Cossins, E.A. and P.Y. Chan. Glycine stimulates polyglutamyl folate synthesis in N. crassa.

In previous studier (Cossins, Chan and Combepine 1975 FEBS Lett. 54: 286) w. reported that exogenous glycin. (1 mM) stimulated folot. synthesis and increased the specific activities of key enzymes of C_1 metabolism during exponential growth of the wild typ Lindegren (+) (FGSC #853). Mycelia receiving glycin. also had increased ability to cleave this amino acid and to utilize C-2 for synthesis of adenine, serine ond methionine. Mutants partially deficient in serine hydroxymethyltransferase and locking the glycine cleavage reaction did not show an increase in net folot. synthesis when 1 mM glycine was supplied (Combepine, Cossins and Chan 1976 In Chemistry and Biology of Pteridines, deGruyter, Berlin, p 330). In th. present studies we have examined the effect of glycin. on folot. biosynthesis in greater &toil.

Conidia were harvested ond used to inoculate (10^6 conidia/ml) sterile media (Vogel's and Vogel's + 1 mM glycine) which were then vigorously aerated at 25° C for periods up to 22 hr. Folot. was extracted by boiling the mycelia for 10 min in 0.6% K-ascorbate (pH 6.0). Individual folate samples were separated by DEAE-cellulose (Chan, Shin and Stokstad 1973 Con. J. Biochem. 51: 1617) and the fractions were assayed using Lactobacillus casei (ATCC 7469). Aliquots (0.5 ml) of each fraction were also hydrolyzed with a γ-glutamyl carboxypeptidase prepared from pea seedlings (Roos and Cossins 1971 Biochem. J. 125: 17) and reassayed using L. casei and Pediococcus cerevisiae (ATCC 8081). In other instances mycelial extracts were treated with th. above enzyme prior to column chromatography by the method of Sotobayashi, Rosen ond Nichol (1966 Biochemistry 5: 3878).

Freshly harvested conidia contained 7.8 μg of total folate/g dry wt and of this, 6.9 μg represented polyglutamyl folates. After 22 hr growth, mycelia harvested from Vogel's medium contained 25.7 μg of tot.1 folate/g dry wt of which 17.2 μg were polyglutamyl derivatives. After 22 hr in the supplemented medium th. corresponding concentrations were 75.6 μg and 48.6 μg respectively. When expressed on a per culture basis th. amounts of polyglutamyl folate after 22 hrs were 49.8 μg and 97.7 μg for th. unsupplemented and supplemented cultures respectively. Clearly, growth in the presence of 1 mM glycine practically tripled the concentration of polyglutamates and approximately doubled their amount per culture.

Figure 1 shows that the bulk of this increased polyglutamate synthesis was due to a derivative (peak d) collected in fractions 120-140. Glycine also increased the levels of another polyglutamate (peak c) which was collected in fractions 86-104. Growth of L. casei on peak d was increased about 10 fold by γ-glutamyl carboxypeptidase treatment of these fractions. A smaller but significant increase was also noted when this treatment was applied to peak c. Clearly both peaks contain conjugated derivatives. Furthermore, both peaks occupy positions in the elution sequence like those of authentic hexa- and heptaglutamates (Chan et al., 1973). The nature of the C_1 substituent group of the derivative in peak d was investigated in two ways. Firstly, this peak, after enzyme treatment, supported the growth of P. cerevisiae (approx. 85% of the response shown by L. casei) indicating that the derivative was the tetrahydro type and either unsubstituted or formylated. Secondly, mycelial extracts were treated with excess carboxypeptidase prior to chromatography. This treatment converts all polyglutamyl folates to mono- and diglutamyl derivatives (Roos and Cossins, 1971). Chromatography revealed large quantities of a folate which supported growth of both assay bacteria and which cochromatographed with authentic tetrahydrofolate.

From these assays we conclude that glycin. mainly stimuates the synthesis of on unsubstituted tetrahydrofolate which is highly conjugated. This compound may be a hexa- or heptaglutamate and authentic markers or. now being used to elucidate this point. As this principal folate derivative was rapidly synthesized by actively growing cells it is conceivable that it plays a central role in C_1 metabolism. Such a role could include the utilization of exogenous glycin. for C_1 unit biogenesis.

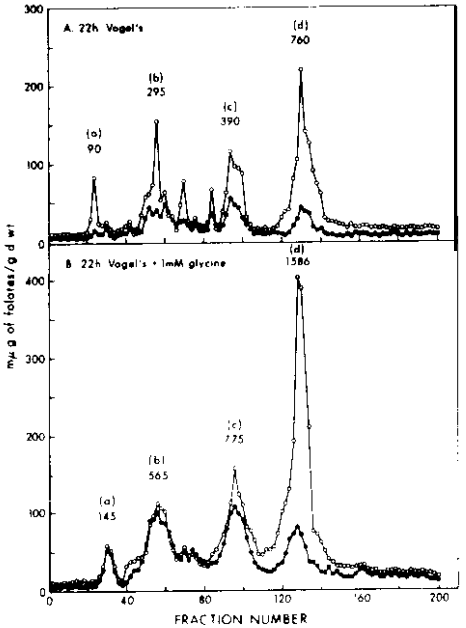

Figure 1. DEAE-cellulose column chromatography of folate derivatives. Mycelial extracts were prepared after 22 hr growth in Vogel's defined medium (A) and in Vogel's supplemented with 1 mM glycin. (B). Columns of DEAE-cellulose (0.9 x 30 cm) were eluted with a gradient of 100 ml of 0.01 M K-phosphate (pH 6.0) containing 0.6% K-ascorbate and 500 ml of 0.5 M K-phosphate (pH 6.0) also containing 0.6% K-oscorbat.. Fractions of 2.5 ml were collected in tubes containing 0.1 ml of 10% K-ascorbat. (pH 6.0). Folates were assayed using L. casei before (●) and after (○) treatment of the fractions with γ-glutamyl carboxypeptidase.

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