Rates of glycolate synthesis and metabolism during photosynthesis of *Euglena* and microalgae grown on low CO₂*

A. Yokota and S. Kitaoka
Department of Agricultural Chemistry, University of Osaka Prefecture, Sakai, Osaka 591, Japan

**Abstract.** The rate of glycolate excretion in *Euglena gracilis* Z and some microalgae grown at the atmospheric level of CO₂ was determined using aminooxyacetate (AOA). The extracellular O₂ concentration was kept at 240 μM by bubbling the incubation medium with air. Glycolate, the main excretion product, was excreted by *Euglena* at 6 μmol·h⁻¹·(mg chlorophyll (Chl))⁻¹. Excretion depended on the presence of AOA, and was saturated at 1 mM AOA. A substituted oxime formed from glyoxylate and AOA was also excreted. Bicarbonate added at 0.1 mM did not prevent the excretion of glycolate. The excretion of glycolate increased with higher O₂ concentrations in the medium, and was competitively inhibited by much higher concentrations of bicarbonate. Aminooxyacetate also caused excretion of glycolate from the green algae, *Chlorella pyrenoidosa*, *Scenedesmus obliquus* and *Chlamydomonas reinhardtii* grown on air, at the rates of 2–7 μmol·h⁻¹·(mg Chl)⁻¹ in the presence of 0.2–0.6 mM dissolved inorganic carbon, but the cyanobacterium, *Anacystis nidulans*, grown in the same way did not excrete glycolate. The efficiency of the CO₂-concentrating mechanism to suppress glycolate formation is discussed on the basis of the magnitude of glycolate formation in these low-CO₂-grown cells.

**Key words:** Aminooxyacetate — Chlorophyta (microalgae) — CO₂-concentrating mechanism — Cyanobacteria — *Euglena* — Glycolate excretion — Photosynthesis (CO₂-concentrating mechanism).

---

* This is the 16th paper in a series on the metabolism of glycolate in *Euglena gracilis*. The 15th paper is Yokota et al. (1985c).

**Introduction**

A mechanism that concentrates CO₂ in cells and the enzyme carbonic anhydrase (EC 4.2.1.1) are induced in green algae and cyanobacteria grown on low CO₂ such as in the air (Badger et al. 1980; Kaplan et al. 1980; Miller and Colman 1980; von Caemmerer et al. 1983; Tsuzuki 1983; Shiraiwa and Miyachi 1985; Yokota and Canvin 1986). Carbon dioxide or HCO₃⁻ present in the medium at low concentrations is concentrated 10- to 1000-fold within the cells by this mechanism, and the cooperation of the mechanism with carbonic anhydrase makes possible high rates of photosynthesis in medium equilibrated with CO₂ at its concentration in the air. Increasing information on the CO₂-concentrating mechanism has led to the idea that synthesis and metabolism of glycolate or photorespiration are suppressed when the CO₂-concentrating mechanism is operating, which results in lowering the oxygenase activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) (Ogren 1984). However, there has been no quantitative information on the extent to which the CO₂-concentrating mechanism suppresses the oxygenase activity of the enzyme, particularly in the presence of air-levels of CO₂ and O₂.

Some reports are available on synthesis of glycolate by unicellular green algae grown on air during photosynthesis. Kaplan and Berry (1981) and Becker and Fock (1983) found that low-CO₂-grown cells of *Chlamydomonas* excreted glycolate in the presence of low extracellular concentrations of CO₂. The glycolate excreted, however, is only some of the glycolate synthesized in the cells in the absence of a metabolic inhibitor (Tolbert 1985). *Chlamydomonas* can metabolize glycolate at the rate of 8 μmol·h⁻¹·(mg chlorophyll (Chl))⁻¹ in
the light (Spencer and Togasaki 1981). The experiments did not show the exact rate of glycolate synthesis in the cells. On the other hand, Tolbert (1985) and Tolbert et al. (1983, 1985) reported that low-CO₂-grown cells of *Chlamydomonas* excreted glycolate at high rates in the presence of amino-oxacetate (AOA; "H₃N—O—CH₂—COO⁻"); a strong inhibitor of the glycolate pathway in green algae. However, the O₂ and CO₂ concentrations in their incubation medium were not well defined.

The present study was designed to explore these problems. Cell suspensions of *Euglena* and some algae were used greatly diluted, and extracellular [O₂] was kept at 240 μM by bubbling the suspension with air, while the metabolism of glycolate formed was completely inhibited with AOA. In this paper, we report the results obtained with these strategies, and discuss the physiological significance of the CO₂-concentrating mechanism in CO₂ and O₂ metabolism in these cells during photosynthesis in air, i.e. under conditions of growth in low CO₂.

**Materials and methods**

*Organisms and their culture.* *Euglena gracilis Z* was cultured photoautotrophically on air at pH 5.5 (Yokota et al. 1983). *Chlorella pyrenoidosa* (C-104), the wild-type of *Scenedesmus obliquus* and *Anacystis nidulans* D₁ were grown in air in Allen’s medium buffered at pH 8.0 with 25 mM 4-(2-hydroxyethyl)-1-piperazinencetan-1-sulfonic acid (Hepes; Sigma Chemical Co., St. Louis, Mo., USA)-NaOH buffer (Yokota and Canvin 1985). *Chlamydomonas reinhardtii* Dangeard was cultured in Allen’s medium buffered with 25 mM Hepes-NaOH buffer at pH 8.0 (Yokota and Canvin 1985, 1986). *Chlorella pyrenoidosa* was cultured in Allen’s medium buffered at pH 5.5 (Yokota et al. 1985a). The organic acids eluted were described in Yokota et al. (1985a). The experimental conditions were the same as those used in the glycolate-excretion studies except that 250 units of carbonic anhydrase (bovine erythrocytes; Sigma) per 1 ml of incubation medium was included where indicated.

*Chlorophyll determination.* Chlorophyll of *Euglena* was determined by the method of Arnon (1949). For the green algae and the cyanobacterium, the cells were extracted with methanol, and the Chl concentration in methanol was determined as described in Yokota and Canvin (1985, 1986). Calibration was done with known concentrations of NaHCO₃.

*High-pressure liquid chromatography (HPLC) of excreted products.* After experiments on glycolate excretion, the incubation medium was concentrated as above, unless otherwise stated. The concentrate was passed through a Dowex 50 (H⁺-type) column (8 mm diameter, 40 mm long) to convert elution products to their free forms and to remove AOA. The eluate was analyzed by HPLC. The conditions of the HPLC were as described in Yokota et al. (1985a). The organic acids eluted were monitored by measurement of absorbance at 210 nm, unless specified otherwise.

**Photosynthetic O₂ evolution.** The O₂ concentration was monitored with an O₂ electrode (Yellow Spring Instruments Co., Yellow Spring, O., USA or Rank Brothers, Cambridge, UK). The experimental conditions were the same as those used in the glycolate-excretion studies except that 250 units of carbonic anhydrase (bovine erythrocytes; Sigma) per 1 ml of incubation medium was included where indicated.

**Determination of dissolved inorganic carbon (DIC).** Dissolved inorganic carbon in the incubation medium was determined by injecting a portion of the medium into a 100-ml syringe containing 50% H₂SO₄ and measuring the CO₂ concentration in the syringe with a calibrated infrared CO₂ analyzer (Nihon Kagaku Kogyo Co., Osaka, Japan) (Yokota and Kitaoka 1985; Yokota and Canvin 1986). Calibration was done with known concentrations of NaHCO₃.

**Results**

*Experiments with low-CO₂-grown Euglena.* When, low-CO₂-grown *Euglena* was incubated with or without 2 mM AOA in the dark for 30 min and the cell suspension illuminated (and was bubbled with air throughout the experiments), glycolate was excreted linearly with time, at the rate of 6 μmol·h⁻¹·(mg Chl)⁻¹. The excretion was strictly dependent on illumination and was satu-