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Radiation inactivation analysis of amino acid transport systems in Neurospora crassa

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Abstract
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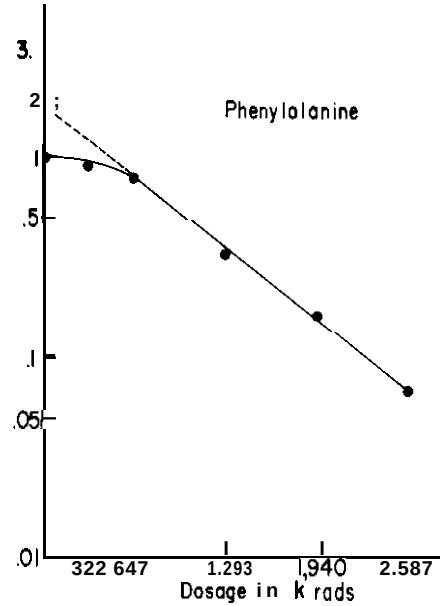
Permease systems, i.e., enzymes responsible for the transfer of met&liter into the cell, can be studied only in an intact cell. Therefore, standard methods of molecular weight determination are ruled out. However, since we were interested in obtaining approximate molecular weights for some amino acid 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 500μ 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 $A/Ao = -(\text{constant})(\text{dosage})$, where A/Ao = 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, tryptophon and leucine are near two. This would indicate that probably two enzymes ore 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 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, $MW = 0.72 \times 10^{12} / D37$, 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 phenylalanine transport (as well as the tryptophan and leucine systems) in Neurospora crassa is mediated by two enzymes of fairly high but not unreasonable molecular weight. The exact function of the two enzyme components is as yet unknown.

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