Glycolate Excretion & Uptake by Chlorella

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Glycolate has been identified as one of the early products of photosynthesis in green plants (5). Tolbert and Zill have shown that glycolate is excreted by Chlorella cells during photosynthesis, and they postulated an equilibrium between the glycolate and bicarbonate ions at the cell membrane to account for this excretion (12). In 1958 Tolbert reported a similar release of glycolate to the medium by isolated spinach chloroplasts (11). Other investigations have shown that maximal rates for glycolate formation occur at low CO₂ concentrations and high O₂ pressures (1, 13).

This laboratory has been interested in the effect of cellular manganese upon the uptake of CO₂ (9, 10) and the metabolism of glycolate by Chlorella. This study was undertaken to establish more clearly the effect of O₂, CO₂, and cellular manganese upon glycolate excretion and uptake, including various pretreatments.

Methods & Materials

Chlorella pyrenoidosa cells (Emerson strain, obtained from Dr. Jack Myers of the University of Texas) were grown from small inocula in Warburg and Burk medium (6) and harvested after one week. Manganese-deficient cultures were grown on manganese-free Warburg and Burk media after subculturing to guarantee a low level of manganese, (lower than 10⁻⁷ M by EPR measurements & of the order of 2 x 10⁻⁸ M to 5 x 10⁻¹⁰ M as determined by bioassay (6).) At these manganese levels there was a negligible Hill response to quinone under conditions described by Brown et al. (3). Cells obtained from a 15 minute centrifugation at 1500 x g were transferred with distilled water to culture tubes, recentrifuged at 1500 x g and resuspended in 5 ml potassium phosphate solution. Cell densities of 50 ml/ml were used for all experiments. Solutions of KH₂PO₄ or K₂HPO₄ were used for experiments in acid (pH 4.5) and alkaline media (pH 8.5) respectively. These potassium phosphate solutions were 0.02 M.

Culture tubes (15 mm x 120 mm) containing 5 ml of the cell suspensions described above were gassed by means of individual glass capillaries (OD=9 mm, ID=2 mm) which extended to within several mm of the bottom of the tube. This arrangement gave good exposure of the high density suspensions to light and gas. Gas flow rates were 60 ml/minute. A water bath at 25°C, containing the sample tubes, was illuminated from both sides by six 300 W photoflood lamps to give light intensities of 5000 ± 800 ft-c at the sample locations. The potassium phosphate solution was presaturated (30 min) with the gas to be used during the subsequent photosynthesis period. The washed cells were then added to this solution.

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In some experiments cell suspensions were given pretreatment periods of 2 hours prior to the regular photosynthesis period. When added, the final concentration of glycolate was 1.3 mM (approx 100 mg/l).

During the photosynthesis period, samples of approximately 0.7 ml were removed at appropriate intervals and, following two centrifugations, the first for 10 minutes at 1500 x g and the second for 20 minutes at 20000 x g, the supernatant fluid was analyzed for glycolic acid by the method described by Calkins (4). Absorbancies were measured at 5300 A with a Beckman DU spectrophotometer.

The alcohol extracts (Table I) were obtained by suspending the washed cell pellet in 10 ml of 80% ethanol at 70 C. This suspension was cooled to room temperature, centrifuged, and the supernatant fluid reduced in volume to 2 ml. Final dilution to 6 ml was made with iso-propanol from which 1 ml samples were dried for counting.

**Results**

The excretion of glycolate by Chlorella is influenced by the partial pressures of both CO₂ and O₂, as shown in figure 1. These data are in agreement with the previous report of Warburg and Krippahl (13), that a CO₂ concentration of greater than 1.5% inhibited the excretion of this compound, and also agree with the statement made by Tolbert and Zill (12) that glycolate was not excreted if Chlorella cells were gassed with a mixture of CO₂ and N₂.

The present data show that maximal excretion of glycolate was obtained at approximately 0.2% CO₂ in the air, with the yield falling off rapidly at higher concentrations. The reason for the inhibition seen at higher concentrations of CO₂ is not known. From the data given below on excretion of glycolate in basic or acid media, it appears that the inhibition was not a pH effect, but more likely was due to a direct effect on some enzyme system controlling glycolate metabolism.

The requirement for O₂ concentrations of more than 15% for appreciable excretion of glycolate indicates that an oxidative step may be involved either in the formation of this compound from CO₂ or in its excretion by the cell. In the present experiments, no combination of CO₂ and O₂ has supported glycolate formation unless (a) the CO₂ concentration was lower than 1.5%, and (b) the O₂ concentration was greater than approximately 15%. Bradbeer and Racker (2) have reported that transketolase catalyzes the formation of glycolate from fructose-6-P in the presence of ferricyanide, and that spinach chloroplasts will catalyze the same reaction in the absence of ferricyanide. This chloroplast catalyzed reaction is stimulated by light. Since an oxidation of the active glycolaldehyde moiety is required in these reactions, the chloroplast apparently uses O₂ for this reaction, whereas the isolated enzyme requires ferricyanide. The role of transketolase in the reactions leading to glycolate excretion by Chlorella cells is not clear, since both glycolate and phosphoglycolate are excreted by chloroplasts (7) and phosphoglycolate appears to be the precursor of the glycolate observed (N. E. Tolbert, personal communication).

The effect of O₂ partial pressure upon the uptake of C¹⁴O₂ was examined to see if the increased glycolate formation at high O₂ tensions could be due to increased fixation of CO₂. Table I shows there was less activity found in the alcohol-soluble fraction of Chlorella cells exposed to the high O₂ tension. If the activity contained in the glycolate excreted into the medium is also considered, the radioactivity incorporated in both cases was approximately equal. Thus, the effect of O₂ was to divert a portion of the incorporated C¹⁴O₂ into the glycolate pathway. Analysis of the alcoholic extracts of the cells exposed in the presence of O₂ showed a 3 to 5 times greater concentration of activity in glycine and serine, as would be expected since these compounds are metabolically related to glycolate.

The effect of the manganese level in the cell upon both the uptake and excretion of glycolate is shown in figure 2. Whereas the manganese-deficient cells were capable of consuming glycolate at a significant but lower rate when compared to the normal cells, the deficient cells were not able to perform the normal reactions leading to excretion of glycolate. It is known that manganese is required for the O₂ evolution system in Chlorella (8), and also that glycolate formation from radioactive CO₂ is particularly sensitive to the level of this metal ion (9). The present investigation shows the sensitivity of the production of glycolate to manganese in the cell and again speaks for a special manganese function related to the formation of this compound in the photosynthetic process.

Cells which have been kept in the dark for 2 hours prior to the measured photosynthesis period will ex-
Fig. 1. The effect of $O_2$ and $CO_2$ concentration on excretion of glycolate by Chlorella during 1 hour of photosynthesis. Experimental conditions: $25^\circ$ C, incandescent light of 5000 ft-c, Chlorella density = 50 $\lambda$/ml, potassium phosphate solution (0.02 M, pH 8.5) presaturated 30 minutes with test gas.

Fig. 2. The effect of cellular manganese on excretion and uptake of glycolate by Chlorella. The excretion experiments were run under 0.1 % $CO_2$ in air, while the uptake experiments were run under $N_2$. The other conditions were those of figure 1. Dashed lines, uptake. Solid lines, excretion.
crete glycolate into the medium upon illumination. If suspended in a medium containing added glycolate, they consume this compound when illuminated, as shown in figure 3. The uptake of glycolate by Chlorella in a N₂ atmosphere in the light has been previously reported (11) and our data confirms this finding. Our choice of air for formation-excretion experiments and of N₂ for uptake experiments is based on this result. The formation of glycolate is favored in a basic medium (pH 8.5), whereas the uptake is favored in an acid medium (pH 4.5). The initial rates of formation were approximately the same at the two pH values, but in acid medium a maximal amount of glycolate was produced after about 60 minutes, and thereafter an uptake occurred. This indicates that the system responsible for uptake of glycolate is formed upon illumination, or at least it becomes much more significant after an initial illumination period. This is also shown by the lag period shown in the curves representing uptake of glycolate. A 2-hour illumination period prior to the measured photosynthetic period emphasizes this feature of the system. Figure 4 shows that after the pre-illumination period, the rate of formation of glycolate was very low, and the rate of uptake of the acid was considerably higher than that previously observed. This again indicates that the system responsible for the consumption of glycolate was formed after a short exposure to light. The equilibrium between the glycolate and its metabolic products was shifted, resulting in a lower concentration of the compound in the medium.

The previous experiments suggest that glycolate is rapidly formed during the initial period of illumination, and the high level of this compound then induces the cell to either form or activate a system responsible for glycolate utilization. To test this concept, glycolate was added to cells for varying periods of time in the dark; the cells were collected by centrifugation and resuspended in a medium containing glycolate and the rate of uptake followed. The results of these experiments are given in figure 5, and they show that prolonged exposure (18 hr) of the cells to glycolate did allow the cell to consume glycolate from the medium. However, the rate of glycolate consumption even under these extreme conditions did not approach that observed after an initial illumination period, showing that mere exposure to the compound was not sufficient to allow subsequent uptake of glycolate. This could mean either that glycolate as such cannot directly elicit the response, or that only cellular glycolate is effective and the added glycolate is poorly taken into the cell. The latter explanation is preferred since it has been shown that added glycolate will not enter isolated chloroplasts, while glyoxylyte readily does so (7).

**Discussion**

The results of this investigation show that glycolate is excreted into the medium by Chlorella cells during photosynthesis when the CO₂ concentration is less than 1.5 %, the O₂ concentration is greater than 15 %, and there exists a normal state of manganese nutrition in the cell. The manner in which oxygen and carbon dioxide function in controlling glycolate production by the cell is not known, but as discussed

**Fig. 3.** The effect of dark pretreatment on excretion and uptake of glycolate by Chlorella in 0.02 M potassium phosphate solution, pH 8.5 and pH 4.5. Conditions: 25°C, incandescent light of 5000 ft-c, Chlorella density = 50 µg/µl. Two hours dark pretreatment in N₂ for all samples. Excretion experiments, light and 0.1 % CO₂ in air. Uptake experiments, glycolic acid injected at zero time, samples in light and N₂. Dark control in N₂. Dashed lines, uptake. Solid lines, excretion.

**Fig. 4.** Effect of light pretreatment on excretion and uptake of glycolate by Chlorella in acidic and alkaline media. Two hours light pretreatment in N₂ for all samples. Other conditions as in figure 3. Dashed lines, uptake. Solid lines, excretion.

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*Fig. 5.* Effect of dark incubation of Chlorella and glycolate on uptake of glycolate by Chlorella in the light. Experimental conditions as described in figure 1. Periods of dark pretreatment in N₂ in the presence of glycolate as described on the plot. Uptake measured during light period in N₂.
above, the CO₂ effect is probably not related to the pH of the medium, and the O₂ requirement indicates that an oxidative reaction is required either for the formation or the elaboration of glycolate. The subsequent rapid uptake of glycolate after an illumination period is an interesting phenomenon which requires more investigation. The most logical explanation of this uptake is that glycolate formed photosynthetically (which does not necessarily include excretion of this compound) causes the cell to elaborate a system for glycolate utilization. This could be either an enzyme system involved in glycolate metabolism, or some alteration of the cell wall to allow glycolate to penetrate into the cell. Exposure of the cells to high levels of glycolate for 4 hours was not sufficient to allow the rapid uptake regularly observed after a 2-hour illumination period (fig 5). This indicates that the system responsible for glycolate uptake is controlled by metabolic processes inside the cell, most likely by the internal photosynthetic production of glycolate. It is presumed that the uptake of glycolate is followed by glycolate metabolism in the cell, but further experiments must be done to show this.

Tolbert (11) and Tolbert and Zill (12) have proposed that glycolate is in equilibrium with bicarbonate in the cell, and moves across the cell membrane to counter the ionic effects of bicarbonate absorption. The data given in table 1 and figures 3 and 4 are not consistent with this hypothesis, since depending upon the conditions the cells may either excrete or consume glycolate during a photosynthesis period. These data do not exclude the possibility, however, that some precursor such as phosphoglycolate does function in this manner, and that glycolate excretion and uptake is a secondary process deriving from the phosphoglycolate.

Summary

I. Chlorella cells excrete glycolate into the medium if the carbon dioxide concentration is below 1.5% and the oxygen concentration is above 15%. Experiments with manganese-deficient cells show that this metallic ion is also required for the process.

II. Chlorella cells which have been pre-illuminated or exposed to glycolate in the growth medium have the ability to take up this compound upon illumination. Available evidence indicates that the glycolate formed photosynthetically induces the formation of a system which consumes glycolate in the light. The uptake of glycolate proceeds much faster at pH 4.5 than at 8.5 in phosphate, and is only moderately affected by a condition of manganese deficiency.

III. Glycolate may be either excreted or consumed by the cell depending upon its condition. The evidence available does not support the idea that glycolate per se serves to maintain ionic balance in the cell during the process of bicarbonate uptake in photosynthesis.

Literature Cited