# INACTIVATION OF TRYPTOPHAN SYNTHETASE FROM NEUROSPORA CRASSA DURING DIALYSIS

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Tryptophan-independent strains of Neurospora crassa exhibit an enzymic activity attributed to a single enzyme called tryptophan synthetase. This enzyme system catalyzes the following reactions (Yanofsky and Rachmeler, 1958):

1. Indoleglycerol phosphate (InGP) + serine → tryptophan + triose phosphate

2. Indole + serine → tryptophan

3. InGP ⇄ indole + triose phosphate

In the usual laboratory demonstration of enzymic activity, reaction 2 is assayed by measuring indole disappearance. Extracts of certain tryptophan-dependent mutants of this organism contain a protein (CRM) that is immunologically related to tryptophan synthetase (Suskind et al., 1955). This protein, which is enzymically inactive, will cause the production by rabbits of antibody reactive with the enzyme. CRM inhibits the neutralizing capacity of an antiserum produced with the enzyme as antigen and competes with enzyme for antibody whether the antiserum was prepared against enzyme or CRM.

The comparison of enzymic activity of fresh and aged extracts of tryptophan-independent strains indicates that some of the activity is lost at -20°C. This loss of activity is not accompanied by an equivalent

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decrease in detectable antigen, an anomaly attributable to a possible conversion of enzyme to immunological cross-reacting material.

The loss of enzymic activity during dialysis is accompanied by a concurrent decrease in total immunological activity in two tryptophan-independent strains studied. In a third strain, enzymic activity is lost accompanied by an increase in immunological cross-reacting material. In the latter instance, the total immunological activity remains relatively constant. These findings are the subject of the present communication and support other observations (Wust and Bonner, in press) for an apparent heterogeneity of tryptophan synthetase.

Three strains of N. crassa were used in this study: 74A (St. Lawrence strain); 5256A (Emerson strain); and C-84a (hi-5 strain). Cultures were incubated for 48 hours at 30°C with forced aeration in minimal medium with or without the addition of DL-tryptophan (150 μg/ml) and indole (20 μg/ml). The medium used for the growth of C-84a was supplemented with histidine (75 μg/ml). Extracts from lyophilized, ground mycelia were prepared according to previously described methods (Suskind, 1957).

Antisera had been produced against partially purified enzyme preparations or partially purified CRM obtained from $td_3$ or $td_7$. A particular antiserum was standardized with regard to its enzyme-inhibiting capacity by titrating for anti-enzyme activity using pooled extracts of 2 or 3 of the strains. When such a procedure was employed, a neutralization titer was obtained that was higher than when any one strain alone was assayed. One unit of antibody activity is defined as the amount of antiserum necessary to neutralize one unit of enzyme activity. The difference between the standard titer of a particular serum and the titer determined with any single enzyme preparation probably is owing to immunological cross-reacting material that is non-enzymic. This material apparently does not compete with enzyme for anti-enzyme antibody when pooled extracts of 2 or 3 of the strains are used in the titration. One unit of this material is defined

as the amount of extract that would remove one unit of antibody and that did not have enzymic activity. Since CRM has been defined as that cross-reacting material present in mutant (td) strains, there is reservation about using the designation, CRM, in the present studies.

Dialysis was performed in an Aminco internal stirring unit against 0.1 M potassium phosphate buffer at pH 7.8. Samples were withdrawn periodically and the residual enzymic activity was assayed. In these experiments, one unit of enzyme is defined as the amount of activity that resulted in the disappearance of 0.1 μmole of indole per hour under standard conditions (Yanofsky, 1955).

Representative data on the effect of dialysis on the enzymic activity of the three strains are presented in Figure 1.

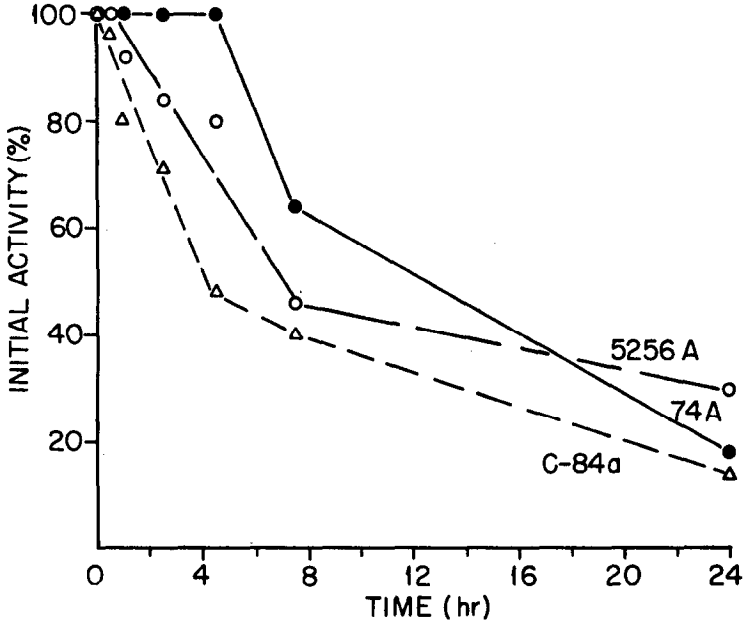


Figure 1. Inactivation of enzymic activity during dialysis at 0°C.

The curves were reproducible with repeated trials. The enzymic activity of strain 74A was more stable to dialysis for the first 4 1/2

hours. The activity then decreased as rapidly as in the extracts of the other two strains. The results of an experiment in which the total immunological activity, which includes the enzymic activity and the nonenzymic portion (cross-reacting material), were determined and are presented in Table 1.

Table 1

Immunological Activity after Dialysis at 0°C*

| | Strain | | | | | |
|---|---|---|---|---|---|---|
| | 5256A | | 74A | | C-84A | |
| Time | Cross-Reacting Material† | Enzyme Activity | Cross-Reacting Material | Enzyme Activity | Cross-Reacting Material | Enzyme Activity |
| (hours) | units per ml | | | | | |
| 0 | 4.0 | 9.8 | 0.5 | 10.3 | 6.4 | 32.8 |
| 2.5 | 4.0 | 8.2 | 0.5 | 10.3 | 6.0 | 24.6 |
| 7.5 | 4.9 | 4.5 | 2.7 | 6.7 | 6.0 | 16.4 |
| 24.0 | 5.0 | 3.0 | 6.4 | 1.8 | 6.8 | 8.0 |
| 30.0 | 4.2 | 2.1 | 7.2 | 0.7 | 6.8 | 3.6 |

*Antiserum used in this experiment = anti-$\underline{td}_3$ having a neutralizing titer of 22 units/ml in a 1:3 dilution when assayed with a pool of 5256A, 74A and C-84a extracts.

†Cross-reacting material = the portion of the extract that removes enzyme-neutralizing antibody but has no enzymic activity.

After 4 1/2 hours, when the enzymic activity of strain 74A began to decrease, the immunological cross-reacting material increased significantly; therefore, the total immunological activity of enzyme and cross-reacting material did not decrease to the same extent as in either of the other two strains. Strains 5256A and C-84a lost not only enzymic but immunological activity. Dialysates of strain 74A and 5256A were concentrated and added to fresh extracts of each of the strains. The stability of the extracts to subsequent dialysis was unaffected; they were inactivated like fresh extracts alone.

These observations emphasize that tryptophan synthetase from one strain does not behave identically with the enzyme obtained from another strain. The differences in stability to dialysis among the three tryptophan-independent strains examined probably reflect differences in genetic background. The fine-structure analysis of the td locus (Bonner et al. 1960) suggests that alterations within its subunits could result in differences in stability to dialysis although the product of gene function still would be tryptophan synthetase activity (Wust and Bonner, in press). Thus the enzyme protein itself, genetically determined, may be different in each strain. Other factors, however, could influence the relative stability of the enzyme. These factors would be the products of other loci producing modifying effects. The conversion of enzyme to an immunological cross-reacting material during dialysis in only one strain, 74A, suggests that in this case at least, the enzyme molecule itself is altered and is different from the enzyme molecules from strains 5256A and C-84a.

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