EFFECT OF GLUTAMIC ACID ON THE FORMATION OF TWO GLUTAMIC ACID DEHYDROGENASES OF NEUROSPORA[1]

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It has been demonstrated that wild-type strains of Neurospora crassa produce two glutamic acid dehydrogenases, one specific for DPN and another for TPN (Sanwal and Lata, 1961 a,b) It was frequently noted during this study that under growth conditions which favoured an increase in the specific activity of the DPN-specific enzyme, there was always a corresponding decrease, within statistical limits, of the specific activity of the TPN-specific enzyme. Since we could not correlate these changes to the ratios of reduced to oxidized pyridine nucleotide coenzymes obtaining in the cells under varying growth conditions (Sanwal and Lata, unpublished), an attempt was made to correlate this phenomenon to the presence of repressors and inducers in the cells under different growth conditions.

The organism used in this study was a wild-type strain of N. crassa (R^+). It was grown from a conidial inoculum on Vogel's medium N (Vogel, 1956) with shaking. Preparation of cell-free

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extracts and assay procedures have already been described (Sanwal and Lata, 1961 b). Table I shows the variations obtained in the activities of the two enzymes during the initial and exponential phase of growth. The DPN-specific enzyme is absent from the conidia and throughout the course of development its activity, relative to that of the TPN-specific enzyme, is low. In mutant strains of Neurospora, which are deficient in amination and do not possess the TPN-specific enzyme (Fincham, 1951; Sanwal and Lata, 1961 a), conidia germinate after a very long lag period and this seems to be due to the initial absence of the DPN-specific enzyme. When it is synthesized in the conidia, mycelial growth starts normally but slowly as compared to the wild-type strains. It will be noted from Table I that with a decrease in the activity of the TPN enzyme after 24 hours, there is a corresponding increase in the DPN enzyme. In 7 separate experiments the pattern shown in Table I was noted consistently.

Table I

THE ACTIVITY OF DPN- AND TPN- SPECIFIC GLUTAMIC ACID DEHYDROGENASES DURING GROWTH ON MINIMAL MEDIUM

Specific activity	Time after incubation		
	0 hr.	24 hrs.	48 hrs.
DPN-enzyme	0	82	119
TPN-enzyme	72	540	482

Yura and Vogel (1959) noted that the activity of pyrroline-5-carboxylate reductase is considerably decreased in Neurospora in the presence of proline in the growth medium. It, therefore, seemed plausible that internally generated glutamate might repress the activity of the TPN-specific enzyme in our strain. To test this theory, the organism was grown in minimal medium, various concentrations of glutamate as the sole source of nitrogen, or a mixture of ammonium nitrate and glutamate, and the activity of the two enzymes was measured in each case. Table II and III show the results of this investigation.

It will be seen that glutamate alone or ammonium nitrate alone does not affect the activity of the two enzymes significantly. When they are present together, however, a very striking decrease in the activity of the TPN-specific enzyme and a corresponding increase in the DPN-specific enzyme is noted. This effect is particularly pronounced towards the end of the exponential phase of growth (48 hours), and is dependent on the concentration of L-glutamate (Table III). Since this effect is shown only when glutamate and ammonia are added together in the growth medium, it is reasonable to assume that the actual substance causing this effect is a compound derived from glutamate and ammonia. We do not yet have a clue to the nature of this substance. By suitable mixing of cell-free extracts we have been able to rule out the possibility of the presence of an activator or inhibitor for the DPN- and TPN-specific enzymes, respectively, in extracts from glutamate and ammonia grown cells. Our preliminary experiments, to be described in detail later,

show that the increase in activity of the DPN-specific enzyme in the presence of glutamate probably reflects the actual increase in the enzyme protein.

Table II

EFFECT OF L-GLUTAMATE ON THE ACTIVITY OF THE TPN- AND DPN-DEPENDENT GLUTAMIC ACID DEHYDROGENASES

Time of incubation	Specific activity of the enzymes on					
	Minimal med.		Glutamate alone (.05 M)		Minimal + glutamate (.05 M)	
	D*	T*	D	T	D	T
24 hours	97	407	114	439	227	208
48 hours	107	530	116	501	839	53

* D = DPN= dependent enzyme; T = TPN- dependent enzyme.

Table III

EFFECT OF VARIOUS SUPPLEMENTS OF GLUTAMATE IN THE MINIMAL MEDIUM ON THE ACTIVITY OF THE TPN- AND DPN- DEPENDENT GLUTAMIC ACID DEHYDROGENASES AFTER 48 HOURS OF GROWTH

Glutamate concentration	Specific activity of	
	DPN-enzyme	TPN-enzyme
0.005 M	112	492
0.01 M	203	211
0.03 M	857	68
0.05 M	897	62

Since there is such close correspondence between the decrease in the TPN-specific enzyme and an increase in the DPN-specific one (Table III), we must consider two possibilities concerning the causes of this effect. One possibility is that under the influence of a substance produced from glutamate and ammonia, the TPN-specific enzyme is converted to the DPN-specific one. It has been shown that several steroid hormones promote the reversible dissociation of beef liver glutamic acid dehydrogenases into subunits (Yielding and Tomkins, 1960), and further that these enzymatically active subunits have a different substrate specificity from that of the aggregate (Tomkins et al., 1961). It has been demonstrated, however, that the DPN- and the TPN-specific enzymes in Neurospora are distinct and separate entities (Sanwal and Lata, 1961 b), under the control of different genetic loci (Sanwal and Lata, 1961 a). These two facts, however, do not completely rule out the possibility of the conversion of TPN-specific enzyme to the DPN-specific one. The second possibility which is more in keeping with the present day ideas (Vogel, 1957; Jacob and Monod, 1961) of the regulation of enzyme synthesis, is that TPN-specific and DPN-specific enzymes are under the control of a single repressor, and that this substance has opposite effects on the two enzymes; the DPN-specific enzyme is continually repressed and this repression is overcome by the substance synthesized from glutamate and ammonia (derepression), while the TPN-specific enzyme is not repressed normally but becomes repressed when the repressor substance combines with the compound produced from glutamate and ammonia

(corepression). Assuming further that a fixed quantity of repressor is available in the cell at any one time, conditions favouring derepression of the DPN-specific enzyme would automatically lead to a corresponding corepression of the TPN-specific enzyme. To support this hypothesis, it will be necessary to demonstrate a regulator cistron involved in the formation of the hypothetical repressor substance. We are currently investigating this phenomenon at the genetic and biochemical level.

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