GLYCOLLATE FORMATION AND EXCRETION BY THE PURPLE PHOTOSYNTHETIC BACTERIUM *RHODOSPIRILLUM RUBRUM*

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1. Introduction

Investigations with over 30 species of eukaryotic algae from six algal divisions have shown a widespread production of glycollate during photosynthesis, followed by its excretion or metabolism [1–6]. Glycollate production of glycollate during photosynthesis, followed its excretion or metabolism [1–6]. Glycollate In contrast, comparatively little is known about glycollate excretion or metabolism in photosynthetic prokaryotes. Among the blue-green algae, glycollate excretion has been found to occur by a *Coccochloris* species [2], *Anacystis nidulans* [8], *Anabaena cylindrica* [9] and *Anaebaena flos-aquae* together with an *Oscillatoria* species [10]. The uptake and metabolism of exogenous glycollate by *A. flos-aquae* [11] and *A. cylindrica* [12] has also been investigated. The only report, to our knowledge, of glycollate production by a photosynthetic bacterium is that by Anderson and Fuller [13] who found glycollate to be the first stable product of photosynthesis by malate photoheterotrophs of *Rhodospirillum rubrum.* We have now investigated glycollate formation by this organism under various environmental conditions and present evidence for both the excretion and further metabolism of this compound. Glycollate production by malate photoheterotrophic *R. rubrum* is stimulated by low oxygen tensions. These data are discussed in the light of current hypotheses on the mechanism of glycollate formation.

2. Materials and methods

Rhodospirillum rubrum (NCIB 8255) was grown photoheterotrophically in completely filled 250 ml medical flat bottles in Ormerod's medium containing malate [14]. Cultures were incubated at 26–28°C and illuminated by tungsten filament bulbs giving a light intensity of 1500 lux at the surface of the bottles.

For glycollate excretion experiments, log-phase cells were harvested by centrifugation at 5000 *g* for 20 min, washed by resuspending in growth medium with or without malate and following a further centrifugation, were finally resuspended at 4 mg dry weight/ml in medium plus or minus malate at pH 7.0, containing 10 mM $NaHCO_3$ and 10 mM α-hydroxy-2-pyridine methane sulphonate (HPMS) where appropriate. The cell suspension was equilibrated from 10 min in the dark and then exposed to 10 000 lux white light provided by a photoflood lamp. The temperature was maintained at 28°C by a fan. In the presence of malate, the cell suspension was sparged at 74 litre/hr by a gas mixture containing, by volume, 0.04% CO_2 and 0, 5, 10, 15, 22, or 30% O_2, in N_2. Excretion experiments were also performed when gassing with 100% O_2. At appropriate times, 1-ml samples were removed into ice-cold centrifuge tubes, the cells quickly spin down and the supernatant decanted. Glycollate present in the supernatant was measured according to Calkins [15]. Dark controls were routinely run by covering the vessels with aluminium foil. Glycollate excretion under autotrophic conditions was followed in the absence of malate when cells were sparged wither either H_2 or a mixture of about 90% H_2/10% O_2 obtained by mixing H_2 and O_2 through a flow meter.

Malate photoheterotrophs, prepared as for the glycollate excretion experiments, were used to test the effect of gassing with oxygen on photosynthesis.

4-ml aliquots of cell suspensions, in Ehrlenmeyer flasks were sparged with the CO_2:N_2:O_2 gas mixtures used in the glycollate excretion experiments, while shaking in an illuminated Warburg manometer bath (10 000 lux) at 28°C. After 1 hr, the flasks were closed by Suba-seal stoppers and gassing stopped. 40 μmoles of $NaHCO_3$, containing 2 μCi of $NaH[^{14}C]O_3$, were syringed into the flasks. Photosynthesis was terminated after 0, 5 or 10 min by the injection of 0.2 ml of 10% (v/v) HCl. The stoppers were removed and after shaking for 3 min aliquots were transferred to vials of Triton X-100 scintillant [16] and counted in a Tracerlab Corumatic 200 scintillation counter.

CO_2:O_2:N_2 gasses were purchased ready mixed from Air Products Ltd. Glasgow, G.B. HPMS was obtained from the Aldrich Chemical Company, Milwaukee, Wisconsin, U.S.A.

3. Results and discussion

Glycollate excretion was not detectable when *R. rubrum* malate photoheterotrophs were gassed in the absence of oxygen (fig. 1). Furthermore no excretion was found under these conditions in the presence of 10 mM HPMS, added at a level which did not affect the total rate of photosynthesis. HPMS functions as an inhibitor of enzymic glycollate oxidation in eukoryotes [4,5,17] and also in the blue-green algae [11,12,18], resulting in increased amounts of glycollate excreted. On the assumption that glycollate oxidation, if it occurs in *R. rubrum*, would be blocked by HPMS, then the absence of excretion under anaerobic conditions with and without HPMS (fig. 1) is due to the absence of detectable glycollate formation under these conditions.

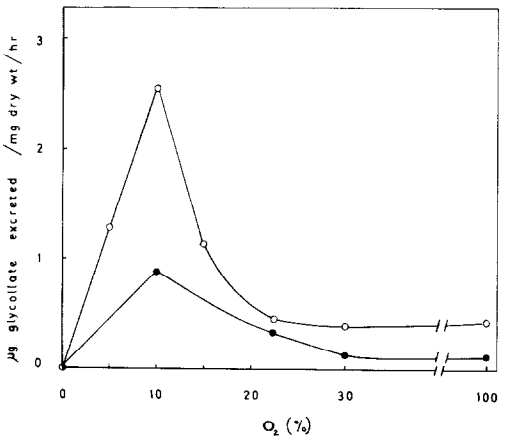

Fig. 1. Glycollate excretion by malate heterotrophic *R. rubrum*. Cells were incubated for up to 1 hr in the light with 10 mM bicarbonate and gassed with a series of increasing O_2 concentrations in N_2/CO_2. For experimental details see materials and methods section. (o—o—o), plus 10 mM HPMS; (•—•—•), minus HPMS.

Glycollate formation in eukaryotes and in blue-green algae is increased by the presence of oxygen [6, 7,9,19]. Since *R. rubrum* is not an obligate anaerobe [20], experiments were performed to determine whether glycollate formation and excretion occurred in the presence of increasing amounts of oxygen. Glycollate excretion in the presence of HPMS occurred during gassing with 5% O_2. Greater rates of excretion occurred in 10% O_2, the rate thereafter decreased to about 15% of the maximal rate, remaining approximately constant in 22, 30 and 100% O_2 (fig. 1). Similar results were obtained in the light in the absence of HPMS, although lower rates of glycollate excretion were consistently found (fig. 1), suggesting that, at each oxygen tension, some of the glycollate produced was excreted and some was further metabolized in the cell.

In all cases where a positive reaction in the Calkins test [15] for glycollate was found, the absorption spectrum of the pink derivative produced was determined. The product formed with cell supernatants taken from the light in the presence of oxygen (fig. 1) had an absorption maximum at 530 nm and corresponded with that formed with authentic glycollate. Incubation in the light without oxygen and at low oxygen tensions in the dark resulted in the formation of an unknown compound giving a pink-brown colour in the Calkins test with an absorption maximum at 480 nm. Cheng et al. [10] have reported the formation of a similar compound in the Calkins test using supernatants from photosynthesising *A. flos-aquae* and *Oscillatoria sp.*

We have found that malate-grown *R. rubrum* can produce glycollate during incubation in the light for 1 hr in the presence of low amounts of oxygen (fig. 1). The source of the carbon metabolized into glycollate may be questioned. Anderson and Fuller [13] originally

reported that malate may be an important source of cell carbon rather than carbon dioxide in malate photoheterotrophs, in which the reductive pentose phosphate cycle is considerably less active compared with autotrophs. However, more recent investigations [21, 22] with turbidostat continuous flow cultures of *R. rubrum* have shown that although levels of reductive pentose phosphate cycle activity may fall during photoheterotrophic growth on malate, depending on growth rate, the reductive pentose phosphate cycle continues to operate as the major mechanism of carbon assimilation in the presence of malate. Nevertheless, since our glycollate excretion experiments were performed with malate as reductant supply for photosynthesis, we have also investigated glycollate excretion during autotrophic conditions. When cells, resuspended in medium minus malate, but containing 10 mM $NaHCO_3$, were sparged with H_2 in the light, no glycollate excretion was detected either in the presence or absence of HPMS. However after gassing with a mixture of about 10% O_2 in H_2, glycollate excretion was readily measureable in the light, giving rates of 1.34 and 0.38 μmoles/mg dry weight/hr in the presence and absence of HPMS respectively. These data therefore demonstrate the production and excretion of glycollate by *R. rubrum* under autotrophic conditions.

In eukaryotic algae, the stimulatory effect of O_2 on glycollate production increases with higher O_2 tensions and does not saturate even at 100% O_2 [7]. However, 60 min incubation of *R. rubrum* in O_2 levels above 10–15% concentration resulted in reduced rates of glycollate formation and excretion (fig. 1). It is known that the introduction of O_2 (in air) to an anaerobic culture of *R. rubrum* in the light causes an immediate cessation of the synthesis of photosynthetic pigments [23]. If O_2, at these concentrations and above also causes an inhibition of photosynthesis, then this would result in a fall in glycollate formation. Indeed, prior gassing with increasing amounts of O_2 for 60 min caused increasing levels of inhibition of subsequent $[^{14}C]O_2$ fixation in the light (fig. 2).

Thus, aerobic conditions in the light stimulate glycollate formation by *R. rubrum*, however, this may not reach high rates due to the inhibitory effect of oxygen on overall photosynthesis. Although glycollate excretion was not detectable by the Calkins procedure in the absence of O_2 (fig. 1), the data of Anderson and

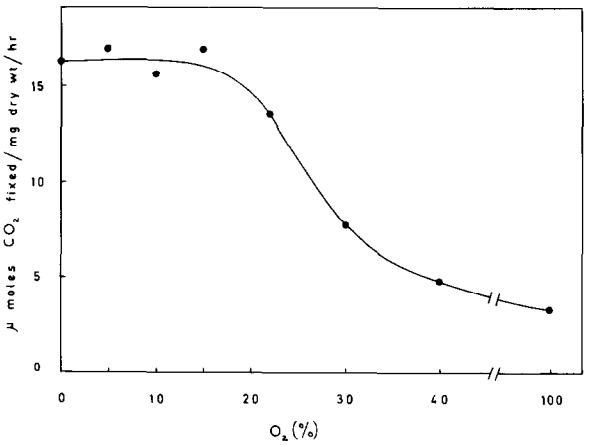

Fig. 2. The effect of gassing for 1 hr by various levels of O_2 on subsequent photosynthetic $[^{14}C]O_2$ fixation by malate heterotrophic *R. rubrum* cells. For experimental details see Materials and methods.

Fuller, who found $[^{14}C]$glycollate during $[^{14}C]_2$fixation in a He atmosphere [13], show that there is not an absolute requirement for O_2 for glycollate production in *R. rubrum*.

Finally, among the hypotheses of the mechanism of glycollate formation [17], that involving the oxygenation of D-ribulose-1,5-diphosphate (RuDP) is receiving considerable current attention [24,25]. This requires RuDP carboxylase to also function as an oxygenase, forming 3-phospho-D-glycerate and the glycollate precursor, phosphoglycollate [26,27]. In view of the data presented herein (fig. 1) an examination of the effect of oxygen on this enzyme in *R. rubrum* should now be undertaken.

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