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NH_2-Terminal analysis of the conidial proteins of N. crassa

H. H. Jervis
Florida State University

A. G. DeBusk
Florida State University

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Abstract

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One of the interests in our laboratory has centered around the initiation of protein synthesis in Neurospora mycelia (Rho and DeBusk 1971 J. Bacteriol. 107: 840, Biochem. Biophys. Res. Comm. 42: 319, and J. Biol. Chem. 246: 6566). The purpose of this study was to analyze the NH_2-terminal amino acids of conidial proteins, thus providing the necessary ground work for elucidation of the protein synthesizing mechanism in this particular stage of fungal growth.

Table 1. The mole percent of DNP-amino acids.

NH_2-terminal amino acids	Conidial fractions			Mycelial	*E. coli* B
	Crude	Ribosomal	Soluble	crude	
Alanine	13.6	17.5	12.4	14.6	23.5
Aspartic acid	11.4	4.1	7.0	11.2	1.4
Glutamic acid	11.5	4.0	6.0	10.4	2.2
Glycine	31.4	30.6	33.6	35.1	2.4
Lysine	-	-	3.5	-	-
Phenylalanine	12.6	22.0	14.1	-	-
Serine	6.9	3.0	6.5	6.8	25.4
Threonine	6.4	10.6	8.2	10.9	6.5
Valine	8.0	8.2	8.7	8.5	-
Methionine	-	-	-	-	38.6

The NH_2-terminal analysis employed the method of Sanger (1949 Biochem. J. 45:536), and the DNP-amino acids were separated by two-dimensional paper chromatography. For the first dimension, an ascending one, a solvent of toluene-pyridine-2-chloroethanol-.8 N NH_4OH (5:1:3:3) was used. For the second dimension, a descending one, a 1.5 M phosphate buffer was employed. The presence of all nine of the amino acids at the amino terminal position was confirmed by employing the 1-naphthylisothiocyanate method of Deyl (1970 J. Chromatogr. 48:231). We believe that this technique will be of particular value in further studies as it is a modification of the Edman method and will allow sequential degradation analysis from the NH_2-terminal to be employed.

Since phenylalanine appeared as a novel NH_2-terminal amino acid in the conidial protein preparations, cells grown on ^{14}C-L-phenylalanine were also used to confirm these results. The radioactivity appeared at the DNP-phenylalanine spot and at no other. The existence of phenylalanine as a unique NH_2-terminal amino acid in the completed protein of Neurospora conidia provides a convenient handle by which this class of molecules may be followed during fungal development.

- - - Genetics Group, Department of Biological Sciences, Florida State University, Tallahassee, Florida 32306.