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NON-COMPACT CONFORMATION OF OVINE MSEL-NEUROPHYSIN

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Received 20 September 1976

1. Introduction

Neurophysins [1] are proteins which bind specifically the neurohypophysial hormones (for reviews, see [2–5]). In mammals, two types of neurophysins, called MSEL-neurophysins and VLDV-neurophysins according to the amino acids in position 2, 3, 6 and 7, have been distinguished [6–8]. The amino acid sequences of ovine [7–8] and bovine [8–9] MSEL-neurophysins have recently been determined. The molecules, nearly identical, comprise 95 residues arranged in a single polypeptide chain. Apparently, in solution at physiological pH values neurophysins are present as dimers rather than monomers [3]. We describe here experiments suggesting a non-compact or flexible conformation for neurophysins at pH about 8.0. In contrast to many native proteins, all the basic residues of 'native' neurophysin are accessible to trypsin; on the other hand, all the disulfide bridges are virtually reduced by dithiothreitol without the help of urea.

2. Results

2.1. *Accessibility of the basic residues to trypsin*

About 0.5 μmol of ovine MSEL-neurophysin, purified as previously described [6], are dissolved in 0.7 ml of 0.1 M ammonium bicarbonate buffer, pH 8.0. Trypsin (3% w/w) is added and the mixture is maintained for 80 min at 37°C. The solution is concentrated and the material is subjected to peptide mapping under conditions previously described [10]. Four tryptic peptides, T_1, T_3, T_7 and T_8, previously characterized in the fully unfolded protein [6,8], are identified by their positions on the fingerprint and their amino acid compositions (table 1). A non-migrating 'core' is eluted, oxidized with performic acid and submitted to peptide mapping. The tryptic units T_2, T_4, T_5 and T_6 can then be recognized by their positions and their amino acid compositions (table 1). Apparently trypsin has split 'native' neurophysin at the level of Arg-8, Lys-18, Arg-20, Arg-43, Arg-66, Arg-86 and Arg-93 as it does when acting on reduced or oxidized protein and this suggests a non-compact conformation accessible to enzymic attack.

2.2. *Accessibility of the disulfide bridges to reduction*

The peculiar susceptibility of neurophysins to partial reduction in the absence of urea has already been observed by Menendez-Botet and Breslow [11]. However in our hands, all the disulfide bridges seem accessible to reduction with dithiothreitol in the absence of urea. 0.5 μmol of ovine MSEL-neurophysin are dissolved in 0.75 ml of 0.1 M borate–KCl buffer pH 8.1. One milligram dithiothreitol dissolved in 0.2 ml of the same buffer is added and the mixture is maintained for 110 min at pH 8.1. Then alkylation is carried out by addition of 1 mg of iodoacetamide dissolved in 0.2 ml of water and the pH is maintained at 8.0 for 30 min. The reaction is stopped by lowering the pH to 3.5 and the reagents are removed by gel filtration on a column of Sephadex G-10 equilibrated with 0.1 M acetic acid. About 60% of the neurophysin subjected to reduction are recovered after lyophilization. The derivative is then submitted to tryptic hydrolysis. 0.3 μmol are dissolved in 1 ml of 0.05 M ammonium bicarbonate and trypsin (3% w/w) is allowed to act for 80 min at 37°C. Tryptic fragments are separated by peptide mapping as previously described [10]. The 8 tryptic peptides, which are usually found when trypsin acts on fully reduced

Table 1
Amino acid composition of tryptic peptides obtained by the action of trypsin on native ovine MSEL-neurophysin[a]
(Values given in residues per mole of peptide)

	Cystine-free peptides isolated before performic acid-oxidation				Cystine-containing peptides isolated after performic acid-oxidation of the 'core'			
	T_1 (197)	T_3 (276)	T_7 (215)	T_8 (274)	T_2 (87)	T_4 (124)	T_5 (96)	T_6 (106)
Lys					0.75 (1)		0.77 (1)	0.96 (1)
His								
Arg	0.94 (1)	1.00 (1)	1.04 (1)	0.46 (1)		0.77 (1)	0.83 (1)	
Asp	1.17 (1)					0.84 (1)		1.90 (2)
Thr						0.82 (1)		0.73 (1)
Ser	0.57 (1)					0.83 (1)	1.96 (3)	1.25 (1)
Glu	0.94 (1)		0.82 (1)		1.28 (1)	2.00 (2)	4.41 (5)	3.02 (3)
Pro			0.87 (1)		1.90 (2)	0.70 (1)	2.42 (3)	0.72 (1)
Gly		0.60 (1)	2.08 (2)		3.03 (3)	4.20 (4)	4.26 (4)	1.34 (1)
Ala	0.87 (1)					2.00 (2)		3.33 (3)
Val				1.00 (1)		0.83 (1)		0.94 (1)
Met[b]	(1)							
Ile			0.87 (1)			0.93 (1)	0.63 (1)	1.00 (1)
Leu	2.00 (2)				1.00 (1)	2.00 (2)	1.00 (1)	
Tyr[b]							(1)	
Phe			1.00 (1)			1.96 (2)		
Cys[c]					1.49 (2)	2.45 (4)	1.87 (3)	3.67 (5)
Number of residues	8	2	7	2	10	23	23	20

[a] Amounts of peptides recovered from 500 nmol of neurophysin are indicated: T_1 (197) = 197 nmol, etc.
[b] Methionine (not stabilized as methionine sulfone) and tyrosine are destroyed after hydrolysis of peptides eluted from paper
[c] Determined as cysteic acid; a partial destruction is observed with peptides eluted from paper

carboxamido-methylated neurophysin prepared in the presence of urea [8], can be detected with the derivative prepared in the absence of urea. It can be concluded that all the 7 disulfide bridges of the molecule are accessible to reduction without urea. Similar results are obtained when reduction without urea is applied using the 'crude' ovine neurophysin–neurohypophysial hormone complex. In the complex, MSEL-neurophysin accounts for 80% of the total neurophysins and there is approximately one mole of neurohypophysial hormone for one mole (mol. wt 10 000) of neurophysin. Therefore the bound peptides do not protect neurophysins against reduction.

3. Discussion

The accessibility, on one hand, of the 7 basic residues to trypsin, on the other, of the 7 disulfide bridges to dithiothreitol in the absence of urea strongly suggests that the conformation of ovine MSEL-neurophysin is not compact. Globular proteins are usually resistant to trypsin hydrolysis and only a few disulfide bridges can be reduced without urea. The great number of proline residues [7] can be partially responsible for the non-compact conformation of neurophysins. An analysis of the far-ultraviolet circular dichroism spectrum of bovine MSEL-neurophysin (Neurophysin-II) has suggested that this protein has 40% β-structure and about 5% α-helix [3]. The ready dimerization of neurophysin might be explained by an extended conformation of the monomer. On the other hand, the specific binding of neurohypophysial hormones may be due to some kind of induced fit because of the flexible conformation of neurophysins.

Acknowledgement

The authors wish to thank Miss Marie-Hélène Simon and Mrs Monique Lidonne for their skilled technical assistance. This study was supported in part by grants from CNRS (ERA No. 563) INSERM (No. 73-1-488-22), and Fondation pour la Recherche Médicale.

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