxygenase catalyzes the oxidation of ribulose 1,5 bisphosphate to phosphoglycolate by molecular O₂. Phosphoglycolate is then converted to glycolate (Randall et al. 1971). In the previous study (Passera 1975) photosynthetic CO₂ assimilation, radiocarbon distribution and photorespiration level in 5, 15 and 25 d old barley leaves were determined. CO₂ fixation decreased progressively with increasing age of the leaves owing to the loss of carboxylating enzyme activity and to the increased rate of photorespiration. The aim of the present work was to inquire into the source of photorespired glycolate and to verify whether processes involved in CO₂ fixation and CO₂ loss exhibit cyclic changes during the development of barley leaves.

Material and Methods

Barley seeds (Hordeum vulgare L. cv. Astrix) were germinated at 25°C on moist towels. After 24 h, seeds were transferred into pots containing quartz sand.

Received April 8, 1977; accepted June 21, 1977
Plants were grown in a controlled environment with 12/12 h light/dark, 21/15 °C cycle, humidity 70—82%. Irradiance was 120 W m$^{-2}$ (400 to 700 nm) at leaf surface. Daily watering was carried out with inorganic solution (Kessler and Brugger 1957). For each sample, the primary leaves were harvested 30 min after daily illumination began.

Fixation of $^{14}$CO$_2$, liberated from 1 ml of 6 mM NaH$^{14}$CO$_3$ solution (specific activity 0.83 mCi mmol$^{-1}$), was tested according to Chen et al. (1971) on 250 mg leaves at 25 °C, 150 W m$^{-2}$ irradiance, for 10 min, after 30 min preillumination.

Determinations of ribulose-1,5-bisphosphate (RuBP) carboxylase and phosphoenolpyruvate (PEP) carboxylase activity were carried out as previously described (Passera and Albuio 1975). The method of Hewitt and Nicholas (1964) was employed to determine nitrate reductase activity. Glycolate oxidase activity was assayed according to Downton and Slatyer (1971), glycolate phosphatase according to Rehefeld et al. (1969). RuBP oxygenase activity was assayed by measuring O$_2$ uptake at 25 °C by the O$_2$ electrode (YSI 53) procedure as reported by Badger and Andrews (1974). RuBP carboxylase, PEP carboxylase and RuBP oxygenase activities were measured on a single sample at a single time. All determinations were run in triplicate.

**Results**

$^{14}$CO$_2$ Assimilation and RuBP and PEP Carboxylase Activity

Leaves of different ages show different rates of photosynthesis. The maximum photosynthetic rate is usually attained before the leaf is fully expanded; thereafter photosynthesis decreases (Smillie 1962). $^{14}$CO$_2$ assimilation and RuBP carboxylase activities were not constant, but fluctuated with an amplitude decreasing as the leaf aged (Fig. 1). We have reported the results of determinations carried out on alternate days, but daily determinations of enzyme activity confirmed the general pattern of the fluctuation with no changes in the day of appearance of maxima or minima.

The RuBP carboxylase and $^{14}$CO$_2$ fixation peaks occurred on the 9th, 13th and 17th d from sowing (Fig. 1). PEP carboxylase showed peaks coincident with those of RuBP carboxylase and $^{14}$CO$_2$ fixation with exception of the 7th d peak. This peak was responsible for 36% of the total enzymatic carboxylating activity, whereas the second and the third ones, on the 13th and 17th d, were responsible for only 25%. Therefore the shoulder in the $^{14}$CO$_2$ assimilation curve in the youngest leaf (first maximum) can be attributed to a higher contribution of PEP carboxylase activity.

**RuBP Oxygenase, Glycolate Phosphatase, Glycolate Oxidase and Nitrate Reductase Activity**

RuBP oxygenase, glycolate phosphatase, glycolate oxidase and nitrate reductase activity also fluctuated during development of barley leaves (Fig. 2). RuBP oxygenase and glycolate phosphatase revealed three peaks on the 7th, 11th and 17th, and on the 7th, 11th and 19th d, respectively. The first peak did not appear in determinations of glycolate oxidase and nitrate reductase (Fig. 2), but the later occurring peaks were coincident with those of RuBP oxygenase.

1 μCi = 37 kBq.