

COMPLETE AMINO ACID SEQUENCE OF OVINE NEUROPHYSIN-III

David H. SCHLESINGER, Michael ERNST, Ann NICHOLAS*, W. B. WATKINS** and Roderich WALTER***

Department of Medicine, Massachusetts General Hospital, Boston, Mass. 02115, USA

Department of Biophysics, University of Chicago, Chicago, Ill. 60637, USA; **Postgraduate School of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand; *Department of Physiology, University of Illinois at the Medical Center, Chicago, Ill. 60612, USA*

Received 7 July 1975

1. Introduction

The neurophysins are a class of disulfide-rich proteins which reversibly bind oxytocin and vasopressin (for recent reviews see refs. [1] and [2]). Bovine neurophysin II (BNP-II) is the only neurophysin for which the complete covalent structure is known [3,4]. The sequence of porcine neurophysin I (PNP-I) has also been reported [5] and tentative complete covalent structures for bovine and human neurophysin I (BNP-I and HNP-I) have been proposed [6].

In order to gain further insight into the evolutionary history of neurophysins [7] and their structural requirements for binding neurohypophyseal hormones, we determined the sequence of one of the major neurophysin proteins of sheep (ONP-III). The work by Chauvet et al. [8], describing 27 residues of the N-terminal sequence of ONP-III, prompts us to report our data at this time.

2. Material and methods

ONP-III, prepurified by the procedure of Watkins [9], was subjected to final purification by preparative disc electrophoresis as detailed by Schaechtlin et al. [10]. For automated sequential analysis with a Beckman 890 protein sequencer using a double cleavage Quadrol program [11], one sample was oxidized by treatment with performic acid [12] and another reduced and S-alkylated with ^{14}C -iodoacetic acid [13]. Peptides suitable for manual Edman degradation were secured from a third sample of ONP-III, which was S-alkylated with iodoacetic acid and maleated [14]

at the N-terminal amino group and the N^ϵ groups of the Lys residues; one aliquot of this modified protein was cleaved at the carboxyl side of Arg residues by treatment with trypsin [15] and another was fragmented at the carboxyl side of Glu residues by staphylococcal protease [16] as described [14]. The resulting peptides were purified by chromatography on a Bio Gel P-6 column under previously described conditions [14]. This previous report also details the amino acid analysis and manual Edman degradation procedures used as well as the approach for the C-terminal analysis of ONP-III following carboxypeptidase digestion of a sample of the native protein. PTH amino acids derived from automated and manual Edman degradation were determined by gas [17] and thin-layer chromatography [18].

3. Results

The amino acid composition (reported as moles of amino acid per minimum molecular weight) of ONP-III purified by disc electrophoresis indicates that the protein is comprised of 94 residues: Asp(3), Thr(2), Ser(6), Glu(13), Pro(8), Cys(14), Gly(15), Ala(7), Val(3), Met(1), Ile(4), Leu(6), Tyr(1), Phe(3), Trp(0), Lys(2), His(0), Arg(6). Digestion of ONP-III with carboxypeptidase A resulted in the release of valine. No amino acids were released when ONP-III was digested with carboxypeptidase B. However, equal amounts of valine and arginine were released with a mixture of carboxypeptidases A and B indicating that the C-terminal dipeptide sequence of ONP-III is Arg-Val-COOH.

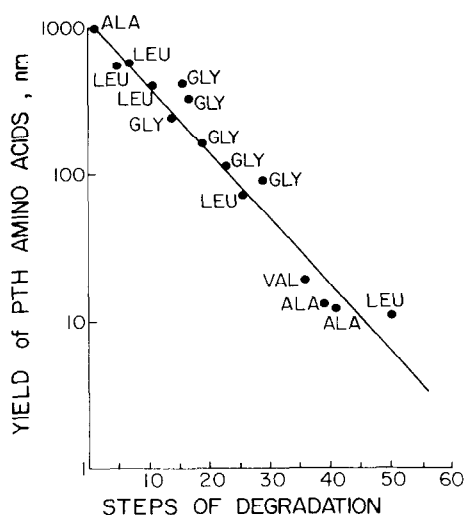


Fig.1. Yields of PTH amino acids at selected steps of the automated Edman degradation of S-alkylated ovine neurophysin III (ONP-III).

Quantitative data for the automated degradation on S-alkylated ONP-III are presented in fig.1. Positive identifications, obtained at every residue through position 54, were confirmed by the degradation of performic acid-oxidized material. The first 27 residues determined by automated analysis confirm the findings of Chauvet et al. [8].

Further information (fig.2) was obtained from manual Edman degradation of peptides derived from tryptic digestion of S-alkylated and maleated ONP-III. Ion-exchange chromatography resolved these peptides into three peaks. Three peptides (MT-1A, MT-1B, and

MT-1C) present in the first peak were subjected to manual sequence analysis as a mixture for 15 cycles. Peptide MT-1A was placed in the ONP-III sequence at positions 21–35 on the basis of sequence identity with results obtained by automated analysis and MT-1B, with its N-terminus placed at position 44, overlaps through residue 54 with results from the automated analysis and then extends the sequence through residue 58. MT-1C can be placed at positions 67 through 81 by homology with the invariant region common to all neurophysin proteins sequenced to date [7].

From the second and third peak eluting from the ion-exchange chromatography one peptide each (referred to as MT-2 and MT-3, respectively) was isolated and their complete sequence determined by the manual procedure (fig.2). Peptide MT-2 was placed at positions 9–20 in the ONP-III sequence since it had an identical sequence to that portion of the protein determined by automated sequencing. Peptide MT-3, possessing a single Phe residue but not sharing a sequence common to the two Phe-containing regions in the N-terminal half of ONP-III, was tentatively placed near the C-terminus in which the third Phe residue is located in all other neurophysins.

The above five sequenced tryptic peptides and the maleated N-terminal peptide of ONP-III account for all 6 maleated tryptic peptides expected from a tryptic digestion of maleated ONP-III, with its 6 Arg residues as revealed by amino acid composition. Completion of the sequences of the tryptic fragments located in the second half of the ONP-III structure is one way to elucidate the amino acid sequence of this protein.

For this purpose, a second aliquot of the mixture

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MT-1A:	NH ₂ -Cys-PHE-GLY-PRO-SER-ILE-CYS-CYS-GLY-ASP-GLU-LEU-GLY-CYS-PHE														
MT-1B:	NH ₂ -Cys-GLN-GLU-GLU-ILE-TYR-LEU-PRO-SER-PRO-CYS-GLN-SER-GLY-GLN														
MT-1C:	NH ₂ -Cys-ALA-ALA-ALA-GLY-ILE-CYS-CYS-ASN-SER-GLU-ALA-CYS-VAL-THR														
MT-2:	NH ₂ -GLN-CYS-LEU-PRO-CYS-GLY-PRO-GLY-GLY-LYS-GLY-ARG-COOH														
MT-3:	NH ₂ -GLU-GLY-ILE-GLY-PHE-PRO-ARG-COOH														

Fig.2. N-terminal sequences of tryptic peptides of S-alkylated and maleated ONP-III.

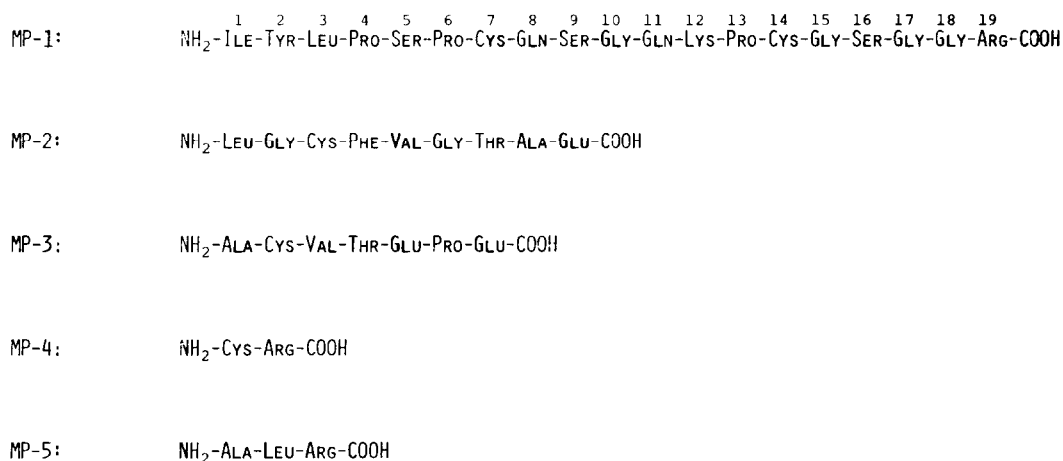


Fig.3. N-terminal sequences of fragments obtained from maleated tryptic ONP-III peptides after digestion with staphylococcal protease.

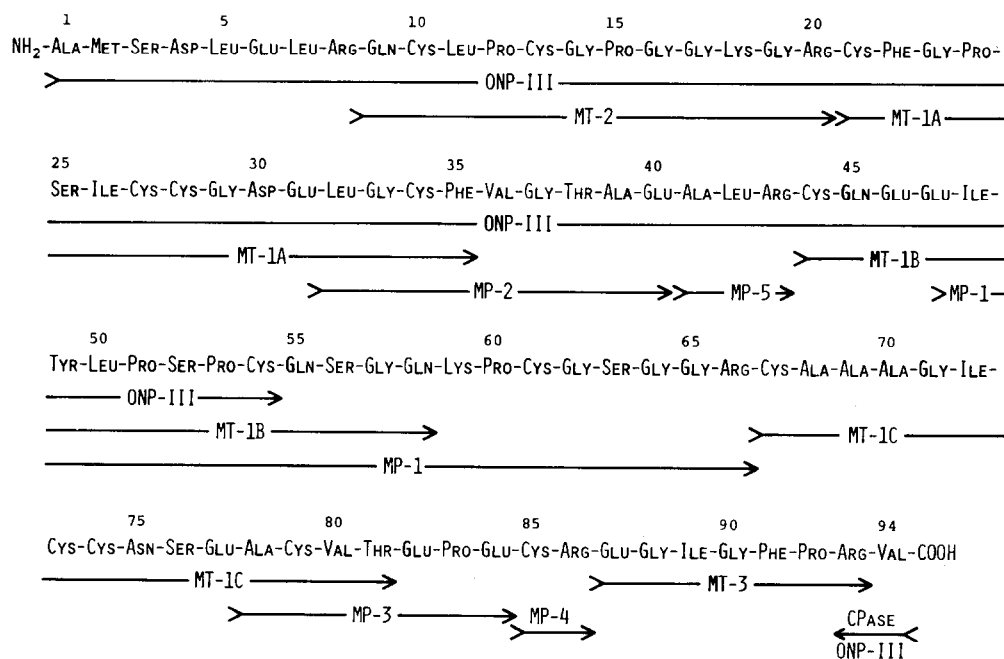


Fig.4. Complete amino acid sequence of ovine neurophysin-III (ONP-III). Sequence determined by automated Edman degradation of S-alkylated ONP-III (—ONP-III—); MP represent staphylococcal protease peptides of maleated ONP-III; MT represent tryptic peptides of maleated ONP-III; CPase represents residues released from ONP-III by digestion with carboxypeptidases A and B.

of MT-1 peptides was maleated in order to block all N-terminal amino groups, making them unavailable for Edman degradation, and then digested with staphylococcal protease, which cleaves the peptide chain specifically at the carboxyl side of Glu residues [16]. The five peptides produced from this digestion (designated MP-1 through MP-5), were purified by gel filtration on Bio Gel P-6 [4] and then sequenced from N- to C-terminus by the manual Edman degradation (fig.3). The N-terminus of MP-1 (derived from MT-1B) is placed at position 48 of ONP-III (fig.4) and extends knowledge of the sequence through the Arg residue at position 66. This analysis also supports placement of the N-terminus of peptide MR-1C at position 67 in ONP-III; as indicated MT-1C completes the sequence of ONP-III through position 81. The N-terminal tetrapeptide of MP-3 overlaps the four C-terminal residues of MT-1C. Peptide MP-3 was sequenced through the Glu-Pro sequence, not cleaved by the protease, to its C-terminal Glu residue at position 84 in ONP-III. MP-2 and MP-5 are readily placed in known portions of the ONP-III sequence, and both are derived from MT-1A. The only remaining 'maleated protease' peptide not placed in the sequence is the dipeptide Cys-Arg (designated MP-4, fig.4) which must be placed at positions 85 and 86 in ONP-III, since it can only be the C-terminal dipeptide of MT-1C (although the sequence of this peptide was not completed); the C-terminal sequences of MT-1A and MT-1B have already been placed. By process of elimination, the peptide MT-3 can be definitively placed at positions 87 through 93 in ONP-III. Selective digestion studies of ONP-III with carboxypeptidases A and B reveal Arg-Val to be the C-terminal sequence of the protein. Proline in the third position from the C-terminus of ONP-III (and in the penultimate position of MT-3) explains the fact that only Val and Arg residues are liberated by the carboxypeptidases.

4. Discussion

Two important points emerge from the analysis of the amino acid sequence of ONP-III (fig.4) and on comparison of its sequence with the neurophysin sequences of other species.

Comparison of the known neurophysin protein

sequences reveals that the N-terminal nonapeptide sequence and approximately a 12-residue sequence at the C-terminus exhibit considerable variability, while the central portion of neurophysins is surprisingly homologous [7,19]. The ONP-III sequence resembles most closely that of BNP-II [3]. In fact, the N-terminal sequences of both proteins are for 70 residues practically identical with two notable exceptions. Firstly, while BNP-II possesses a Glu residue in position 34, ONP-III has a Cys residue in this site as do all other neurophysins of known sequence. This has important implications for the covalent structure of the ONP-III; despite the great homology in amino acid sequences of the ONP-III and BNP-II, their topography will differ. Secondly, the substitution of Asn at position 48, present in BNP-II and all other neurophysins of known sequence, by Ile in ONP-III, represents the first detection of a mutation in this constant region. This alteration, though it can result from a point mutation, is of interest because of its proximity to the single tyrosine residue (position 49) in neurophysins. This Tyr residue has been suggested to be near one of the sites of interaction of neurohypophyseal hormones with neurophysin [20-22].

Acknowledgments

This study was supported by USPHS Grant No. AM-18399, contract NO1-CB-53868, and the Medical Research Council of New Zealand.

References

- [1] Neurophysins: Carriers of Peptide Hormones (1975) (Walter, R. ed.), Ann. N. Y. Acad. Sci. 248, 1-512.
- [2] Breslow, E. (1974) Adv. Enzymol. 40, 271-333.
- [3] Walter, R., Schlesinger, D. H., Schwartz, I. L. and Capra, J. D. (1971) Biochem. Biophys. Res. Commun. 44, 293-298.
- [4] Schlesinger, D. H., Frangione, B. and Walter, R. (1972) Proc. Natl. Acad. Sci. US 69, 3350-3354.
- [5] Wu, T. C., Crumm, S. and Saffran, M. (1971) J. Biol. Chem. 246, 6043-6063.
- [6] North, W. G., Walter, R., Schlesinger, D. H., Breslow, E. and Capra, J. D. (1975) Ann. N. Y. Acad. Sci. 248, 408-422; see also ref. [2], pp. 291-292.
- [7] Capra, J. D. and Walter, R. (1975) Ann. N. Y. Acad. Sci. 248, 397-407.

- [8] Chauvet, M. -T., Chauvet, J. and Acher, R. (1975) FEBS Lett. 52, 212–215.
- [9] Watkins, W. B. (1973) J. Endocrinol. 59, 17–29.
- [10] Schaechtelin, G., North, W. G. and Walter, R. (1975) Ann. N. Y. Acad. Sci. 248, 365–377.
- [11] Edman, P. and Begg, G. (1967) Eur. J. Biochem. 1, 80–91.
- [12] Schlesinger, D. H., Niall, H. D. and Wilson, D. (1974) Biochem. Biophys. Res. Commun. 61, 282–289.
- [13] Anfinsen, C. B. and Haber, E. (1961) J. Biol. Chem. 236, 1361–1363.
- [14] Schlesinger, D. H., Goldstein, G. and Niall, H. D. (1975) Biochemistry, in press.
- [15] Keil, B. (1971) in: The Enzymes Vol. III, p. 250. (Boyer, P. D., ed.), Academic Press, N. Y.
- [16] Houmard, J. and Drapeau, G. R. (1972) Proc. Natl. Sci. US 69, 3506–3509.
- [17] Pisano, J. J. and Bronzert, T. J. (1969) J. Biol. Chem. 244, 5597–5607.
- [18] Edman, P. (1970) in: Protein Sequence Determination p. 211. (S. Needleman, ed.), Springer Verlag, Berlin.
- [19] Capra, J. D., Kehoe, J. M., Kotelchuck, D., Walter, R. and Breslow, E. (1972) Proc. Natl. Acad. Sci. US 69, 431–434.
- [20] Furth, A. J. and Hope, D. B. (1970) Biochem. J. 116, 545–553.
- [21] Balaram, P., Bothner-By, A. A. and Breslow, E. (1972) J. Am. Chem. Soc. 94, 4017–4018; (1973) Biochemistry 12, 4695–4704.
- [22] Griffin, J. H., Alazard, R. and Cohen, P. (1973) J. Biol. Chem. 248, 7975–7978.