Ritari, S. J., W. Sakami, C. W. Black and J. Rzepka. The synthesis of polyglutamate forms of folate by N. crassa.

The synthesis of folylpolyglutamates by N. crassa has been investigated with an assay based on the determination of the conversion of L-glutamate-U-^{14}C to folylpolyglutamate-^{14}C. Folate was isolated from deproteinated (TCA) incubation mixtures by adsorption on columns of Darco G-60 (5 mg): the charcoal adsorbed folates, but not glutamate, from acid solution. After removing traces of glutamic acid -^{14}C with a wash solution containing acetic acid, mercaptoethanol and glutamic acid, folylpolyglutamate-^{14}C was eluted with 2 ml of an aqueous-alcoholic solution of ammonia and counted in a liquid scintillation counter. The recovery of $H_4PteGlu$-^{14}C from incubation mixtures containing from 10 to 150 nmoles of the folate per ml was 97 ± 3%.

Table 1. Polyglutamate synthase activities of $(NH_4)_2SO_4$ fractions of N. crassa.

$(NH_4)_2SO_4$ fraction	Glutamate-^{14}C incorporated into folate* when incubated with			
	$H_4PteGlu_1$	$H_4PteGlu_2$	$H_4PteGlu_3$	$H_4PteGlu_4$
0-35%	0.67	2.82	1.46	1.15
45-60%	17.20	1.08	0.60	-
Crude extract	6.53	-	1.33	1.06

*mμMoles/hr/mg protein

Clear extracts of N. crassa 74-OR8-1a that had been dialyzed against Tris buffer and passed through columns of Dowex 1X4 (Cl^-, 100-200 mesh) to remove folate and nucleic acids were found to possess folylpolyglutamate synthase activity. When incubated at 37°C under N_2 with ATP, Mg^{++}, KCl, 2-mercaptoethanol, CoA, Tris buffer, pH 8.5, and either $H_4PteGlu_1$ or $H_4PteGlu_3$, they incorporated L-glutamate-^{14}C into folylpolyglutamate (see Table). Coenzyme A stimulated the reaction but was not required for activity. Fractionation of the extract with ammonium sulfate between 0-35, 35-45, 45-60 and 60-100% saturation demonstrated that the activities with the two folates were properties of different enzymes. The 0-35% fraction w[illegible] most active with $H_4PteGlu_2$, $H_4PteGlu_3$ and $H_4PteGlu_4$,

whereas the 45-60% fraction possessed greatest activity with the monoglutamate (see Table). Further studies in which the activities with the three polyglutamates were found to be absent from an extract of an me-6 strain demonstrated that they are the properties of a single enzyme (Ritari et al. 1973 Neurospora Newsl. 20, companion note, immediately following).

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