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Abstract

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In N. crassa folylpolyglutamates ($H_4PteGlu_n$) are important in methionine synthesis as demonstrated by 5-$CH_3H_4PteGlu_n$* activation of cystathionine γ-synthase (Selhub et al. 1971 Proc. Natl. Acad. Sci. 68: 312) and by the occurrence of polyglutamate-deficient mutants that display methionine auxotrophy (Ritari et al. 1973 Neurospora Newsl. 20: 27). Recently we have reported sharply reduced incorporation of p-amino-

*The recomnendations of the IUPAC-IUB Commission are followed (1967 Arch. Biochem. Biophys. 118: 511) in designation of folate derivatives, e.g. 5-$CH_3H_4PteGlu_n$ = 5-methyltetrahydropteroyl poly-γ-glutamate.

benzoate into $H_4PteGlu_n$ and decreased folate concentrations when N. crassa was cultured in L-methionine supplemented media (Chan and Cossins 1980 Arch. Biochem. Biophys. 200: 346). We have now examined folylpolyglutamate synthetase activity in the wild type (FGSC #853) and a met-6 mutant (FGSC #1330) with emphasis on the nature of the palyglutamyl folate products (conjugated folates).

Mycelia were cultured and harvested after growth for 24 and 96 h (Cossins et al. 1976 Biochem. J. 160: 305). In the case of the mutant, 0.2 mM L-methionine was provided, an amount that was growth-limiting and depleted within 48 h. Folate levels were determined with Lactobacillus casei before and after hydrolysis (Cossins et al. 1976). Synthetase activities were measured using dialyzed extracts (Ritari et al. 1975 Anal. Biochem. 63: 118) with the products being fractionated as noted in Table 2.

Preliminary experiments showed that in the mutant, methionine levels (free and protein) increased for at least 48 h despite media depletion of this amino acid. Methionine synthesis occurred at this stage as serine[3-^{14}C] was rapidly incorporated into methionine. However, mutant conidia showed poor germination and growth if methionine was not supplied initially. The data in Table 1 show that similar levels of methionine were recovered from both strains at 24 and 96 h but differences were apparent in total and polyglutamylfolate. At 24 h the mutant essentially lacked conjugated folates. A comparison of total and conjugated folate in the mutant at 96 h suggests that the folate pool was still largely non-conjugated, a situation different from the wild type. This difference was also apparent when polyglutamate synthesis was examined in vitro. In terms of $H_4PteGlu$ incorporated into polyglutamate, the mutant had greater activity than the wild type. In terms of glutamate added to the folate substrate however the wild type displayed more ability at 24 h. The nature of this difference was apparent when ^{14}C and ^{3}H substrates were employed, combined with fractionation of the products (Table 2). In the wild type, synthetase activity produced 3 labelled peaks with ^{3}H:^{14}C ratios of essentially 1, 2 and 5. These products would correspond to di-, tri- and hexaglutamates, the latter being the principal polyglutamate

TABLE 1

A comparison of growth, levels of methionine, folates and folylpolyglutamate synthetase in the wild type and met-6 mutant at 24 and 96 h of culture.

	Wild type		met-6 mutant	
	24 h	96 h	24 h	96 h
Growth (mg dry wt.)	75	255	55	160
Exogenous met (µmo1e/culture)	n.d.	n.d.	2.0	n.d.
Endogenous met (µmole/culture)	3.8	14.0	2.5	12.4
Conjugated folate (µg/culture)	3.6	2.40	0.02	1.4
Total folate (µg/culture)	4.6	3.3	3.4	6.0
Synthetase activity (nmole/mg protein/h)				
$H_4PteGlu$ incorporated	0.45	0.30	0.70	0.85
glutamate incorporated	1.40	0.78	0.70	0.85

Data are mean values of at least three determinations and were derived from analysis of 100 ml cultures. The standard synthetase reaction system contained 2.5 µmole ATP, 5 µmole $MgCl_2$ 15 µmole KCl, 100 µmole Tris-HCl (PH 8.5), 1.5 µmole glutamate-[^{3}H] sp. act 3300 dpm/nmole, 0.1 µmole $H_4PteGlu$ and dialyzed extract containing 1.2-1.5 mg protein. The final volume was 0.5 ml and the reaction was terminated by boiling, after 2h incubation at 37°C. In both strains synthetase activity was ATP- and $H_4PteGlu$-dependent. n.d. = not detected.

chain length found in N. crassa (Chan and Cossins 1980). The mutant extracts only produced $H_4PteGlu_2$. These products were not changed by longer incubations but mixing extracts from each strain resulted in a pronounced shift in the label from $H_4PteGlu_2$ to $H_4PteGlu_6$.

TABLE 2

The nature of conjugated folate produced by wild type and mutant extracts

Strain	Elution volume from DEAE cellulose (ml)	Glutamate-[^{3}H] incorporation (nmole)	$H_4PteGlu$[2-^{14}C] incorporation (nmole)	Ratio $^3H:^{14}C$
Wild type	43 - 47	0.40	0.38	1.05
	53 - 58	1.70	0.78	2.18
	65 - 74	5.40	1.06	5.10
met-6 mutant	43 - 48	2.10	2.00	1.05
	53 - 58	n.d.	n.d.	
	65 - 74	n.d.	n.d.	

Assays were performed as in Table 1 but included ^{14}C-1abelled $H_4PteGlu$ (3400 dpm/nmole). Polyglutamates were eluted from columns of Bio-Rad Cellex D using the gradient elution method of Taylor and Hanna 1977 Arch. Biochem. Biophys. 181: 331.
n.d. = not detected.

The mutant may synthesize limited amounts of methionine after prolonged growth by virtue of the folate pool. Homocysteine methylation in N. crassa is about 70 times mare rapid with 5-$CH_3H_4PteGlu_2$ than the corresponding monoglutamate (Burton et al. 1969 Biochem J. 111: 793). The met-6 mutation could alter the properties of a single folylpolyglutamate synthetase as noted for some multiple auxotrophs of animal cell lines (Taylor and Hanna 1979 Arch Biochem. Biophys. 197: 36) or possibly prevent the formation of an enzyme catalyzing $H_4PteGlu_2 \rightarrow H_4PteGlu_6$ (Ritari et al. 1973). An adequate assessment of these possibilities requires enzyme purification combined with verification of the polyglutamate products. - - - - Department of Botany, University of Alberta, Edmonton, Canada T6G 2E9.