

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

tures 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 $\text{H}_4\text{PteGlu-}^{14}\text{C}$ from incubation mixtures containing from 10 to 150 nmoles of the folate per ml was $97 \pm 3\%$.

Table 1. Polyglutamate synthase activities of $(\text{NH}_4)_2\text{SO}_4$ fractions of N. crassa.

$(\text{NH}_4)_2\text{SO}_4$ fraction	Glutamate- ^{14}C incorporated into folate* when incubated with			
	$\text{H}_4\text{PteGlu}_1$	$\text{H}_4\text{PteGlu}_2$	$\text{H}_4\text{PteGlu}_3$	$\text{H}_4\text{PteGlu}_4$
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

* $\mu\text{Moles/hr/mg protein}$

The synthesis of folylpolyglutamates by N. crassa has been investigated with an assay based on the determination of the conversion of L-glutamate- $\text{U-}^{14}\text{C}$ to folylpolyglutamate- ^{14}C . Folate was isolated from deproteinated (TCA) incubation mix-

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 $\text{H}_4\text{PteGlu}_1$ or $\text{H}_4\text{PteGlu}_3$, 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 was most active with $\text{H}_4\text{PteGlu}_2$, $\text{H}_4\text{PteGlu}_3$ and $\text{H}_4\text{PteGlu}_4$,

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 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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