EFFECTS OF PYRIDOXAL PHOSPHATE AND SERINE IN CONVERSION OF INDOLE GLYCEROL PHOSPHATE TO INDOLE BY EXTRACTS FROM TRYPTOPHAN MUTANTS OF NEUROSPORA CRASSA+

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Tryptophan synthetase from Neurospora crassa catalyzes the following three reactions (Yanofsky and Rachmeler, 1958; DeMoss and Bonner, 1959):

(1) Indole-3-glycerol phosphate (InGP) + serine → tryptophan + triosephosphate

(2) Indole + serine → tryptophan

(3) Indole-3-glycerol phosphate (InGP) ⇌ indole + triosephosphate

It has been reported (DeMoss and Bonner, 1959) that mutation affects different catalytic abilities of the enzyme and may lead to loss of ability to catalyze reactions (1) and (2), but retention of enzymatic activity in reaction (3). It has also been shown that mutation of this sort is characterized by accumulation of indole, but differences were found between two such mutants on examination of their extracts. The extract of one mutant (td 2) catalyzes reaction (3) only with added pyridoxal phosphate (BalP), while the extract of the second mutant (td 71) requires the addition of serine to BalP. Thus, two mutants were previously known to show cofactor requirements judged in terms of reaction (3), whereas extracts of wild type do not require either BalP or serine. This suggests that mutation can affect enzyme structure and create diverse phenotypic changes for reaction (3) in terms of BalP- and serine- requirements in

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addition to the simultaneous loss of reaction (1) and (2). Therefore, it is of interest to determine what different types of mutants can be obtained, if a large number of similar mutants as judged in terms of indole accumulation are selected.

Fourteen mutants which accumulate indole were selected from ST 74A after U.V. treatment, followed by the selective method of Woodward et al. (1954). Mutants were grown for 68 to 72 hours at 37°C in Fernback flasks containing 1 liter of minimal medium (Vogel, 1956) supplemented with 150 mg. L-tryptophan. Crude extracts were prepared from lyophilized mycelia by the method of Yanofsky (1955). A 50 per cent ammonium sulfate precipitate was used throughout this study. Activity was measured in terms of indole formation in the reaction mixture (Table 1) after 60 min. incubation at 37°C. The amount of protein was adjusted to detect at least .01 μmole indole formation. Protein determination was made by the method of Lowry et al. (1951).

In Table 1, specific activities are presented for reaction (3) with and without BalP and serine in extracts of 14 mutants in addition to two previously known mutants (td 2 and td 71). It is seen that td 97 is like td 71 which requires both BalP and serine, while td 99 like td 2 is stimulated by only BalP. The remaining mutants constitute a group similar to wild type, i.e., the reactions catalyzed by their extracts were not appreciably stimulated by either BalP, or BalP and serine. The bulk of mutants in this category are characterized by having a low specific activity. Two exceptions to this; td 96 and td 104 both show a high specific activity without any cofactor added. These differences in specific activity are not due to different amounts of the enzyme produced, since it has been found (Suyama, unpublished data) that all of these mutants possess CRM, a protein serologically related to wild type enzyme (Suskind et al., 1955; Suskind, 1957) and no correlation between specific activities of the reaction and CRM has been observed. Therefore, these differences are apparently due to different

TABLE 1

Effects of pyridoxal phosphate and serine in conversion of indole-glycerol-phosphate to indole by extracts from 16 indole accumulating mutants.

	Reaction rate (μmoles indole/hr/mg prot)			
Mutants	Complete+	Minus BalP	Minus Ser	InGP (only)
td 2	0.009	0.002	0.009	0.002
td 71	0.018	0.004	0.004	0.003
td 96++	0.019	0.024	0.024	0.024
td 97++	0.016	0.002	0.003	0.002
td 98	0.001	0.001	0.001	0.001
td 99	0.006	0.004	0.006	0.004
td 100	0.012	0.004	0.010	0.004
td 101	0.002	0.002	0.002	0.002
td 102	0.002	0.002	0.002	0.002
td 103	0.001	--	--	0.001
td 104	0.090	0.086	0.086	0.080
td 107	0.002	0.002	0.002	0.002
td 109	0.001	--	--	0.001
td 110	0.001	0.001	0.001	0.001
td 113	0.002	--	--	0.002
td 116	0.001	--	--	0.001

+ complete reaction mixture constitutes 1 ml; 0.32 μmoles InGP, 20 γ BalP, 80 μmoles serine, 100 μmoles buffer at pH 7.8 and 1 μmole glutathione.

++two mutants are leaky and 10 per cent of wild type activity of reaction (2) was detected.

alterations in the enzyme structure as a result of mutation.

It had been suspected that mutants which showed only a serine requirement might occur. However, the fact that no such mutant was found, indicates that BalP may be a prerequisite for serine to act as a cofactor in reaction (3).

Since three types of mutations which are associated with phenotypic changes of the reaction (3) concerning cofactor requirement were found, it is of interest to determine specific genetic areas which are responsible for such alterations. A genetic fine structural analysis by use of these mutants has been made and significance of these mutations in relation to the enzyme kinetic properties will be discussed elsewhere. (Bonner, et al., 1960; Suyama and Bonner, 1960).

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