Radiation inactivation analysis of amino acid transport systems in Neurospora crassa

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Abstract
Radiation inactivation analysis of amino acid transport systems in *Neurospora crassa*

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Increased activity of the first two enzymes of tryptophan biosynthesis.

As reported earlier (Carsiotis and Lacy 1965 J. Bacteriol. 89: 1472), the last two enzymes of the tryptophan biosynthetic pathway (indoleglycerol phosphate synthetase and tryptophan synthetase) are elevated two-fold or more in all histidine mutants of Neurospora crassa.

We have now completed surveying these same mutants and found a similar increase in the first two enzymes of the Pathway (anthranilate synthetase and PR-transferase), thereby providing proof that the entire pathway is elevated in these mutants.

The mechanism of this elevation is currently under investigation. - - - Department of Microbiology, University of Cincinnati College of Medicine, Cincinnati, Ohio and Department of Biological Sciences, Goucher College, Towson, Maryland.


As reported earlier, the last two enzymes of the tryptophan biosynthetic pathway (indoleglycerol phosphate synthetase and tryptophan synthetase) are elevated two-fold or more in all histidine mutants of Neurospora crassa.

A reinvestigation of the effect of zinc deficiency noted by Nelson et al. (1951 J. Biol. Chem. 188: 397) has revealed that all enzymes of the tryptophan biosynthetic pathway are increased in histidine mutants, not in the wild type. An explanation of this effect is being sought currently. - - - Department of Microbiology, University of Cincinnati College of Medicine, Cincinnati, Ohio.

Radiation inactivation analysis of amino acid transport systems in Neurospora crassa.

Radiation inactivation analysis of amino acid transport systems in Neurospora crassa reveals that the uptake of the amino acids is mediated by a number of enzymes. The activity of these enzymes is decreased when nitrogen is limiting for growth. In some cases, the uptake experiments are done under conditions such that very little protein synthesis is occurring and remains proportional to dose.

The data obtained from thirteen experiments employing phenylalanine were averaged and plotted in the accompanying figure. The fraction of the remaining activity, as compared to the control, is plotted as the ordinate, the dosage as the abscissa. If the equation in A/A0 = (constant)(dosage), where A/A0 = remaining activity holds, a straight line should be obtained.

This non-linearity would indicate that more than one enzyme is involved in the transport of the amino acids that more than one “hit” is necessary to inactivate the transport system. If the straight line portion of the curve is extrapolated back to the zero dosage, the intercept values for phenylalanine, tryptophan and leucine are near two. This indicates that probably two enzymes are functioning in the transport of these particular amino acids.

It must be emphasized that the uptake experiments are done under conditions such that very little protein synthesis is occurring and remains proportional to dose.

Our original purpose in beginning these studies was to approximate the molecular weight of the transport enzyme. Since our data indicate a multiplicity of enzymes, we cannot as yet determine individual molecular weights for the two enzymes. However, since D37 falls on the straight line portion of all the curves, we felt it would be of value to complete our calculations for the “radiation sensitive size” of the transport complex.

Using the formula developed by Hutchinson and Pollard, MW = 0.72 x 10^12 / D37, where D37 is the dose in rads which will produce 37% activity, we have found a “radiation sensitive molecular weight” for the transport complex for phenylalanine of 575,000; tryptophan, 817,000; leucine, 893,000; and glycine, 817,000.

The data obtained would indicate that the transport (as well as the tryptophan and leucine systems) in Neurospora crassa is mediated by a number of enzymes of fairly high molecular weight. The exact function of the two enzyme components is as yet unknown.

This work was supported in part by a contract (AT-40-I-2690) between the Division of Biology and Medicine, U. S. Atomic Energy Commission and the Institute of Molecular Biophysics, Florida State University. - - - Institute of Molecular Biophysics and Genetics Laboratories, Department of Biological Sciences, Florida State University, Tallahassee, Florida.