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Glycollate oxidase in *Chlorella pyrenoidosa*

The formation of glycollic acid during the photosynthetic fixation of $^{14}CO_2$ was noted by BENSON AND CALVIN[1]. It was further shown that glycollate production by algae was greatly enhanced by high light intensity, low CO_2 concentration and high O_2 partial pressure, and under these environmental conditions much of the glycollate formed was released into the surrounding medium[2–5]. Several green algae, all of which excrete glycollate, have been shown to lack the enzyme glycollate oxidase (glycollate: oxygen oxidoreductase, EC 1.1.3.1)[6]. It was concluded that in the absence of glycollate oxidase no biochemical pathway was present for the further metabolism of glycollate, resulting in glycollate excretion[6]. Besides glycollate formation during the photosynthetic fixation of CO_2, glycollate was an early product of the photoassimilation of acetate by Chlorella[7,8]. When *Chlorella pyrenoidosa* photoassimilated [^{3}H]acetate kinetic experiments revealed that, after 10 sec, over 45% of ^{3}H in the soluble fraction of the cells was in glycollate[7]. Similar experiments with [^{14}C]acetate also demonstrated a rapid light-driven formation of glycollate[8]. The early incorporation of ^{14}C and ^{3}H into glycollate during the photoassimilation of [^{3}H-^{14}C]acetate demonstrated a rapid

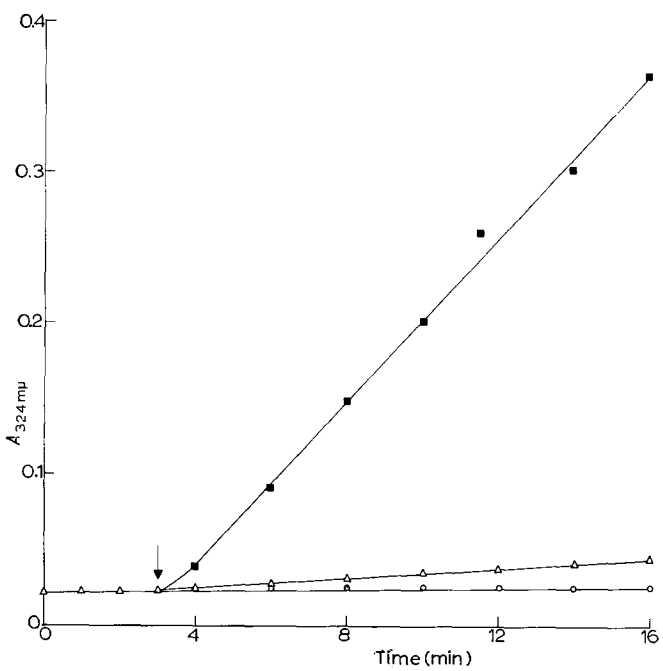

Fig. 1. Glycollate oxidase in *C. pyrenoidosa*. The reaction mixture contained, in a final volume of 3.0 ml, the following components, in μmoles: potassium phosphate (pH 8.3), 200; phenylhydrazine·HCl (adjusted to pH 6.8), 10; cysteine·HCl, 10; 0.5 ml enzyme extract containing 0.1 mg protein (fraction obtained from 20% ammonium sulphate saturation). Reaction started by the addition of 30 μmoles sodium glycollate. ■, complete reaction mixture; △, glycollate omitted; ○, enzyme boiled.

synthesis of glycollate, but we were unable to demonstrate glycollate excretion under a wide range of environmental conditions with this strain of *C. pyrenoidosa*. Thus, it is probable that the further metabolism of glycollate takes place in this organism and this would be dependent on the presence of glycollate oxidase.

C. pyrenoidosa (strain 211/8P Cambridge culture collection) was grown photoautotrophically, carbon starved and harvested for use exactly as described before[9,7]. The harvested cells were placed in a mortar, frozen at −12° in a deep-freeze and then disrupted by grinding with fine acid-washed sand, accompanied by the gradual addition of 0.1 M phosphate (pH 8.3) containing 20 mM cysteine. After centrifugation at 500 × *g* for 5 min to remove intact cells and cell debris, the supernatant was centrifuged at 13 000 × *g* for 20 min to remove larger particulate matter. Glycollate oxidase present in the supernatant was partially purified by ammonium sulphate fractionation. The bulk of the enzyme was precipitated by 20% ammonium sulphate saturation and this increased the specific activity, *i.e.* μmoles glyoxylate produced per mg protein per h, 17-fold. Glycollate oxidase was assayed spectrophotometrically by measuring the increased absorbance at 324 mμ resulting from the formation of glyoxylate phenylhydrazone[6]. The enzyme was also assayed by following the decrease of glycollic acid in the reaction mixture. Aliquots were removed at intervals and glycollic acid determined by the method of CALKINS[10].

The results of a glycollate oxidase assay are given in Fig. 1. In a typical experiment the oxidation of 340 μg of glycollate per mg protein per h was accompanied by the formation of 300 μg glyoxylate per mg protein per h. Although HESS AND TOLBERT[6] were unable to detect glycollate oxidase in the algae which they investigated, this enzyme has been shown to be present in *C. pyrenoidosa* (Fig. 1). The presence of glycollate oxidase and the absence of glycollate excretion in *C. pyrenoidosa* supports the conclusion of HESS AND TOLBERT[6] that in many green algae glycollate excretion results from the absence of glycollate oxidase.

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1 A. A. BENSON AND M. CALVIN, *J. Exptl. Botany*, 1 (1950) 63.
2 N. E. TOLBERT AND L. P. ZILL, *J. Biol. Chem.*, 222 (1956) 895.
3 O. WARBURG AND G. KRIPPAHL, *Z. Naturforsch.*, 15B (1960) 197.
4 J. A. BASSHAM AND M. KIRK, *Biochem. Biophys. Res. Commun.*, 9 (1962) 376.
5 C. P. WHITTINGHAM AND G. G. PRITCHARD, *Proc. Roy. Soc. London, Ser. B*, 157 (1963) 366.
6 J. L. HESS AND N. E. TOLBERT, *Plant Physiol.*, 42 (1967) 371.
7 M. J. MERRETT AND K. H. GOULDING, *J. Exptl. Botany*, 18 (1967) 128.
8 K. H. GOULDING AND M. J. MERRETT, *J. Exptl. Botany*, 18 (1967) 620.
9 K. H. GOULDING AND M. J. MERRETT, *J. Exptl. Botany*, 17 (1966) 678.
10 V. P. CALKINS, *Ind. Eng. Chem.*, 15 (1943) 762.

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