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GLYCINE CLEAVAGE AND GENERATION OF ONE-CARBON UNITS IN *EUGLENA GRACILIS**

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Key Word Index—*Euglena gracilis* (Strain Z); Euglenales; synchronized cultures; glycine cleavage, methylenefolate formation and utilization; pathways of folate metabolism; effects of high and low CO_2 concentrations.

Abstract—*Euglena gracilis* Klebs (strain Z) was maintained in division synchronized autotrophic culture, receiving either air (low CO_2) or 5% CO_2 in air (high CO_2) at 25°. These growth conditions, which alter cellular glycollate oxidation and formylfolate biosynthesis, were assessed for possible effects on ability to cleave glycine. Glycine cleavage was examined by comparing incorporations of glycine-[1-^{14}C] and -[2-^{14}C] into products of folate metabolism. Cells grown in high and low CO_2 converted greater amounts of C-1 to labelled CO_2 than C-2. The C-2 of glycine was more extensively incorporated into RNA, adenine, serine and methionine than the carboxyl carbon in both types of culture. Degradations of serine derived from protein were consistent with the conclusion that glycine acted as a source of methylenefolate under these growth conditions. It is concluded that the glycine cleavage reaction will augment folate generated through the 10-formyltetrahydrofolate synthetase (EC 6.3.4.3) reaction when conditions favour glycollate formation and metabolism. During growth in high CO_2, glycine decarboxylase and serine hydroxymethyltransferase (EC 2.1.2.1) are visualized as the major enzymes for generation of metabolically important folates.

INTRODUCTION

The glycine cleavage reaction (glycine synthase; EC 2.1.2.10), detected in *Peptococcus glycinophilus* by Sagers and Gunsalus [1] has more recently been the subject of detailed study. The reaction, which is folate dependent (equation 1), is widely distributed in bacteria [2, 5], animals [6, 8] and plants [9, 18]. In *P. glycinophilus* [19, 22],

Equation 1

Glycine + H_4PteGlu + NAD ⇄
5,10-CH_2H_4PteGlu + CO_2 + NH_3 + NADH.

Arthrobacter globiformis [23, 25] and rat liver mitochondria [2, 26] this reversible reaction is collectively catalysed by 4 protein components. Although such systems form glycine *in vitro*, physiological conditions favour the cleavage reaction [2]. In this respect the reaction is acknowledged as an important source of 5,10-methylenetetrahydrofolate for utilization in a variety of folate-dependent syntheses [27].

Interest in this reaction in plants was generated by the detailed work of Tolbert's group [11, 28]. These workers showed that glycine cleavage was an integral part of the glycollate pathway. Subsequently evidence accumulated that this decarboxylation was a source of photorespiratory CO_2 [15, 16, 29]. In common with other eukaryotes, the plant enzyme is mitochondrial [15, 17, 30, 31] and cleavage is accompanied by ATP synthesis [13, 32]. Methylenefolate arising from C-2 of glycine is largely incorporated into serine by a mitochondrial serine hydroxymethyltransferase [12, 13, 16, 33]. However, pea mitochondria [12] produce labelled formyl and methylfolates from glycine [2-^{14}C]. It can therefore be argued that at least in some cases the enzyme is not tightly coupled to serine synthesis but produces 5,10-methylenetetrahydrofolate for use in other folate-dependent pathways.

In *Euglena* there is evidence that C_1 units are generated in the 10-formyltetrahydrofolate synthetase (EC 6.3.4.3) reaction [34]. Growth in 5% CO_2 in air (high CO_2) or in the presence of hydroxysulphonate reduced this synthesis and the ability to oxidize glycollate [34]. However, high CO_2-grown cells retained a characteristic division synchrony and, after 4 cell cycles of treatment, contained elevated levels of serine hydroxymethyltransferase and 5-CH_3H_4PteGlu$_n$. Considering the close metabolic relationships between serine and glycine and evidence for operation of the glycollate pathway in *Euglena* [35, 37] it follows that high CO_2 may increase the role of these amino acids as sources of methylenefolate.

This possibility has been considered in the present work. Contributions of glycine to folate metabolism [18] have been assessed by comparing the incorporations of C-1 and C-2 into RNA, DNA, adenine, serine and methionine. Cells growing in high or low CO_2 had ability to cleave glycine, and C_1 units resulting from this were utilized in a variety of folate-dependent pathways.

RESULTS

Glycine metabolism during growth in high and low CO_2

Euglena cells rapidly took up glycine in the light or dark. This compound, however, was only utilized as a sole source of carbon when the cells were illuminated. This apparent photoheterotrophy has been noted in an

* The abbreviations used for derivatives of folic acid are those suggested by the IUPAC-IUB Commission listed in (1967) *Biochem. J.* **102**, 15, e.g. 5,10-CH_2H_4PteGlu = N^5,N^{10}-methylenetetrahydropteroyl monoglutamate.

earlier report by Murray *et al.* [38]. Division synchronized cells maintained in high or low CO_2 decarboxylated glycine in the dark (Table 1) but in each case C-2 contributed little label to the evolved CO_2. In more detailed feeding experiments in the light (Table 2) it was clear that the glycine carbon was extensively metabolized. More total ^{14}C was taken up and incorporated by the high CO_2-grown cells. This treatment also affected the distribution of ^{14}C, increasing that entering protein, RNA and DNA but decreasing the amount in the sugar and other fractions. In both cultures C-2 of glycine labelled the nucleic acid, protein, amino acid and acetone soluble fractions more heavily than C-1. When the latter fractions were examined after column chromatography

Table 1. Decarboxylation of glycine by darkened *Euglena* cells

Culture conditions	$^{14}CO_2$ evolved (dpm/10^6 cells) Air-grown	5% CO_2 in air-grown
Glycine-[1-^{14}C]	20600	21700
Glycine-[2-^{14}C]	4200	4130

Cells (10^6) were cultured in air and high CO_2 respectively and at the 10th hr of the light period were placed in the dark for 30 min at 30°. Labelled glycine (1 μCi, 0.02 μmol of glycine [1-^{14}C] or -[2-^{14}C], respectively) was then added and incubation in the dark continued for 1 hr.

Table 2. Metabolism of glycine-[1-^{14}C] and glycine-[2[^{14}C] in the light by air-grown and 5% CO_2-grown cells

	Air				High CO_2			
	Glycine-[1-^{14}C]		Glycine-[2-^{14}C]		Glycine-[1-^{14}C]		Glycine-[2-^{14}C]	
	dpm/10^9	% of ^{14}C incorporated	dpm/10^9 cells	% of ^{14}C incorporated	dpm/10^9 cells	% of ^{14}C incorporated	dpm/10^9 cells	% of ^{14}C incorporated
Amino acids*	56200	19.1	86700	18.4	46800	14.8	99200	17.8
Organic acids	18200	6.2	18500	3.9	8800	2.8	16200	2.9
Sugars	14300	4.9	12100	2.6	3800	1.2	8700	1.6
RNA	14700	5.0	20200	4.3	18800	5.9	32200	5.8
DNA	5400	1.8	6600	1.4	18500	5.8	25600	4.6
Protein	98900	33.6	156000	33.1	179000	56.4	279000	49.9
Acetone solubles	86800	29.5	172000	36.4	41900	13.2	98200	17.6
Total ^{14}C incorporation	295000		472000		318000		559000	

* After correction for residual glycine [^{14}C]; see Table 3.
Cells (10^9) were cultured and harvested as in Table 1. After equilibration in the light for 30 min at 25°, 5 μCi (0.1 μmol) of glycine-[1-^{14}C] or -[2-^{14}C] respectively were added. Feeding was terminated after 30 min.

Table 3. Labelling of free amino acids and adenine following glycine-[1-^{14}C] and -[2-^{14}C] feeding in light

	Air				5% CO_2			
	Glycine-[1-^{14}C]		Glycine-[2-^{14}C]		Glycine-[1-^{14}C]		Glycine-[2-^{14}C]	
Compounds	dpm/10^9 cells	Sp. act.*	dpm/10^9 cells	Sp. act.	dpm/10^9 cells	Sp. act.	dpm/10^9 cells	Sp. act.
Gly	4590	59000	6490	72600	7440	74600	14100	132000
Ser	4370	48300	11100	120000	3210	20400	8990	63500
Met	890	N.C.	930	N.C.	1030	N.C.	720	N.C.
Asp	3290	60500	2410	53600	5290	61100	3830	40600
Glu	8720	39200	10400	43300	5070	16700	11700	39900
Ala	6690	21800	7040	19100	1370	6570	2960	13000
Adenine	1090	21400	5080	77000	3610	86000	10900	125000

* Sp. act. in dpm/μmol. N.C. = not calculated due to small pool size.
Experimental details as in Table 2. Individual compounds were separated by use of ion exchange chromatography.

Table 4. Labelling of protein amino acids following glycine-[1-^{14}C]and -[2-^{14}C] feeding in light

	Air				5% CO_2			
	Glycine-[1-^{14}C]		Glycine-[2-^{14}C]		Glycine-[1-^{14}C]		Glycine-[2-^{14}C]	
Protein Amino Acids	dpm/10^9 cells	Sp. act.*	dpm/10^9 cells	Sp. act.	dpm/10^9 cells	Sp. act.	dpm/10^9 cells	Sp. act.
Gly	126000	8700	123000	9640	215000	13600	224000	17500
Ser	44500	5540	72300	9410	54500	6170	102000	14600
Met	840	360	13200	4550	720	260	6880	3550
Asp	3650	260	4600	330	2840	220	3810	330
Glu	5000	290	10300	620	2890	170	15100	950
Ala	4680	270	6000	380	3120	170	12600	790

* Sp. act. expressed in dpm/μmol. Experimental details as in Table 2.

Table 5. Intramolecular labelling of protein serine following glycine-[1-^{14}C] and -[2-^{14}C] feeding in light

Carbon Position		Air				5% CO_2			
		Glycine-[1-^{14}C]		Glycine-[2-^{14}C]		Glycine-[1-^{14}C]		Glycine-[2-^{14}C]	
		dpm recovered	C^{14} distribution %	dpm recovered	C^{14} distribution %	dpm recovered	C^{14} distribution %	dpm recovered	C^{14} distribution %
COOH	(1)	1840	67.7	440	9.4	22100	79.9	1740	4.5
$CH.NH_2$	(2)	410	15.1	2420	51.8	2010	7.3	26560	69.4
CH_2OH	(3)	470	17.3	1810	38.8	3570	12.9	9990	26.1

Samples of protein serine isolated after glycine-[^{14}C] feedings (Table 2) were degraded using periodate [52].

(Tables 3 and 4) this heavier labelling by C-2 was also apparent for a number of individual compounds. In this respect, adenine, serine and methionine (free and protein) showed the greatest difference in sp. act. Comparing data for glycine-[1-^{14}C] and -[2-^{14}C], it is clear that the free glycine pools of high and low CO_2-grown cells, contained more ^{14}C when glycine-[2-^{14}C] was supplied (Table 3). This tendency was more pronounced in the high CO_2-grown cells. Chemical degradations of such glycine samples revealed some randomization of ^{14}C so that *ca* 6% of the radioactivity following glycine [2-^{14}C] feeding was recovered in the carboxyl carbon. The intramolecular distribution of ^{14}C in protein serine (Table 5) also showed some randomization so that both carbons of glycine contributed to all 3 carbons of this product. However, the C-2 of glycine made greater contributions to the C-3 of serine than the carboxyl carbon. In air-grown cells C-2 and C-3 of serine were almost equally labelled when glycine [2-^{14}C] was supplied, but for high CO_2 cells protein serine was more heavily labelled in the 2 position.

Formate metabolism in high and low CO_2-grown cells

From the above data it is deduced that glycine is utilized in folate metabolism. From the nature of these metabolic products it is clear that formyl, methylene and methyl groups must be derived from glycine cleavage. As earlier work [34] suggested that *Euglena* also uses formate in these syntheses it was of interest to examine the utilization of formate-[^{14}C] in more detail. This substrate is rapidly oxidized in *Euglena*, so feeding experiments were carried out for short periods (10 min) in darkness to prevent the refixation of ^{14}C that would occur in the light via photosynthesis. Under such conditions formate was rapidly incorporated into a variety of products. The nature of these (Tables 6 and 7) suggested that formate was incorporated as a C_1 compound rather than via CO_2 fixation. The incorporation of label into the major products was lower when the cells had been grown in high CO_2 (Tables 6 and 7). In air-grown and in 5% CO_2-grown cells large amounts of formate carbon entered protein serine and methionine despite the relatively short feeding period. Labelling of the free serine, glycine, aspartate and adenine pools was also evident in these experiments.

Table 6. Metabolism of formate-[^{14}C] by *Euglena gracilis* in darkness after growth in the presence of high and low CO_2

Fraction	Air		5% CO_2	
	dpm/1.5 × 10^8 cells	% of ^{14}C incorporated	dpm/1.5 × 10^8 cells	% of ^{14}C incorporated
Amino acids	217000	40.3	13000	43.9
Organic acids	72500	13.5	43800	14.8
Sugars	40400	7.5	12200	4.1
RNA	46000	8.5	15700	5.3
DNA	3100	0.6	5100	1.7
Protein	119000	22.2	63000	21.3
Acetone soluble	40900	7.6	26400	8.9
Total incorporation	539000		296000	

Cells (1.5 × 10^8) were cultured and harvested as in Table 1 followed by equilibration for 30 min in darkness at 25°. Labelled formate (5 μCi, 0.08 μmol) was then added and incubation continued for 10 min. During the equilibration and feeding periods the cell suspensions were bubbled with air.

Table 7. Incorporation of formate-[^{14}C] into adenine and into free and protein amino acids by darkened cells

Compounds	FREE AMINO ACIDS				PROTEIN AMINO ACIDS			
	Air		5% CO_2		Air		5% CO_2	
	dpm/1.5 × 10^8 cells	Sp. act.*	dpm/1.5 × 10^8 cells	Sp. act.	dpm/1.5 × 10^8 cells	Sp. act.	dpm/1.5 × 10^8 cells	Sp. act.
Gly	6840	92000	20300	226000	ND	ND	ND	ND
Ser	17900	347000	27300	384000	29500	13500	20500	2350
Met	ND	—	ND	ND	29500	71300	11200	15800
Asp	16500	878000	11300	341000	ND	—	ND	—
Adenine	11600	723000	18000	727000	—	—	—	—

* Specific radioactivities are expressed in dpm/μmol.
ND = radioactivity not detected. Experimental details as in Table 6.

DISCUSSION

The ability of *Euglena* to incorporate C-2 of glycine into adenine, serine, methionine and nucleic acids (Tables 2–4) and to produce CO_2 from the carboxyl carbon (Table 1) shows that extensive cleavage of this substrate occurs under autotrophic conditions. The earlier findings that this species can utilize glycine as a sole source of carbon in the light and that such culture is accompanied by release of ammonium [38] clearly supports this conclusion. Evidence for glycine cleavage was obtained after growth in high or low CO_2 and when labelled substrate was supplied in light or darkness (Tables 1 and 3). The nature of this reaction in *Euglena* remains to be elucidated by studies *in vitro*. However, the products of glycine metabolism strongly suggest that cleavage resulted in generation of methylene folate and that this product participates in folate metabolism irrespective of carbon flux through the glycollate pathway. Furthermore cleavage does not appear to be tightly coupled to serine synthesis in *Euglena*. The data in Tables 3 and 4 show that C-2 of glycine participated in the synthesis of adenine and methionine to much greater extents than the carboxyl carbon. During growth in low CO_2 it is logical that operation of the glycollate pathway would be accompanied by a drain of C_1 units to support these syntheses. Thus carbon flow through a glycollate → glyoxylate → glycine → C_1 sequence could be of central importance in these cells where intensive synthetic activity precedes cell division [39, 41]. This folate would also augment that generated through the 10-formyltetrahydrofolate synthetase reaction [34].

As noted above, cells cultured in 5% CO_2 in air also utilized C-2 of glycine in folate metabolism. However, this treatment, which curtails formation [42] and oxidation of glycollate [34, 36], would tend to diminish glycine synthesis from C_2 compounds. Cells grown in 5% CO_2 in air contain greater concentrations of free glycine and serine (Table 3), have elevated serine hydroxymethyltransferase activity [34] and, by analogy with other photosynthetic systems [42], would form greater amounts of C_3 compounds by carboxylation of ribulose-1,5-diphosphate. It is therefore conceivable that high CO_2 would favour a flow of photosynthetic intermediates into serine [43, 45] which with glycine, could serve as the main sources of methylene folate. This route would have added significance during growth in high CO_2 as ability to generate more oxidized folates from formate will be reduced (Table 7; ref. [34]).

Degradations of protein serine (Table 5) showed that some randomization of ^{14}C occurred during glycine feeding. Earlier workers have reported similar effects on serine [46] and carbohydrate [47] labelling patterns in other species. These data suggest active turnover of the precursor pools, with ^{14}C re-entering the glycollate pathway via photosynthesis. In low CO_2-grown cells the labelling pattern of serine (Table 5) implies that essentially all of the C_1 units needed for the β-carbon arose from C-2 of glycine. In high CO_2 however dilution of the β-carbon occurred. This could occur if a relatively large unlabelled pool of serine exchanged with labelled glycine in the serine hydroxymethyltransferase reaction.

A comparison of data in Tables 3, 4 and 7 suggests that formate was a better precursor of adenine and methionine than glycine. Although formate was supplied in the dark, our earlier studies [34] showed a similar flow in illuminated cells. Interpretation of such data is difficult without knowledge of the precise localization of these biosynthetic pathways. However, if the glycine cleavage reaction in *Euglena* is mitochondrial [32] and the 10-formyltetrahydrofolate synthetase reaction is cytosolic [48, 49], a channeling of formyl and methylene folates into distinct pathways could be visualized.

EXPERIMENTAL

Materials. Sodium formate-[^{14}C], glycine-[1-^{14}C] and glycine-[2-^{14}C] were purchased from Amersham-Searle, Des Plaines, Illinois. Other chemicals, were obtained from Sigma, St. Louis and from Fisher Scientific, Edmonton. Cylinders of air containing 5.0 ± 0.1% CO_2 were supplied by Matheson of Canada Ltd., Whitby, Ontario.

Culture of Euglena. Division synchronized cultures were grown in Na citrate medium [50] as previously described [34]. Culture densities were measured with a haemocytometer after HCHO fixation. Cells (*ca* 10^5/ml) were harvested by centrifugation at the 10th hr of the light period. After washing in fresh sterile culture media the cells were resuspended to give the densities shown in the tables.

Decarboxylation of glycine-[^{14}C] *in vivo.* This was studied in Warburg flasks fitted with 2 sidearms and a centre well. Cell suspension (3 ml, harvested as above) containing 10^6 cells were placed in the main compartment. The centre well contained 0.2 ml of 20% KOH and a small filter paper wick. The flasks were incubated in the dark at 30° for 30 min. Glycine-[1-^{14}C] or -[2-^{14}C] was then added from the sidearm (see Table 1) and incubation was continued with shaking for 1 hr. The reaction was stopped by addition of 0.4 ml of 4N H_2SO_4 from the second sidearm. After shaking for a further 1 hr, the contents of the centre well were assayed for ^{14}C by liquid scintillation counting [54].

Glycine and formate feeding expts. These were carried out in 20 ml test tubes containing 5 ml of cell suspension. The tubes were aerated with air or 5% CO_2 in air throughout. Before addition of labelled glycine the cells were illuminated (3500 lx) for 30 min at 25°. Following this equilibration period 5 μCi of glycine-[1-^{14}C] or -[2-^{14}C] were added and incubation in the light continued for 30 min. In formate-[^{14}C] feeding expts, equilibration and incubation with the label were carried out in darkness (Table 6). After feeding, the cells were removed by rapid centrifugation, washed in fresh ice-cold medium and killed with 5 ml 20% TCA. Nucleic acids and protein were fractionated by the method ref. [51]. H_2O soluble compounds were separated by ion-exchange chromatography [18] and protein was hydrolysed in 6N HCl at 110° for 16 hr under N_2. Individual amino acids were isolated using amino acid analyser as described previously [18]. Serine-[^{14}C], recovered from the protein was degraded using periodate [52]. Labelled glycine samples were degraded with ninhydrin [53]. Radioactivities were measured by liquid scintillation counting [54]; counting efficiencies were *ca* 70%.

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