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RELATIONSHIPS BETWEEN GLYCOLLATE AND FOLATE METABOLISM IN *EUGLENA GRACILIS**

KIM-LOON LOR and EDWIN ALBERT COSSINS
Department of Botany, University of Alberta, Edmonton, Canada, T6G 2E9

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Key Word Index—*Euglena gracilis* (Strain Z); Euglenales; synchronized cultures; folate derivatives; enzymes of C-1 metabolism; effects of high and low CO_2 concentrations.

Abstract—When division synchronized cultures of *Euglena gracilis* Klebs (strain Z) were aerated with 5% CO_2 in air the specific activity of glycollate dehydrogenase was only 13% of that in cultures receiving unsupplemented air. The concentrations of 10-formyltetrahydrofolate synthetase (EC 6.3.4.3) and formylfolate derivatives were also lowered by this treatment. In contrast, the specific activity of serine hydroxymethyltransferase (EC 2.1.2.1) and the concentration of methylfolates were raised by supplying CO_2-supplemented air. These effects on enzyme levels were reversed when air was supplied following a period of CO_2 treatment. The levels of glycollate dehydrogenase, 10-formyltetrahydrofolate synthetase and formylfolate derivatives were decreased when cells were aerated in media containing 5 mM α-hydroxy-2-pyridinemethane sulphonate. Cell free extracts had the ability to decarboxylate glyoxylate, producing *ca* equal amounts of CO_2 and formate from C-1 and C-2 respectively. Cells receiving 5% CO_2 in air had a decreased ability to incorporate formate-[^{14}C] into serine and methionine. It is concluded that during growth at low CO_2 concentrations glycollate metabolism will provide substrate for the formyltetrahydrofolate synthetase reaction.

INTRODUCTION

Glycollate is an important product of photosynthesis in several algae and higher plants [1]. In these species there is also good evidence that the glycollate pathway is quantitatively important in the flow of CO_2 into hexoses [2, 3]. The intermediary formation of serine from glycine in this pathway implies that at least one folate-dependent reaction participates in the metabolism of recently fixed carbon. It is generally agreed [4] that two glycine molecules are used in the overall production of one serine molecule within the glycollate pathway. Studies of spinach leaf preparations resulted in the proposal [5] that glycine was initially cleaved by glycine decarboxylase to yield 5,10-CH_2-H_4PteGlu*, ammonia and CO_2. A second molecule of glycine then condenses with this folate derivative in a reaction catalyzing by serine hydroxymethyltransferase to form serine and H_4PteGlu. As these are separate reactions it is conceivable that some of the 5,10-CH_2-H_4PteGlu arising from C-2 of glycine may also enter into the synthesis of purines, thymidylate and methionine; a situation well documented for bacteria, fungi and animals [6].

There is also evidence, in some photosynthetic species, that potential C_1 units might arise from glycollate and glyoxylate. For example, Zelitch [7] found that envelope-free spinach chloroplasts oxidatively decarboxylate glyoxylate to yield formate from C-2. Leaf peroxisomes of spinach and spinach-beet also form formate when incubated with glycollate or glyoxylate [8]. In addition, Davies *et al.* [9, 10] have presented evidence that pyruvic decarboxylase catalyzes glyoxylate decarboxylation in several plant species. As formyltetrahydrofolate synthetase is widely distributed in higher plants and algae [11–15], it follows that such formate may participate in folate-mediated reactions. In fact, Halliwell [16] has calculated that spinach leaf homogenates contain sufficient levels of this synthetase to activate all of the formate produced from glyoxylate or glycollate.

Codd and Merrett [17, 18] have examined the production and metabolism of glycollate during the cell cycle of *Euglena gracilis*. Prior to division the cells contained high levels of glycollate dehydrogenase and glycerate was formed from bicarbonate by the reactions of the glycollate pathway. In later studies, Lor and Cossins [15] found that cultures of *E. gracilis* contained greatest concentrations of 10-HCO-H_4PteGlu$_n$ and formyltetrahydrofolate synthetase prior to cell division. Formylfolates may therefore arise during operation of the glycollate pathway in this species.

The glycollate dehydrogenase of *E. gracilis*, in common with other algae, is repressed when the cells are cultured in air supplemented with 5% CO_2 [18]. Furthermore, the metabolism of glycollate is effectively blocked by α-HPMS [18]. Clearly if glycollate is a precursor of formyl folates these treatments should affect their concentration and possibly also the activity of formyltetrahydrofolate synthetase. These and related possibilities have been examined in the present work.

Besides causing repression of glycollate dehydrogenase and 10-formyltetrahydrofolate synthetase, culture in air supplemented with 5% CO_2 altered the composition of the folate pool. This treatment also reduced the flow of formate carbon into serine and methionine. It is concluded that glycollate and C_1 metabolism in *Euglena*

* The abbreviations used for derivatives of folic acid are those suggested by the IUPAC–IUB Commission as listed in (1967) *Biochem. J.* **102**, 15, e.g. 5, 10-CH_2-H_4PteGlu =N^5, N^{10}-methylenetetrahydropteroyl monoglutamate. Polyglutamyl derivatives are designated: Glu$_n$. α-HPMS = α-hydroxy-2-pyridinemethane sulphate.

are closely interrelated during the autotropic growth. Part of this work has appeared as a congress report [19].

RESULTS

Glycollate dehydrogenase and enzymes of C_1 metabolism after culture in high and low CO_2

Preliminary experiments showed that cells receiving air supplemented with 5% CO_2 (high CO_2) during the 14:10 hr light–dark cycle retained the characteristic synchrony of air-grown cells [15]. Growth rates were, however, greater with this CO_2 treatment as *ca* 25% more divisions occurred during each dark period. High CO_2 also affected the levels of glycollate dehydrogenase, reducing both sp. act. and units per cell (Table 1); a situation already reported for random cultures of *E. gracilis* and *Chlamydomonas* [21]. This treatment also reduced formyltetrahydrofolate synthetase activity throughout the light period (Fig. 1). In contrast, serine hydroxymethyltransferase levels were increased by high CO_2 (Fig. 2). When high CO_2-treated cells are given air (low CO_2) at the start of the 4th cell cycle the level of these enzymes rapidly changed (Fig. 3). It is clear from these data that glycollate dehydrogenase and formyltetrahydrofolate synthetase levels rose more rapidly in cells receiving air. However, the levels of serine hydroxymethyltransferase remained low in the transferred cells but rose in the cultures receiving high CO_2.

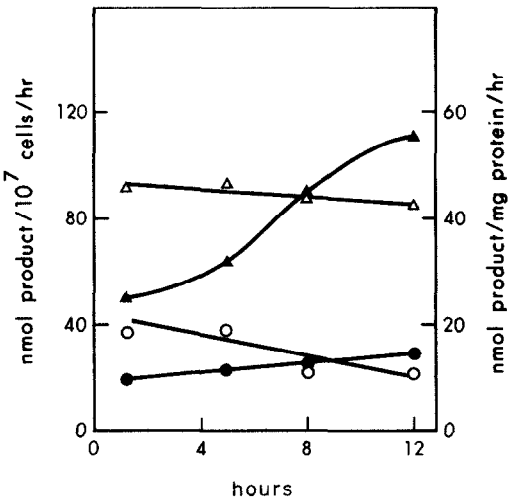

Fig. 2. Serine hydroxymethyltransferase activity during growth in high and low CO_2. Extracts were prepared during the light phase of the 4th cell cycle of growth in high (△, ▲) and low (○, ●) CO_2. Data are expressed as in Fig. 1.

Table 1. Levels of glycollate dehydrogenase after culture in high and low CO_2

Culture conditions	Enzyme activity Units/mg protein	Units/10^7 cells
Air (low CO_2)	2.66	2
5% CO_2 in air	0 35	0.22

Cells were grown synchronously at 25° and received air (low CO_2) or 5% CO_2 in air. After 10 hr (4th cell cycle of treatment) samples were withdrawn, harvested by centrifugation and cell-free extracts were assayed for enzyme activity. Dehydrogenase units are defined in the Experimental.

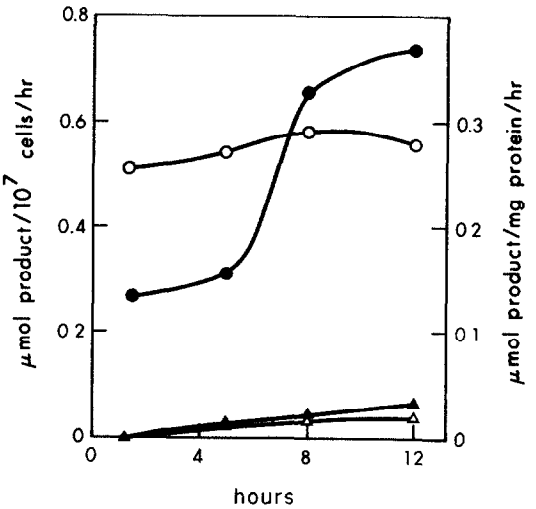

Fig. 1. Formyltetrahydrofolate synthetase activity during growth in high and low CO_2. Extracts were prepared during the light phase of the 4th cell cycle of growth in high (△, ▲) and low (○, ●) CO_2. Data are expressed as specific enzyme activities (▲, ●) and activities per 10^7 cells (△, ○) respectively.

Effects of α-HPMS on enzyme levels

As high CO_2 lowered glycollate dehydrogenase and formyltetrahydrofolate synthetase activities it is conceivable that substrate for the latter enzyme may be derived from the metabolism of glycollate. To examine this, cells were cultured in the presence of α-HPMS, a compound known to inhibit glycollate oxidation [1]. The inhibitor (5 mM) was added to air-grown cells (5×10^4 cells/ml) at the start of the light phase. After 10 hr, glycollate dehydrogenase and formyltetrahydrofolate synthetase levels were only 30% of the controls (Table 2). In contrast serine hydroxymethyltransferase activity on a per cell or protein basis was not changed by this treatment.

Folate pool sizes after high CO_2 and α-HPMS treatments

As high CO_2 and α-HPMS decreased formyltetrahydrofolate synthetase, the possible effects of these treatments on formylfolate pool sizes were examined. Detailed microbiological assays showed that cultures receiving high CO_2 for one and four cell cycles respectively contained essentially the same total concentrations of folates as air-grown cells. Conjugase treatments of these native folates showed that in both cases slightly more than 50% of the extracted folates were polyglutamyl derivatives. When closer comparisons were made after ion-exchange chromatography it was clear that cells receiving high CO_2 contained less formylfolates and more methylfolates than air-grown cells (Table 3). This tendency was more pronounced during the 4th cell cycle of treatment.

This effect on conjugated formylfolate pool size, was also seen after 10 hr of culture in the presence of α-HPMS

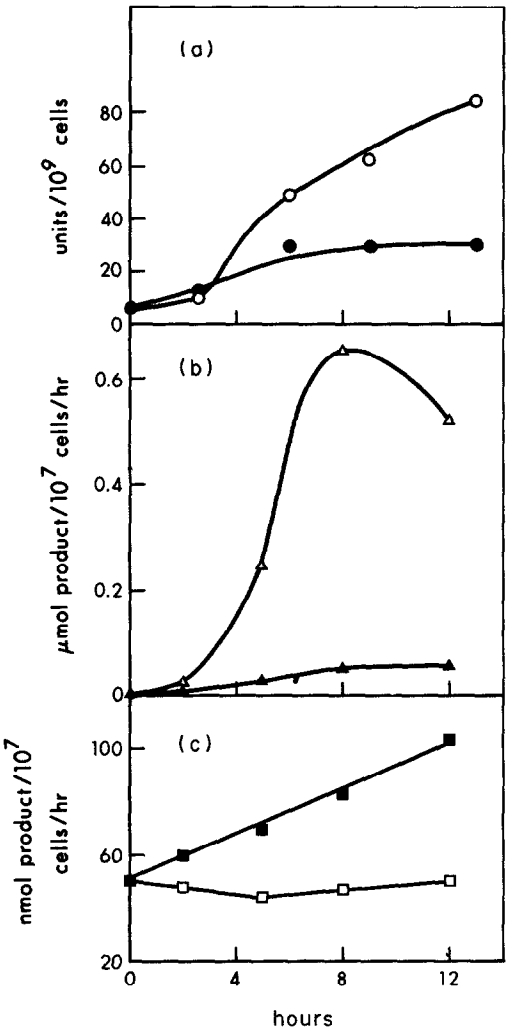

Fig. 3. Changes in enzyme activities on transfer from high CO_2 to air. Cells were cultured in high CO_2 for 3 cell cycles. Air was supplied (○, △, □) at the start of the 4th cell cycle. Cultures receiving high CO_2 throughout the experimental period (●, ▲, ■) served as controls. Extracts were prepared during the light period of the 4th cell cycle and assayed for: (a) glycollate dehydrogenase; (b) formyltetrahydrofolate synthetase; and (c) serine hydroxymethyltransferase.

Table 2. Effects of α-HPMS on glycollate dehydrogenase and enzymes of C_1 metabolism

Enzyme	Specific activity Control	α-HPMS	Activity/10^7 cells Control	α-HPMS
Glycollate dehydrogenase (units)	2.66	0.76	2	0.6
Formyltetrahydrofolate synthetase (μmol product/hr)	0.3	0.09	1.6	0.6
Serine hydroxy-methyltransferase	18.6	17.6	49.8	49.5

Synchronous cultures were grown with aeration (low CO_2) to a density of 5×10^4 cells/ml. At the start of the next light phase, 5 mM α-HPMS was added and 10 hr later the cells were harvested. Cell free extracts were dialyzed (see Experimental) before assay of enzyme activities.

(Table 4). After a longer period of growth, marked reduction in the concentrations of conjugated methylfolates were also observed. As a result total folate contents of the α-HPMS-treated cells were substantially lower than the controls.

Production and oxidation of formate in vitro

As treatment which would alter the production and metabolism of glycollate [17, 18] appeared to affect formylfolate synthesis it was of interest to determine whether formate could arise from glyoxylate *in vitro*. Initially cell free extracts of air-grown cells were examined for ability of decarboxylate carrier-free glyoxylate-[1-^{14}C]. The release of labelled CO_2 appeared to be enzyme catalyzed, displayed a requirement for Mn^{2+} but proceeded without additions of thiamine pyrophosphate (Table 5). Decarboxylation was accompanied by formation of *ca* equal amounts of labelled formate which was only detectable when glyoxylate-[2-^{14}C] served as substrate (Table 6). The sp. act. of this reaction was low and comparable in extracts prepared from cells treated with air or high CO_2. In other studies it was found that the rate of decarboxylation was linear for at least 1 hr under the assay conditions of Table 5. In such work average reaction rates per 10^7 cells of 0.84 and 0.81 nmol CO_2 per hour respectively, were recorded for extracts of high and low CO_2 treated cells.

Dialyzed cell free extracts also had the ability to oxidize formate-[^{14}C]. Additions of NAD or NADP gave only small increases in the rate of $^{14}CO_2$ production but boiling the cell free extract resulted in complete loss of activity. When extracts from air and high CO_2-grown cells were compared (Fig. 4) greater activities were found in the latter.

Metabolism of formate-[^{14}C] by high and low CO_2-grown cells

The above enzyme and folate data suggest that cells receiving high CO_2 had less ability to generate formylfolates. To examine this possibility pulse feeding experiments were conducted with formate-[^{14}C]. It was found that ^{14}C was rapidly incorporated into a variety of products. In short feeding periods the protein and free amino acid fractions contained *ca* 50% of the formate carbon incorporated; consequently these fractions were examined as summarized in Table 7. Data are given for serine and methionine which contained the greatest concentrations of formate carbon. It is clear that the free serine pool was essentially saturated with ^{14}C by 5 min of formate feeding and that greater amounts of label entered this pool in the air-grown cells. Radioactivity was not detected in free methionine but this may reflect the smallness of this pool (<0.5 nmol/10^7 cells, air or CO_2-treated) as substantial amounts of ^{14}C accumulated in protein methionine. The sp. radioact. of protein serine and methionine were also greater in the air-grown cells.

Labelling of serine in these experiments could conceivably involve two major pathways. For example serine-[3-^{14}C] would arise if the major reactions were catalyzed by formyltetrahydrofolate synthetase, methylenetetrahydrofolate dehydrogenase and serine hydroxymethyltransferase. Serine predominantly labelled in the carboxyl group would arise if formate carbon was extensively oxidized and then refixed via photosynthetic

Table 3. Concentrations of folate derivatives after culture in high and low CO_2

Cell cycle	Derivative	Folate concentration (ng PteGlu equivalents/10^7 cells)			
		Air (low CO_2)		5% CO_2 in air (high CO_2)	
First	HCO-H_4PteGlu$_{1-2}$	4.1		4.6	
	HCO-H_4PteGlu$_\eta$	94.7		86.4	
	5-CH_3-H_4PteGlu	2.1		1.3	
	5CH_3-H_4PteGlu$_\eta$	23.1		35.3	
	5-HCO-H_4PteGlu$_3$ + 5-CH_3-H_4PteGlu$_3$	36.8		38.3	
	Total folate recovered:	160.8		165.9	
Fourth	HCO-H_4PteGlu$_{1-2}$	3.4	(1.3)	1.9	(0.6)
	HCO-H_4PteGlu$_\eta$	152.3	(76.5)	111.2	(65.0)
	5-CH_3-H_4PteGlu	0.8	(0.8)	0.6	(0.4)
	5-CH_3-H_4PteGlu$_\eta$	13.2	(17.0)	50.1	(49.0)
	5-HCO-H_4PteGlu$_3$ + 5-CH_3-H_4PteGlu$_3$	20.6	(6.5)	30.0	(7.1)
	Total folate recovered:	190.3	(102.1)	193.8	(122.1)

Cells were maintained in synchronous culture through 4 cell cycles, in the presence of high and low CO_2 respectively. At 7 hr of the first cycle and at 10 and 15 hr of the 4th cycle, ascorbate extracts were prepared for folate analysis. Extracts were chromatographed on DEAE-cellulose before and after γ-glutamyl carboxy-peptidase treatment. The assay organism was *L. casei* Data in brackets are for extracts prepared at 15 hr of the 4th cell cycle: i.e. first hr of dark phase.

Table 4. Concentrations of folate derivatives after treatment with α-HPMS

Derivative	Folate concentrations (ng PteGlu equivalents/10^7 cells)			
	Light phase		Dark phase	
	Control	α-HPMS treated	Control	α-HPMS treated
HCO-H_4PteGlu$_{1-2}$	3.3	4.7	1.3	2.3
HCO-H_4PteGlu$_\eta$	102.7	44.1	76.5	54.3
5-CH_3-H_4PteGlu	0.8	1.1	0.8	0.8
5-CH_3-H_4PteGlu$_\eta$	9.8	8.8	17	2.8
5-HCO-H_4PteGlu$_3$ + 5-CH_3-H_4PteGlu$_3$	20.6	9.8	6.5	2.3
Total folate recovered:	137.2	68.5	102.1	62.5

Cells were grown synchronously with aeration (low CO_2) to a density of 5×10^4 cells/ml. At the start of the next light phase, α-HPMS (5 mM) was added. Extracts for folate analysis were prepared after 10 hr of the light phase and after 1 hr of the following dark phase. Other details as in Table 3.

Table 5. Requirements for glyxoylate decarboxylation *in vitro*

Reaction system	$^{14}CO_2$ produced(nmol)
Complete	0.17
$-Mn^{2+}$	0.05
+ Thiamine pyrophosphate	0.18
Complete; boiled extract	0.02

Extracts were prepared (10th hr of the 4th cell cycle) from division synchronized cells receiving air. The complete reaction system contained in 1.7 ml, 100 μmol K Pi buffer (pH 7.5), 10 μmol $MgCl_2$, 0.1 μCi glyoxylate [1-^{14}C] sp. act. (1 μCi/0.13 μmol), 350 units bovine catalase and 300 μg protein. The reaction was terminated after 1 hr at 30°.

Table 6. Stoichiometry of formate and CO_2 production from glyoxylate

Substrate	Cell free extracts prepared from			
	Low CO_2-treated cells		High CO_2-treated cells	
	$^{14}CO_2$ (nmol)	Formate-[^{14}C] (nmol)	$^{14}CO_2$ (nmol)	Formate-[^{14}C] (nmol)
Glycoxylate-[1-^{14}C]	6.5	n.d.	7.2	n d.
Glyoxylate-[2-^{14}C]	0.04	7.6	0.05	8 1

Extracts of cells cultured in high and low CO_2 respectively, were prepared at the 10 hr of the 4th cell cycle of treatment. Assay conditions were as described in Table 5 with the exception that 1 μCi of the substrates was employed (0.13 μmol glyoxylate) and 1.5 mg *Euglena* protein.

pathways. These alternatives were therefore assessed by chemical degradations (Table 8).

Serine isolated from the protein of air-grown cells contained 51 and 40% of its radioactivity in C-3 and C-2. Labelling of C-2 suggests that the glycine pool for the serine hydroxymethyltransferase reaction also contained ^{14}C. This could occur if 5,10-CH_2-H_4PteGlu, derived from labelled formylfolate, acted as substrate for a reversible glycine cleavage reaction [22, 23]. In contrast C-2 of serine from high CO_2-grown cells contained only 9% of the total with C-3 accounting for 60%. This increased labelling of the carboxyl group in these samples suggests that formate carbon partly entered this product after oxidation and refixation.

Table 7. Incorporation of formate-[^{14}C] into serine and methionine

Amino acid product	Pulse period	Air-grown (low CO_2) cpm/3 × 10^7 cells 2 min	5 min	cpm/nmol 2 min	5 min	5% CO_2 in air-grown (high CO_2) cpm/3 × 10^7 cells 2 min	5 min	cpm/nmol 2 min	5 min
Serine									
free		800	730	14.8	13.5	580	608	3.4	3.6
protein		4420	5350	164	198	1804	3630	69.4	139
Methionine									
protein		4110	4900	1054	1256	2110	5680	351	947

Cells were cultured synchronously in high and low CO_2 respectively and were harvested during the 4th cell cycle as described in the Experimental. Serine and methionine were isolated after pulse feeding formate-[^{14}C] for 2 and 5 min periods.

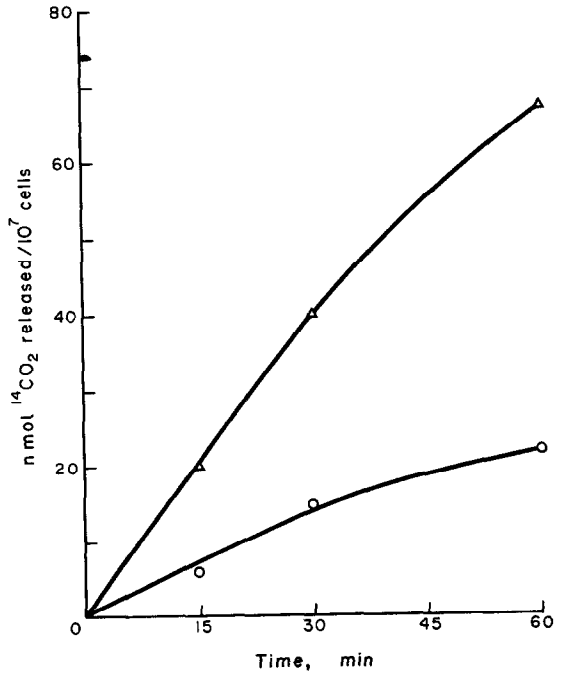

Fig. 4. Oxidation of formate by extracts of high and low CO_2-treated cells. Cells were grown synchronously in air (○) or 5% CO_2 in air (△) and were harvested at the 10th hour of the 4th cell cycle. Extracts were prepared and dialyzed (see Experimental) before assay. The complete reaction system (1.2 ml) contained: 50 μg NAD, 0.1 μCi: Na formate-[^{14}C] (2 μmol) and 1.5 mg *Euglena* protein. After incubation at 30° the reaction was terminated by addition of 2 N HCl.

Table 8. Intramolecular distribution of ^{14}C in protein serine

Carbon number	Air-grown (low CO_2) cpm recovered	^{14}C distribution (%)	5% CO_2 in air-grown (high CO_2) cpm recovered	^{14}C distribution (%)
1	246	9	958	31
2	1152	40	272	9
3	1433	51	1845	60

Protein serine was isolated from cells after 5 min pulse feedings with formate-[^{14}C] as described in Table 7. Samples were degraded using periodate (see Experimental).

DISCUSSION

In previous studies of folate metabolism during the cell cycle of *Euglena* [15] we observed increases in formyltetrahydrofolate synthetase which accompanied a net synthesis of formylfolate derivatives. This relationship is also appararent in the present data. For example the dramatic effects of high CO_2 and α-HPMS treatments on enzyme activities (Figs 2 and 3) and on formylfolates (Tables 3 and 4) implies that formyl group biogenesis in *Euglena* may be principally catalyzed by the synthetase. In this regard the availability of CO_2 appears to control this reaction. From the data in Figs 1 and 3 it follows that such control involves repression and derepression of enzyme synthesis. Although CO_2 may have a direct effect in modulating enzyme levels, the data for α-HPMS-treated cells (Table 2) suggest that a product of glycollate oxidation may be necessary to maintain high enzyme activities. We have not examined this possibility but it is noteworthy that Whiteley [24] found the corresponding synthetase of *Micrococcus aerogenes* to be induced by formate.

The above conclusions regarding the importance of the formyltetrahydrofolate synthetase reaction are strengthened by the results of the formate-[^{14}C] feeding experiments (Tables 7 and 8). Comparison of the sp. radioact. for air-grown and high CO_2-grown cells shows that less formate carbon entered serine and methionine when the latter treatment was applied. The heavy labelling of protein methionine shows that C_1 units, arising from formate, were not exclusively used in the glycollate pathway. In fact, earlier studies of Venkataraman *et al.* [25] have clearly shown that a wide variety of products become labelled when formate-[^{14}C] is supplied to *Euglena* for longer periods. These included products related to C_1

Extracts of both air-grown and high CO_2-grown cells had the ability to produce CO_2 and formate (Table 6) by a reaction which was enzymatic (Table 5). The rates of decarboxylation were low. However, the concentrations of glyoxylate used were low compared to earlier work [7], so these rates are probably suboptimal. Other comparisons with higher plant systems point to possible differences in the nature of this decarboxylation in *Euglena*. For example the *Euglena* glycollate dehydrogenase uses cytochrome *c* [26] and organelles of this species lack catalase [27–29]. Hence the decarboxylation of glyoxylate in *Euglena* may not involve the partially peroxidatic reactions noted for higher plant systems [7, 8]. Study of

this reaction and its intracellular localization in photoautotrophic cells of this species are therefore warranted.

The substantially higher levels of serine hydroxymethyltransferase after growth in high CO_2 (Figs. 2 and 3) suggest that this enzyme is not solely involved in catalyzing serine formation within the glycollate pathway. By analogy with other systems [6] it is logical that this enzyme will have some role in the generation of C_1 units during the cell cycle. This role would be enhanced in the high CO_2-grown cells when ability to form C_1 units at the formyl level of oxidation becomes limited. In this connection it remains to be determined whether methylene folate might also arise from glycine in *Euglena* and if so whether such a contribution is altered by the availability of CO_2.

In conclusion we visualize high CO_2 conditions favouring the synthesis of serine from C_3 intermediates of photosynthesis. In such cells C_1 units and glycine will be mainly produced through the serine hydroxymethyltransferase reaction. During growth in low CO_2 glycollate formation and oxidation will be favoured [30] and C_1 metabolism will include the formyltetrahydrofolate synthetase reaction. The extent to which the product of this reaction enters the glycollate pathway may vary during the cell cycle [15, 18]. Conceivably such participation could be altered by changing rates of glycollate excretion [18], production of methylenefolate from glycine and the needs of other biosynthetic pathways for C_1 units.

EXPERIMENTAL

Materials. Sodium formate-[^{14}C], glyoxylate-[1-^{14}C], glyoxylate-[2-^{14}C], and L-serine-[3-^{14}C] were purchased from Amersham-Searle, Des Plaines, Illinois. α-HPMS was obtained from Terochem Laboratories Ltd., Edmonton, Alberta. PteGlu and H_4PteGlu were purchased from Sigma, St. Louis. Other chemicals, of the highest quality commercially available, were obtained from Sigma. ICN Pharmaceuticals, Inc., Cleveland, Ohio and Fisher Scientific, Edmonton. Cylinders of air containing $5 \pm 0.1\%$ CO_2 were supplied by Matheson of Canada Ltd., Whitby, Ontario.

Culture of Euglena. *Euglena gracilis* Klebs (strain Z) ATCC 12716 was obtained from the American Type Culture Collection. Cells were initially maintained on slants of 1% Difco Bacto-Agar and 1% Bactopeptone with monthly transfer. Division synchronized cells [31], were grown autotrophically on a mineral salts medium (pH 6.8) modified after ref. [32], as described previously [15]. Cultures were aerated (600–700 ml/min) with moistened sterile air (low CO_2) or air containing 5% CO_2 (high CO_2). The culture temp. was 25° throughout light : dark cycles of 14:10 hr. The light intensity was 3500 lx provided by cool white fluorescent lamps. Cell divisions occurred exclusively in the dark period as described earlier [15]. Culture densities were measured in a Coulter counter model B after fixation in formaldehyde.

Extraction and chromatography of folate derivatives. For assay of folates, 300 ml of culture (5×10^4 to 10^5 cell/ml) were withdrawn and the cells harvested by centrifugation at 4000 *g* for 10 min. Extracts were then prepared [15] and subjected to column chromatography [33]. Individual folates were assayed microbiologically using *Lactobacillus casei* (ATCC 7469) and *Pediococcus cerevisiae* (ATCC 8081) as previously described [34]. Polyglutamyl folates were assayed with *L. casei* after treatment of the ascorbate extracts with a γ-glutamylcarboxypeptidase from pea cotyledons [34].

Enzyme studies. Cells (5×10^4 to 1×10^5/ml) were harvested by centrifugation at different stages of the 4th cell cycle following culture in high and low CO_2 respectively. After suspension in 2 ml of 5 mM KPi buffer (pH 6.9) containing 5 mM-mercaptoethanol the cells were sonicated and the extract was centrifuged at 18 000 *g* for 20 min. Low MW compounds were removed by dialysis against KPi buffer (pH 6.9) for 12 hr at 2° or by passage through a 1 × 5 cm column of Sephadex G-15. Formyltetrahydrofolate synthetase was assayed by the method of ref [11] and serine hydroxymethyltransferase by that of ref. [35]. Glycollate dehydrogenase was assayed colorimetrically at 25° using, 2,6-dichlorophenolindophenol as redox dye [36]. One unit of activity was taken as the amount of enzyme causing a decrease in *A* of 0.01/min at 590 nm. Formic dehydrogenase of desalted cell-free extracts was measured by following the NAD-dependent decarboxylation of formate-[^{14}C]. The reaction systems, in stoppered Warburg flasks, contained NAD, 50 μg, Na formate-[^{14}C], 2 μmol (0.1 μCi) and cell-free extract in a total vol. of 1.2 ml. The substrate was added after a 10 min equilibration period at 30°. Labelled CO_2 evolved during the reaction was absorbed in the centre well on fluted filter paper moistened with 0.1 ml of 20% KOH. The reaction was terminated at periods up to 1 hr by addition of 0.5 ml 2 N HCl from the sidearm. The flasks were shaken for an additional 3 min period to allow complete absorption of labelled product. Protein concns were determined by the method of ref. [37] using crystalline egg albumin (ICN Pharmaceuticals, Inc.), as reference standard.

Enzymatic decarboxylation of glyoxylate. The method of ref. [7] was followed using conventional Warburg flasks to facilitate CO_2 collection. The reaction system (1.7 ml) contained KPi buffer (pH 7.5), 100 μmol; 0.1 μCi of glyoxylate-[1-^{14}C] (1 μCi/0.131 μmol; $MnCl_2$ 10 μmol; bovine liver catalase, 350 units (Sigma and desalted cell-free extract containing 300 μg protein. The sidearm contained 0.5 ml of 2 N HCl and the centre well contained a fluted filter paper moistened with KOH as described above. The reaction was carried out with shaking at 30° and was terminated by addition of HCl. Boiled enzyme controls were included in each series of assays. The production of formate from C-2 was verified using glyoxylate-[2-^{14}C] followed by TLC on 20 × 20 cm Si gel GF plates with EtOH–NH_4OH–H_2O (20:1:4) as solvent. The R_f values for glyoxylate and formate were 0.34 and 0.55 respectively.

Formate [^{14}C] feeding experiments. Euglena cells were grown for 3 cell cycles in high and low CO_2 respectively. After 7 hr of the 4th cycle the cells (3×10^7) were harvested by centrifugation, and resuspended in 15 ml of fresh media. These cultures were then aerated with high or low CO_2 and were illuminated (3500 lx) for 3 hr at 25°. Following this equilibrium period 10 μCi of Na formate-[^{14}C] (50 μCi/μmol) were added and incubation continued at 25° for up to 5 min with aeration. Evolved $^{14}CO_2$ was absorbed in a trap containing 15 ml of 20% KOH soln. The cells were killed by addition of 2 ml of 1.5 N $HClO_4$ and were sonicated for 2 min at 4°. After centrifugation at 18 000 *g* for 10 min, the residue was washed with 3 ml of H_2O at 2°. The pH of the combined supernatants was adjusted to 6.3 by addition of $KHCO_3$ and following a further centrifugation (18 000 *g* for 10 min) the extracts were fractionated with ion exchange resins [38]. Perchlorate insoluble material was suspended in 3 ml of 6 N HCl and hydrolyzed *in vacuo* for 4.5 hr at 145°. Protein amino acids were recovered from the hydrolyzate and fractionated using an amino acid analyzer [39]. Radioactivities were measured by scintillation counting [40]. Samples of serine-[^{14}C], collected from the analyzer, were degraded using periodate [41].

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