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PARTIAL SEQUENCE OF A CHLOROFORM-METHANOL SOLUBLE POLYPEPTIDE FROM NEUROSPORA MITOCHONDRIAL MEMBRANES

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INTRODUCTION

A chloroform-methanol soluble polypeptide with a molecular weight of 8 500 (HP 8 500) has been isolated from the mitochondrial membrane of the nuclear mutant cni-1 of Neurospora crassa[1]. Labeling studies in the presence of cycloheximide resp. chloramphenicol have shown that this polypeptide is translated on mitochondrial ribosomes. It has an unusual amino acid composition with a very high proportion of nonpolar residues (Table 1). The polarity calculated according to the definition of Capaldi and Vanderkooi[2] is 0.25.

Although the functional role of the polypeptide remains to be solved, studies on its primary structure have been undertaken for the following reasons:

1) The extremely hydrophobic character of the HP is suggestive of a close connection with the lipid bilayer of mitochondrial membranes. Amino acid sequence will provide information on the structural basis of this interaction.

2) The HP is used as a model for the application of solid-phase methods[3-5] to amino acid sequence analysis of hydrophobic proteins.

RESULTS

1. Cyanogen bromide fragments

HP 8 500 dissolved in 80% formic acid was cleaved with cyanogen bromide (500-fold molar excess over methionine). The resulting fragments were separated by gel chromatography on Bio-Gel P-30 in 80% formic acid (Fig. 1). Incomplete cleavage of at least two of the three methionyl bonds leads to overlapping fragments in addition to the theoretically expected peptides (Table 1, Fig. 2). Peptides C (18 residues) and D (9 residues) were obtained in pure form; E_1 (4 residues) and E_2 (9 residues) were resolved by rechromatography on Bio-Gel P-2.

Peptides C, D, and E_2 were sequenced by automated solid-phase Edman degradation on aminopropyl glass after attachment via their

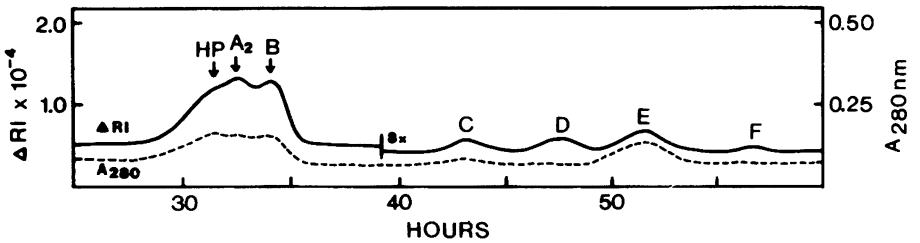

Fig. 1. Chromatography of the cyanogen bromide fragments on 0.8 x 150 cm Bio-Gel P-30 in 80% formic acid (1.0 ml/hr). For the detection of peptides without 280 nm absorption a differential refractometer was used (ΔRI).

TABLE 1

AMINO ACID COMPOSITION OF HP 8 500 AND ITS CYANOGEN BROMIDE FRAGMENTS

The composition of HP 8 500 (from ref. 1) is corrected for destruction and incomplete hydrolysis. Compositions of peptides are from 24 hrs hydrolysis without any correction. Amino acids signed (+) were identified but not quantitated. N-terminal amino acids were determined by dansylation.

Amino acid	HP 8 500	B	D	E_1	E_2
Lys	2 (2.07)		1 (1.11)	1 (1.14)	
Arg	2 (1.93)	2 (2.26)			
Asp	4 (4.13)	3 (3.34)	1 (0.97)		
Thr	3 (2.53)	1 (1.26)		1 (0.90)	
Ser	5 (5.00)	2 (2.34)	1 (0.94)		2 (2.09)
Homoserine		1 (+)	1 (+)		1 (+)
Glu	5 (5.44)	2 (2.46)	1 (0.89)		2 (1.96)
Pro	2 (1.63)	1 (1.26)			
Gly	11 (11.05)	10 (10.23)	1 (1.08)		
Ala	14 (14.23)	11 (10.58)		1 (1.33)	2 (1.96)
Val	6 (6.10)	3 (2.95)	2 (1.76)		
Met	3 (3.21)				
Ile	6 (6.09)	5 (4.53)			1 (1.08)
Leu	11 (11.06)	9 (8.90)	1 (1.25)		
Tyr	2 (2.05)	1 (1.24)			1 (+)
Phe	6 (5.86)	5 (5.23)		1 (0.96)	
Total	82	56	9	4	9
N-terminal amino acid	Tyr	Gly	Val	Ala	Tyr

C-terminal homoserine lactone residues[4,5]. The amino acid phenylthiohydantoins from the degradations were identified by GLC and chemical ionization mass spectrometry (for details see ref. 6). 35 nmoles of peptide C resp. 45 nmoles of peptide D were sufficient for sequence determination. The C-terminal tetrapeptide E_1 was sequenced both by solid-phase degradation (after C-terminal coupling with a carbodiimide[5]) and manually by the dansyl-Edman procedure. From the cyanogen bromide fragments a partial sequence for HP was deduced (Fig. 2) which was confirmed and extended by solid-phase degradation of the intact polypeptide.

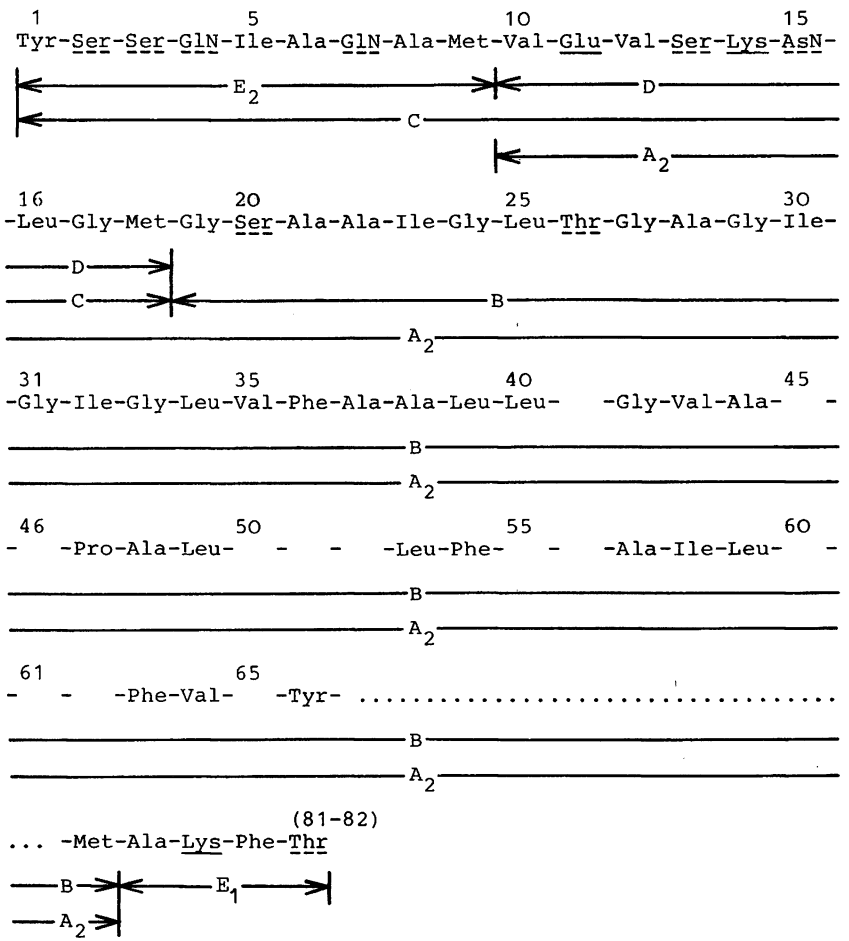

Fig. 2. Partial sequence of HP 8 500. Xxx = polar residues without charge; Xxx = charged residues.

2. Automated solid-phase degradation of the intact polypeptide

Intact HP 8 500 was coupled to p-phenylene diisothiocyanate activated aminopropyl glass (DITC-glass[5]) via its two lysine residues (positions 14 and "79") in more than 90% yield. The covalently bound HP (200 nmoles) was degraded over 80 steps. The amino acid phenylthiohydantoins were determined by GLC and by computerized mass spectrometry (A. Schwab et al., unpublished) in electron impact mode. The second tyrosine was found in step 66. Residues 1 - 40 and several of the later ones were unequivocally identified (Fig. 2).

DISCUSSION

In the partial sequence so far obtained (Fig. 2), polar residues seem to occur preferentially in the N-terminal and C-terminal regions, whereas the midpart contains a long series of nonpolar residues. At least this hydrophobic segment might be located within the lipid bilayer of the membrane.

We hope to complete the amino acid sequence of HP by quantitated solid-phase degradations of the intact polypeptide as well as of the large cyanogen bromide fragment B. In the case that these degradations should leave gaps, appropriate means for a further fragmentation of the polypeptide will have to be found.

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