AMINO ACID SEQUENCE OF BOVINE NEUROPHYSIN-II: A REINVESTIGATION

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Received November 21,1975

SUMMARY: The entire amino acid sequence of bovine neurophysin-II has been redetermined by manual Edman degradation of tryptic peptides obtained from performic acid-oxidized neurophysin. Electrophoretically homogeneous bovine neurophysin-II was found to be a mixture of two species of protein molecules both containing 95 amino acid residues. The only difference between the two species of the neurophysin molecules is a single amino acid substitution at residue 89. Of the bovine neurophysin-II used in this work 70% of the protein material contained valine and 30% contained isoleucine at residue 89 in their sequences. The redetermined sequences of bovine neurophysin-II shown in Fig. 2 differ substantially from the reported sequence of bovine neurophysin-II but resemble closely that of porcine neurophysin-I and ovine neurophysin-III (Fig. 3).

During a re-examination of the difference between the locations of half-cystinyl residues of the published sequence of porcine neurophysin-I (1,2) and that of bovine neurophysin-II (3,4) we noted that the analytic results of tryptic peptides of the bovine neurophysin was at variance with what would be expected from the reported structure (3,4). We have therefore resequenced the entire molecule of bovine neurophysin-II. This report presents our results.

Materials and Methods

Bovine neurophysin-II (BNPII) was isolated from frozen bovine posterior pituitary lobes (Pel-Freeze Biologics, Inc., Rogers, Arkansas) according to the procedures described by Hollenberg and Hope (5) and Rauch et al. (6). This method, with minor modifications, was also used by Breslow et al. (7) and Walter et al. (3) for the preparation of bovine neurophysins. The isolated neurophysin was judged to be homogeneous by the criteria of electrophoresis in starch and polyacrylamide gels and end group amino acid analyses. The

molecular weight of BNP-II was estimated to be near 10,000 daltons by gel filtration on columns of Sephadex G-75 (8) and polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (9). The amino acid sequences of tryptic peptides were determined manually by the subtractive method of sequential Edman degradation (10). Tryptic peptides were produced from performic acid-oxidized BNP-II by trypsin hydrolysis. Larger tryptic peptides (OT-1, OT-2 and OT-4) had to be further hydrolyzed by chymotrypsin, papain, pepsin or HCl to yield shorter fragments amenable to

Table I

Amino Acid Composition of Tryptic Peptides of Oxidized Bovine Neurophysin-II

Amino Acids	OT-1	OT-2	OT-3	OT-4	OT-5	OT-6	OT-7	OT-8	OT-9
Lys			1.1	1.0					
Arg	1.0	1.0		1.0	1.0	1.0	1.0	1.0	1.0
$CySO_3H$	4.8	4.2	2.1	3.1					
Asp	1.9	1.1		1.0	1.0				
$MetO_2$					0.9				
Thr	0.9	1.0							
Ser	0.9	0.9		2.7	0.9				
Glu	3.2	2.1	1.0	5.3	1.1	1.1	1.1		
Pro	0.9	1.1	1.9	2.8		1.0	1.0		
Gly	1.0	4.1	3.2	4.0		2.0	2.0		1.1
Ala	2.9	2.0			1.0				
Val	0.9	0.8				0.9		1.0	
Ile	0.8	0.8					0.9		
Leu		2.0	1	1.0	2.0				
Tyr				0.8					
Phe		1.8				1.0	1.0		
Total Residues	20	23	10	23	8	7	7	2	2
Yields(%)	52	63	72	67	57	29	16	50	69

manual Edman degradation. The general approach developed in our previous work of sequencing porcine neurophysin-I was followed in the present investigation. The methods and procedures used in our laboratory for amino acid analysis, enzyme digestion of peptides, peptide separation and purification and amide groups determination are those as described in detail in a previous publication (1).

Results and Discussion

Amino acid analyses and molecular weight determination indicate that BNP-II contains 95 amino acid residues of the following composition: Lys 2.1, Arg 7.0, Asp 5.0, Thr 1.9, Ser 5.8, Glu 13.2, Pro 8.2, Gly 15.1 Ala 6.1, 1/2 Cys 14, Val 3.7, Met 0.9, Ile 2.3, Leu 6.0, Tyr 0.9, Phe 2.9. The NH_2-terminus was found to be alanine by the cyanate method of Stark

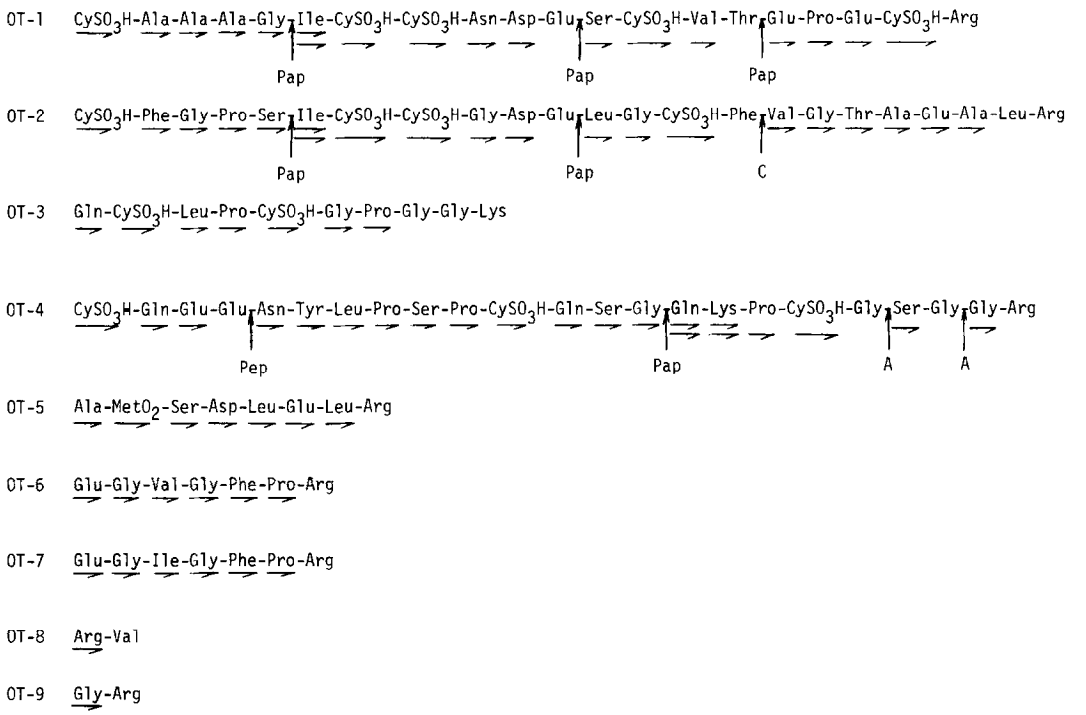

Fig. 1 Amino acid sequences of tryptic peptides of performic acid-oxidized BNP-II. Horizontal arrows below amino acid residues indicate steps of Edman degradation. Vertical arrows indicate the major sites of hydrolysis by chymotrypsin (C), pepsin (Pep), papain (Pap) and dilute HCl (A).

Amino Acid Sequences of Bovine Neurophysin-II

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    1                                   10                                  20
A   Ala-Met-Ser-Asp-Leu-Glu-Leu-Arg-Gln-Cys-Leu-Pro-Cys-Gly-Pro-Gly-Gly-Lys-Gly-Arg-
B   ————————————————————————————————————————————————————————————————————————————
                                    30                                  40
A   Cys-Phe-Gly-Pro-Ser-Ile-Cys-Cys-Gly-Asp-Glu-Leu-Gly-Cys-Phe-Val-Gly-Thr-Ala-Glu-
B   ——————————————————————————————————————————————Gln————————————————————————
                                    50                                  60
A   Ala-Leu-Arg-Cys-Gln-Glu-Glu-Asn-Tyr-Leu-Pro-Ser-Pro-Cys-Gln-Ser-Gly-Gln-Lys-Pro-
B   ——————————————————————————————————————————————————————————————————Arg———
                                    70                                  80
A   Cys-Gly-Ser-Gly-Gly-Arg-Cys-Ala-Ala-Ala-Gly-Ile-Cys-Cys-Asn-Asp-Glu-Ser-Cys-Val-
B   ——————————————————————————————Thr————————Ser-Asn——Glu————————————————————
                                   90
A   Thr-Glu-Pro-Glu-Cys-Arg-Glu-Gly-Val/Ile-Gly-Phe-Pro-Arg-Arg-Val
B   Pro-Asp-Glu——Val-Lys-Pro——Gly-Arg-Gly-Gly-Cys-Phe-Cys-Arg-Val
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Fig. 2 A is the redetermined amino acid sequence of BNP-II: 95 amino acid residue. B is the previously reported sequence of BNP-II (3,4): 97 amino acid residues. The horizontal lines show regions of identity. Altogether the two sequences differ in 21 positions. The positions and the regions of nonidentity are 34, 59, 71, 75-76, 78, 81-83, 85-87, and 89-97.

and Smyth (11). Hydrolysis with carboxypeptidase A followed by carboxypeptidase B revealed the COOH-terminal sequence Arg-Val. Nine peptides were isolated in pure form from a tryptic digest of the oxidized BNP-II. Table I lists the amino acid composition of the tryptic peptides. Peptides OT-6 and OT-7 are heptapeptides with identical composition except for a replacement of a valine (OT-6) by an isoleucine residue (OT-7). The fact that the two peptides were isolated in much lower yields than that of the other peptides (Table I) coupled with the knowledge of their homologeous sequences (Fig. 1) makes it obvious that they represent the same region of the neurophysin molecules.

Fig. 1 shows the amino acid sequences of 9 tryptic peptides. Since OT-5 is the only peptide with an alanyl residue at its NH_2-terminus it must also be the NH_2-terminal peptide of BNP-II. On the basis of end group analysis the sequence of OT-8 is placed at the COOH-terminus of BNP-II. In further derivation of the complete sequence of BNP-II as shown in Fig. 2 the tryptic peptides are arranged according to their homology with porcine

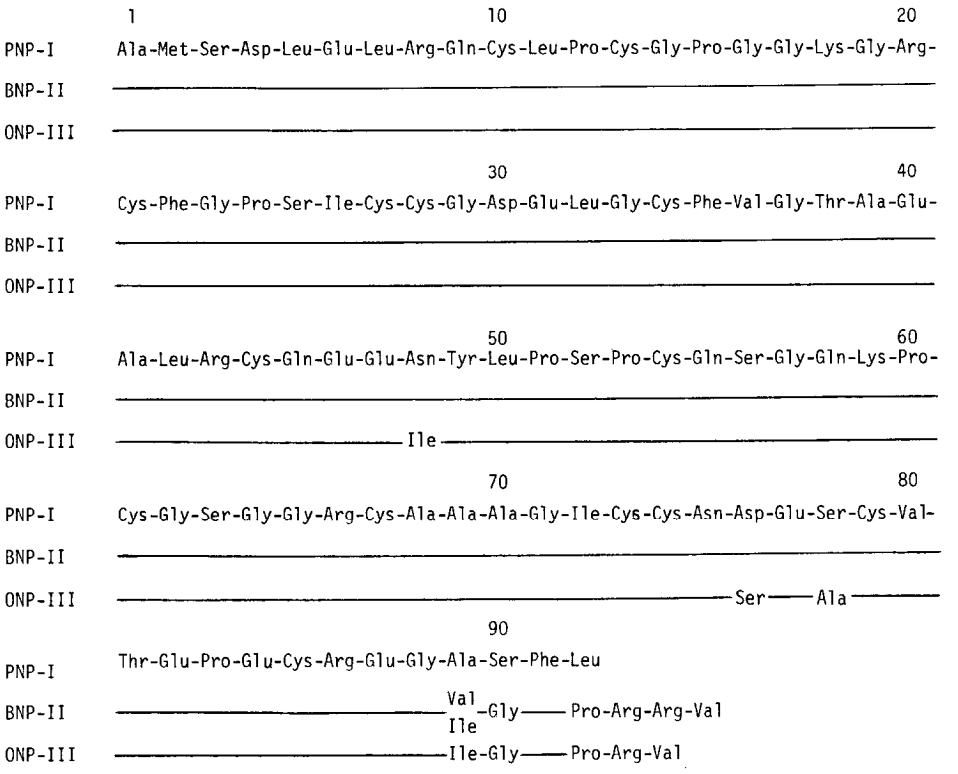

Fig. 3 The amnio acid sequence of porcine neurophysin-I (1,2) bovine neurophysin-II (from the present work) and ovine neurophysin-III (12).

neurophysin-I structure. The sequence of BNP-II determined in this work differs substantially from the reported structure (3,4) but resembles closely that of porcine neurophysin-I (1,2) and ovine neurophysin-III (12). In the redetermined sequence of BNP-II reported here the distribution of the 14 half-cystinyl residues along the polypeptide chain is identical to that of PNP-I and ONP-III (Fig. 3). It is therefore apparent that the disposition of the 7 disulfide linkages of the resequenced BNP-II molecules will be found, when determined, to be different from what has been reported for BNP-II (4).

Finally, BNP-II represents the first case in which two species of neurophysin molecules differing by one amino acid substitution have been detected. Since the BNP-II used in this work was isolated from a pooled

sample of posterior pituitary lobes the distribution of the two forms of BNP-II in individual bovine pituitaries remains to be investigated.

Acknowledgements

The authors wish to thank Dr. Maurice Manning for critical review of the manuscript. This work was supported by the Medical College of Ohio.

References

1. Wuu, T.C., Crumm, S. and Saffran, M. (1971) J. Biol. Chem. 246, 6043-6063.
2. Wuu, T.C., Crumm, S. and Saffran, M. (1973) in Altas of Protein Sequence and Structure (Dayhoff, M.O., ed.) Vol. 5, Supplement I, pp. S-43.
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, D. (1972) Proc. Nat. Acad. Sci. USA 69, 3350-3354.
5. Hollenberg, M.D. and Hope, D.B. (1968) Biochem. J. 106, 557-564.
6. Rauch, R., Hollenberg, M.D. and Hope, D.B. (1969) Biochem. J. 115, 473-479.
7. Breslow, E., Aanning, H.L., Abrash, L. and Schmir, M. (1971) J. Biol. Chem. 246, 5179-5188.
8. Andrews, P. (1964) Biochem. J. 91, 222-233.
9. Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412.
10. Konigsberg, W. (1972) in Methods in Enzymology (Hirs, C.H.W. and Timasheff, S.N., ed.) Vol. 25, pp. 326-332, Academic Press, New York.
11. Stark, G.R. and Smyth, D.G. (1963) J. Biol. Chem. 238, 214-226.
12. Schlesinger, D.H., Ernst, M., Nicholas, A., Watkins, W.B. and Walter, R. (1975) FEBS Letters 57, 55-59.