Radiation inactivation analysis of amino acid transport systems in Neurospora crassa

B. G. DeBusk
J. Mallon
A. G. DeBusk

Follow this and additional works at: https://newprairiepress.org/fgr

This work is licensed under a Creative Commons Attribution-Share Alike 4.0 License.

Recommended Citation

This Research Note is brought to you for free and open access by New Prairie Press. It has been accepted for inclusion in Fungal Genetics Reports by an authorized administrator of New Prairie Press. For more information, please contact cads@k-state.edu.
Radiation inactivation analysis of amino acid transport systems in *Neurospora crassa*

**Abstract**

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


A reinvestigation of the effect of zinc deficiency noted by Nason et al. (1951 J. Biol. Chem. 188: 397) has revealed that all enzymes of the tryptophan biosynthetic pathway are decreased two- to five-fold when zinc is omitted from the growth medium (Vogel's Medium N). The effect is more marked in a histidine mutant than 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.

Obtaining approximate molecular weights for some amino acid permease systems, we have used radiation inactivation of the enzymes as a means to this end.

Neurospora crassa conidia were exposed to X-rays produced by a 3MEV Van der Graaf accelerator operating at 500k amperes for varying times, giving total dosages from 300,000 to 2,500,000 rads. After exposure to X-radiation, the cells were examined for the effect of irradiation on the transport of amino acids. Four amino acids were employed; phenylalanine, leucine, tryptophan and glycine, with particular emphasis on phenylalanine. In each case, the irradiated cells were compared to control cells which had been handled identically except that they were not irradiated.

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 $A/A_0 = \text{constant}(\text{dosage})$, where $A/A_0 = \text{remaining activity holds, a straight line should be obtained.}$

It can be seen from the figure that such is not the case for phenylalanine, nor was it the case for the other three amino acids tested.

This non-linearity would indicate that more than one enzyme is involved in the transport of the amino acids or 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 would indicate 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 proportionally constant after irradiation.

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 $D_{37}$ falls on the straight line portion of 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, $MW = 0.72 \times 10^{12}/D_{37}$, where $D_{37}$ 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 phenylalanine transport (as well as the tryptophan and leucine systems) in Neurospora crassa is mediated by two 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-(40-1)-2690) between the Division of Biology and Medicine, U. S. Atomic Energy Commission and the Institute of Molecular Biophysics and Genetics Laboratories, Department of Biological Sciences, Florida State University, Tallahassee, Florida.