STIMULATION OF FOLATE METABOLISM BY EXOGENOUS GLYCINE IN *NEUROSPORA CRASSA* WILD TYPE

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1. Introduction

Although L-serine is generally regarded as the principal source of methylenetetrahydrofolate (5, 10-CH_2-H_4 PteGlu)* in biological systems, there is increasing evidence that glycine must also be recognized as a precursor of this metabolically important folate. For example, the α-carbon of glycine is converted into the β-carbon of serine in bacteria [1], rat liver [2], avian liver [3], *Saccharomyces* [4] and higher plants [5]. In this respect it is now clear that 5,10-CH_2-H_4 PteGlu arises from glycine as a result of the glycine cleavage system, which has been extensively studied in *Peptococcus glycinophilus* [6–9], but is also present in a variety of eukaryotic species [2–10]. Besides acting as a source of the β-carbon of serine, 5, 10-CH_2-H_4 PteGlu arising from glycine may participate in a variety of other folate-mediated reactions including those of methyl group biogenesis [11] and purine ring formation [12]. In *S. cerevisiae* and *Neurospora crassa* glycine may also enhance production of this folate derivative by elevating the levels of serine hydroxymethyl-transferase [4,13]. However, the effect on general C-1 metabolism of this increased production of 5,10-CH_2-H_4 PteGlu requires elucidation.

* The abbreviations used for folic acid and its derivatives are those suggested by the IUPAC-IUB Commission as listed in Biochem. J. 102(1967)15: e.g. 5-CH_3-H_4 PteGlu = N^5-methyltetrahydropteroylmonoglutamate.

The present studies have shown that exogenous glycine increases the rate of C-1 metabolism in *N. crassa* by increasing synthesis of folate derivatives and by raising the levels of key enzymes for interconversion of C-1 units within the folate pool. [^{14}C]Glycine feeding experiments showed that growth in exogenous glycine stimulated uptake and metabolism of this amino acid. The specific radioactivities of major products following 1-[^{14}C]glycine and 2-[^{14}C]glycine feeding indicated that cultures receiving exogenous glycine during growth extensively cleaved this amino acid, utilizing carbon-2 principally for synthesis of serine, adenine and methionine.

2. Materials and methods

2.1. *Culture conditions*

Neurospora crassa, Lindegren A (FGSC no 853) was cultured aerobically at 25°C in Vogel's defined liquid medium [14] containing 2% sucrose as carbon source. Conidiospores were harvested from mycelia grown on solid medium at 25°C in darkness followed by illumination for a 3 day period [15]. Sterile media (500 ml) with (1 mM) or without a glycine supplement were inoculated (10^6 conidia/ml) and aerated vigorously with sterile air at 25°C for periods up to 25 hr. Samples were harvested after 22 hr growth by rapid filtration for folate analysis, enzyme studies and [^{14}C]glycine feeding.

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2.2. *Analysis of folate derivatives*

Samples of harvested mycelia were washed three times in ice-cold Vogel's medium, suspended in 0.6% potassium ascorbate (pH 6) and heated at 95°C for 10 min [16]. Individual folates present in these extracts were separated by DEAE-cellulose [17] and assayed microbiologically [18] using *Lactobacillus casei* (ATCC 7469) and *Pediococcus cerevisiae* (ATCC 8081). γ-Glutamyl carboxypeptidase treatment of extracts prior to chromatography and basic criteria for identification of derivatives were as previously described [19].

2.3. *Enzyme assays*

Immediately after harvesting, mycelial samples were ground at 2°C in 0.2 M Tris–HCl buffer containing 10 mM KCl, 10 mM EDTA, 1 mM $MgCl_2$ and 0.1 mM dithiothreitol (pH 7.4). After centrifugation (15 000 *g* for 20 min) the extracts were desalted by passage through Sephadex G-15. This latter step was omitted in assays of 5,10-methylenetetrahydrofolate dehydrogenase activity [20]. 10-Formyltetrahydrofolate synthetase was assayed by the method of Hiatt [21] and serine hydroxymethyltransferase by the method of Taylor and Weissbach [22]. Methylenetetrahydrofolate reductase was assayed according to Dickerman and Weissbach [23]. Protein was determined colorimetrically [24].

2.4. *[^{14}C]Glycine feeding experiments*

Mycelia were harvested after 22 hr growth at 25°C in the presence (1 mM) or absence of exogenous glycine. Each 50 ml of hyphal suspension was washed three times in unsupplemented Vogel's medium and finally resuspended in 50 ml of this medium prior to addition of [^{14}C]glycine. In such experiments 10 μCi of [1-^{14}C]glycine (57 μCi/μmole) or 10 μCi [2-^{14}C]glycine (60 μCi/μmole) were added to the cultures followed by incubation at 25°C for 30 min. After rapid filtration and washing in ice-cold water, mycelia were transferred to boiling water for 10 min. Soluble products of glycine metabolism were fractionated and assayed for radioactivity as previously described [25].

3. Results

3.1. *Effect of glycine on levels of folate derivatives*

Mycelia grown for 22 hr in the presence of 1 mM glycine were found to contain 27.0 μg folate/g dry wt which was increased to 75.6 μg folate/g dry wt following carboxypeptidase treatment. In cultures which had not received this glycine supplement during growth the corresponding figures were 8.5 and 25.7 μg folate/g dry wt respectively. Chromatography revealed that glycine markedly altered the composition of the folate pool (fig.1, table 1). In this respect the greatest

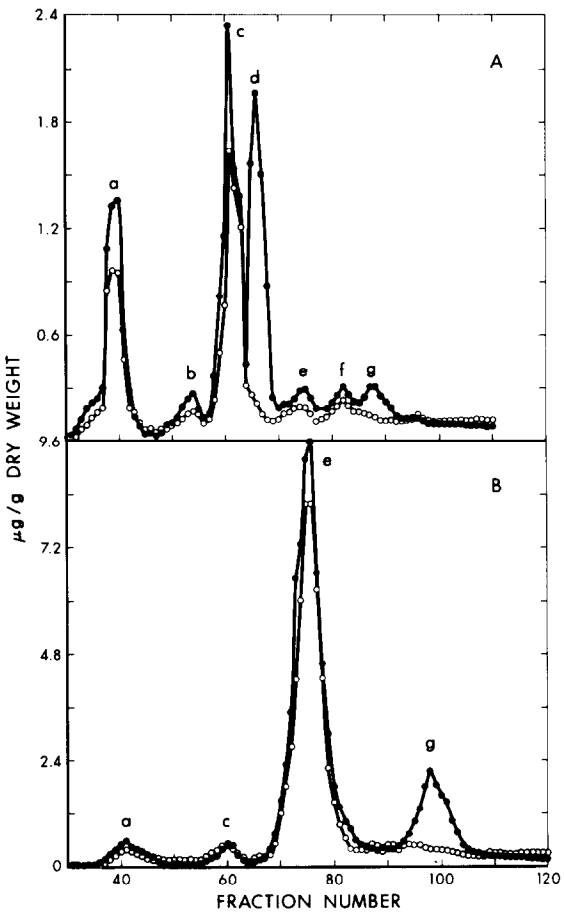

Fig.1. Chromatography of folate derivatives. Extracts were prepared after 22 hr growth in unsupplemented medium (A) and in medium supplemented with 1 mM glycine (B). After γ-glutamyl carboxypeptidase treatment, the extracts were fractionated in DEAE-cellulose. Peaks are identified as: a) 10-HCO-H_4PteGlu; b) 10-HCO-H_4PteGlu$_2$; c) 5-HCO–H_4PTeGlu; d) 5-CH_3-H_4PteGlu; e) H_4PteGlu; f) 5-HCO-H_4PteGlu$_2$ and H_4PteGlu$_2$; g) 5-CH_3-H_4PteGlu$_2$. Growth response of *L. casei* (•) and *P. cerevisiae* (○).

Table 1
The effect of exogenous glycine on individual folate derivatives in *N. crassa* wild type

Derivative	Unsupplemented medium	Glycine supplemented medium
10-HCO-H_4PteGlu	6.2	2.6
10-HCO-H_4PteGlu$_2$	1.3	n.d.
5-HCO-H_4PteGlu	7.85	1.9
5-CH_3-H_4PteGlu	6.25	n.d.
H_4PteGlu	1.30	61.50
5-HCO-H_4PteGlu$_2$ and H_4PteGlu$_2$	1.20	n.d.
5-CH_3-H_4PteGlu$_2$	1.60	10.60
Totals:		
Before γ-GCP treatment	8.5	27.0
After γ-GCP treatment	25.7	75.6

Expressed as μg folic acid equivalents/g dry wt.
Mycelia were harvested after 22 hr growth. Total growth of the unsupplemented culture was 2.9 g dry wt and for the supplemented culture 2.01 g dry wt. Total folate synthesized/culture was 76 μg and 152 μg respectively.
n.d. = not detected. γ-GCP = γ-glutamyl carboxypeptidase.

change involved a derivative (fig.1, peak e) which supported growth of both *L. casei* and *P. cerevisiae* and occupied a position in the elution sequence corresponding to authentic H_4PteGlu. It is clear from table 1 that the increased folate synthesis of glycine-treated cultures was largely attributable to increased synthesis of this derivative. In contrast, growth in the presence of glycine decreased the concentration of formyl derivatives and 5-CH_3-H_4PteGlu was no longer detected. However, polyglutamyl forms of this methyl derivative were formed in greater amounts when glycine was supplied as is evident from the levels of 5-CH_3-H_4PteGlu$_2$ detected after γ-glutamyl carboxypeptidase treatment of these extracts.

Table 2
Specific activities of folate enzymes after 22 hr growth

Enzyme	Culture in: Unsupplemented medium	Culture in: Glycine supplemented medium
Serine hydroxymethyltransferase*	846	970
5,10-methylenetetrahydrofolate reductase*	90	127
5,10-methylenetetrahydrofolate dehydrogenase**	1.9	2.8
10-formyltetrahydrofolate synthetase**	1.3	5.2

* Product formed, pmoles/mg protein/min
** Product formed, nmoles/mg protein/min

Table 3
Specific radioactivities of major products after [1-^{14}C]glycine and [2-^{14}C]glycine feeding

	Unsupplemented medium		Glycine supplemented medium	
Compound (cpm/μmole)	[1-^{14}C]Gly	[2-^{14}C]Gly	[1-^{14}C]Gly	[2-^{14}C]Gly
Aspartate	880	960	908	1110
Glutamate	710	252	124	460
Serine	1360	1660	5800	10 320
Glycine	2900	3300	7000	12 000
Alanine	26	30	108	236
Methionine	2750	2700	15 000	81 200
Adenine	3170	6400	7200	14 000
Carbon dioxide evolved (cpm/mg dry wt)	196	4	514	10
Glycine uptake (cpm/mg dry wt)	9800		27 000	

3.2. *Effect of glycine on enzymes of C-1 metabolism and ability to metabolize [^{14}C]glycine*

The specific activities of four enzymes of C-1 metabolism were determined after 22 hr growth in the presence and absence of 1 mM glycine (table 2). Clearly, growth in the presence of this supplement raised specific enzyme activity in each case, having greatest effect on 10-formyltetrahydrofolate synthetase and least on serine hydroxymethyl-transferase. These effects of glycine were also observed when extracts were prepared after shorter periods of growth.

Besides containing greater levels of folate and key enzymes of C-1 metabolism, glycine-grown cells had greater ability to take up and metabolize this amino acid (table 3). By comparing the incorporations of carbons 1 and 2 it is clear that the carboxyl group was more readily liberated as CO_2 and that this conversion was enhanced by prior growth in medium containing glycine. The specific radioactivities of the major soluble products show that only adenine was preferentially formed from carbon-2 when growth occurred in unsupplemented medium. However, mycelia grown with this glycine supplement extensively incorporated carbon-2 into serine and methionine in addition to forming adenine.

4. Discussion

When the slightly slower growth rate of the glycine supplemented cultures is considered, it is clear that increased folate concentration in such treatment was also accompanied by a doubling in the rate of net folate synthesis (table 1). Carboxypeptidase treatment of these mycelial extracts showed that much of this increase involved polyglutamyl derivatives that appeared to be unsubstituted. However, in this connection it should be noted that 5,10-CH_2-H_4-PteGlu present in these extracts would rapidly dissociate into H_4PteGlu under the conditions employed for extraction and chromatography [26]. Thus in the presence of exogenous glycine a major part of the folate pool may in fact contain C-1 units at the hydroxymethyl level of oxidation. From the enzyme assays and [^{14}C]glycine feeding experiments (tables 2 and 3) it follows that glycine-grown cells would have greater abilities to oxidize and reduce 5,10-CH_2-H_4 PteGlu and to produce this derivative from carbon-2 of glycine. The reason for the general decrease in formyl folates (table 1) is not clear, but could conceivably be related to their rapid utilization in purine ring formation coupled with immediate production of 5,10-CH_2-H_4PteGlu from the H_4PteGlu released.

Although direct assay of the glycine cleavage reaction was not made in this current study, indirect evidence for its occurrence in glycine grown cells was obtained (table 3). As in bacteria [27], the reaction may be induced by exogenous glycine as considerably less cleavage occurred when the mycelia were grown in the absence of this amino acid.

Surprisingly the specific radioactivities of methionine and adenine were higher than that of endogenous glycine

when [2-^{14}C]glycine was supplied to mycelia from the supplemented medium (table 3). This was also observed for adenine produced from [2-^{14}C]glycine by unsupplemented cells. These data strongly suggest that reactions for production of 5,10-CH_2-H_4PteGlu from [2-^{14}C]glycine as well as oxidation and reduction of the C-1 unit are compartmented in *Neurospora*. The specific activity of the endogenous glycine pool was also higher after feeding [2-^{14}C]glycine to supplemented cells. Chemical degradation of [^{14}C]serine samples from such cells has revealed that this may be due to an extensive turnover of glycine molecules. It appears that the enrichment of the glycine pool with ^{14}C is due to conversion of [2,3-^{14}C]serine to glycine by a pathway involving decarboxylation.

A requirement for C-1 units will clearly exist during the exponential growth of *N. crassa*. This requirement will be even greater when exogenous sources of methionine and purines are not available to the cells. Under such conditions excess glycine, derived from serine or available in the growth medium, may serve to meet part of this requirement. Thus the endogenous pool size of glycine, by regulating activity of the glycine cleavage reaction, may effectively augment the supply of 5,10-CH_2-H_4PteGlu when the demand for C-1 units is high. This possibility is currently being investigated in the wild type and in strains which are deficient in serine hydroxymethyltransferase.

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