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Identification of two genes specifying folylpolyglutamate synthases

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Identification of two genes specifying folylpolyglutamate synthases

Abstract

Genes specifying folylpolyglutamate synthases

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The finding that the biosynthesis of folylpolyglutamates by N. crosso involves at least two enzymes (Ritari et al. 1973 Neurospora Newsl.20, preceding note) indicated that two (or mote) loci ore concerned with this process, one specifying a diglutamate synthose and the other on enzyme forming higher polyglutamates. It appeared probable that one locus was me-6, since the folates of the me-6 (35809) strain hod been *analyzed in* this laboratory and found to consist of mono and diglutamates rather than the higher polyglutamates characteristic of wild type N. crassa (Selhub 1970 Ph.D. Thesis, Core Western Reserve Univ.). The identity of the gene coding diglutamate synthose was less obvious. One possibility was that it was mac, even though mac had been considered to be on allele of me-6. Genetic studies supporting this identification hod not excluded the possibility that mac ond me-6 ore closely linked, but non-allelic, loci (Murray 1969 Genetics 61:67).

Table 1. Polyglutamate synthase activities of extracts of strains of N. crassa with different folate substrates.

source of extract	Glutamate-^{14}C incorporated in folate* when incubated with			
	H_4PteGlu$_1$	H_4PteGlu$_2$	H_4PteGlu$_3$	H_4PteGlu$_4$
me-6(35809)A	2.99	-0.05	0.13	0.10
mac(65108)A	0.46	2.59	-	
74-OR8-1a wt.	5.78		2.86	-

*mμMoles/hr/mg protein

In the present study, the folylpolyglutamate synthose activities of N. crosso strains me-6(35809) and 65108(formerly called mac) have been assayed by the procedure of Ritori et al. (ibid.), which involves determination of the ability of the extracts of these strains to convert glutamate-^{14}C into folylpolyglutamate-^{14}C on incubation with various forms of THF. Extracts of the organisms were prepared with a Hughes press. Particulate matter was removed from the suspension of broken mycelia by centrifugation at 105,000 x g for 2 hours in a Spinco ultracentrifuge and the *supernatant solutions* were dialyzed against Tris chloride (0. I'M) buffer, pH 8.1. Folates and nucleic acids were removed by passing the extracts through columns of Dowex 1X4 (Cl-, pH 8. 1). When the crude enzyme preparations were incubated with various forms of tetrahydrofolate, glutamate-^{14}C, ATP, Mg^{++}, K^+ and CoA for 1 hour under anaerobic conditions, the results shown in Table 1 were obtained. The extract of me-6(35809) was able to incorporate glutamate-^{14}C into folylpolyglutamate with H_4PteGlu as substrate but was essential-inactive with H_4PteGlu$_2$, H_4PteGlu$_3$, and H_4PteGlu$_4$. The extract of moc(65108) possessed different activity: while it was able to utilize H_4PteGlu$_2$, it was inactive, or weakly active, with H_4PteGlu. These results indicate that the formotion of the diglutomote is catalyzed by a protein coded by mac and that the ability of the extract of N. crosso to use the di-, tri- and tetraglutamates involves a single enzyme specified by the me-6 locus.

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