COMPLETE AMINO ACID SEQUENCE OF BOVINE NEUROPHYSIN II

Roderich Walter, D. H. Schlesinger*, I. L. Schwartz
and J. D. Capra

Departments of Physiology and Microbiology
Mount Sinai Medical and Graduate Schools
of the City University of New York
New York, N. Y. 10029

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SUMMARY

The complete amino acid sequence of bovine neurophysin II, a 97 amino acid protein which specifically binds the posterior pituitary hormones oxytocin and arginine vasopressin, is proposed.

High yields of neurophysin II are obtained from bovine posterior pituitary lobes. This protein binds neurohypophyseal hormones in general in in vitro experiments (1,2) and appears to be associated in neurosecretory granules with vasopressin specifically (3).

In this paper we describe the complete amino acid sequence of the neurophysin II protein.

MATERIALS AND METHODS

Neurophysin II (NPII) was isolated from bovine pituitary powder (Parke Davis, lot #284346) by a modification of the procedure of Hollenberg and Hope (4), and the purified material was checked for the absence of neurohypophyseal hormones by avian vasodepressor (5) and rat pressor assay (5), and by the lack of precipitation with specific antibodies to oxytocin (6). NPII was tested for homogeneity by disc electrophoresis (7), isoelectric focusing (8) and amino acid analysis (9). A portion of NPII was reduced with β-mercaptoethanol and the sulfhydryl groups were alkylated with ^{14}C-iodoacetamide(10); the radioactive material was desalted by dialysis against water and lyophilized to yield ^{14}C-neurophysin II (^{14}C-NPII). Another portion of NPII was aminoethyl-

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ated with ethyleneimine (11). Peptides were subjected to automated Edman degradations on a Beckman Model 890 protein sequencer, using procedures described previously (12-14). Chymotryptic and tryptic (TCPK treated, Worthington Chem. Company) digestions were performed with 0.33% and 0.5% enzyme to substrate ratios, respectively. The enzyme digest fragments were desalted by gel filtration on Sephadex G-25 in 0.1N formic acid and then subjected to separation by peptide elution employing a pH and ionic strength gradient on a PA-35 Beckman Custom Research resin in a Beckman 120C amino acid analyzer, which was converted to accommodate pyridine acetate buffers. Aminoethylated NPII was treated with carboxypeptidases A and B (Worthington Chem. Co.) and the step-wise release of C-terminal amino acids was determined by amino acid analysis. Amino acid compositions of NPII and NPII fragments were obtained after peptide hydrolysis with 6N HCl at 110° for 22 hrs according to Spackman et al. (9). Accelerated manual Edman degradations were performed by the method of Niall et al. (15), with minor modifications. PTH amino acids were identified by gas (16) and thin layer chromatography (17) and ^{14}C-determination.

RESULTS AND DISCUSSION

The complete amino acid sequence of bovine neurophysin II was derived from automated sequential analysis of ^{14}C-NPII itself as well as of automated and manual sequential analyses of chymotryptic fragments. Supportive evidence came from amino acid compositions of NPII, ^{14}C-NPII and ^{14}C-NPII chymotryptic and tryptic digests (Table 1).

NPII is a 97 amino acid protein with a molecular weight of 10,029 with the following ninhydrin-active components: Lys(2), Arg(7), Thr(2), Ser(6), Asp(5), Glu(14), Pro(8), Gly(16), Ala(6), ½Cys(14), Val(4), Met(1), Ile(2), Leu(6), Tyr(1), Phe(3) and NH_3(8).

When ^{14}C-NPII was programmed for an 80-step automated Edman degradation procedure, the N-terminal amino acid sequence indicated by the arrow ⟶ in Fig. 1 was obtained. Thus the N-terminal sequence Ala-Met-Ser reported earlier is confirmed (18). Next, ^{14}C-NPII was subjected to hydrolysis with chymo-

Table 1.

AMINO ACID COMPOSITIONS OF ENZYME DIGESTS OF BOVINE NEUROPHYSIN II

	Chymotryptic Peptides					Tryptic Peptide
	C_1	C_2	C_3	C_4	C_5	T_1
Lys	1.1(1)	1.1(1)	0.0(0)	0.9(1)	1.3(1)	0.8(1)
His	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)
Arg	1.2(1)	2.4(2)	0.0(0)	4.5(5)	1.2(1)	1.3(1)
CM-Cys*	0.9(1)	3.0(3)	2.4(2)	8.7(9)	1.0(1)	5.1(5)
Asp	0.0(0)	1.2(1)	1.3(1)	3.1(3)	0.0(0)	2.2(2)
Thr	0.0(0)	0.1(0)	0.9(1)	1.1(1)	0.0(0)	1.0(1)
Ser	0.0(0)	1.1(1)	1.2(1)	3.5(4)	0.0(0)	1.8(2)
Glu	0.1(0)	2.4(2)	2.7(3)	9.2(9)	0.0(0)	4.3(4)
Pro	1.3(1)	2.4(2)	1.1(1)	5.2(5)	1.2(1)	1.6(2)
Gly	4.2(4)	4.4(4)	4.1(4)	8.0(8)	4.1(4)	3.2(3)
Ala	0.1(0)	1.0(1)	2.4(2)	3.4(3)	0.0(0)	3.3(3)
½Cys	-	-	-	-	-	-
Val	0.0(0)	0.3(0)	1.0(1)	3.2(3)	0.0(0)	1.6(2)
Met	0.0(0)	0.8(1)	0.3(0)	0.0(0)	0.0(0)	0.0(0)
Ile	0.0(0)	0.1(0)	1.0(1)	1.2(1)	0.0(0)	1.0(1)
Leu	0.1(0)	2.8(3)	2.4(2)	1.1(1)	0.0(0)	0.0(0)
Tyr	0.0(0)	0.0(0)	0.0(0)	1.0(1)	0.0(0)	0.0(0)
Phe	1.0(1)	1.0(1)	1.0(1)	1.4(1)	1.0(1)	0.1(0)
Total Residues	9	22	20	55	9	27

* Carboxymethylcysteine

trypsin. From this enzyme digestion 5 peptides [C_1(residue 14-22); C_2(1-22)· C_3 (23-42); C_4 (43-97); and C_5 (86-94)] were isolated as shown in Fig. 2. The largest chymotryptic fragment (C_4), which was found to be the C-terminal component, gave the amino acid sequence indicated by the arrow --→ in Fig. 1. Nonapeptide C_5, which was determined by manual Edman degradations, gave the sequence 86-94 (⟹, Fig. 1). The C-terminal tripeptide amino acid sequence (see Fig. 1, ⟸) was determined by digestion of aminoethylated NPII with

^{14}C-NEUROPHYSIN II AND ^{14}C-NEUROPHYSIN II PEPTIDES SEQUENCED

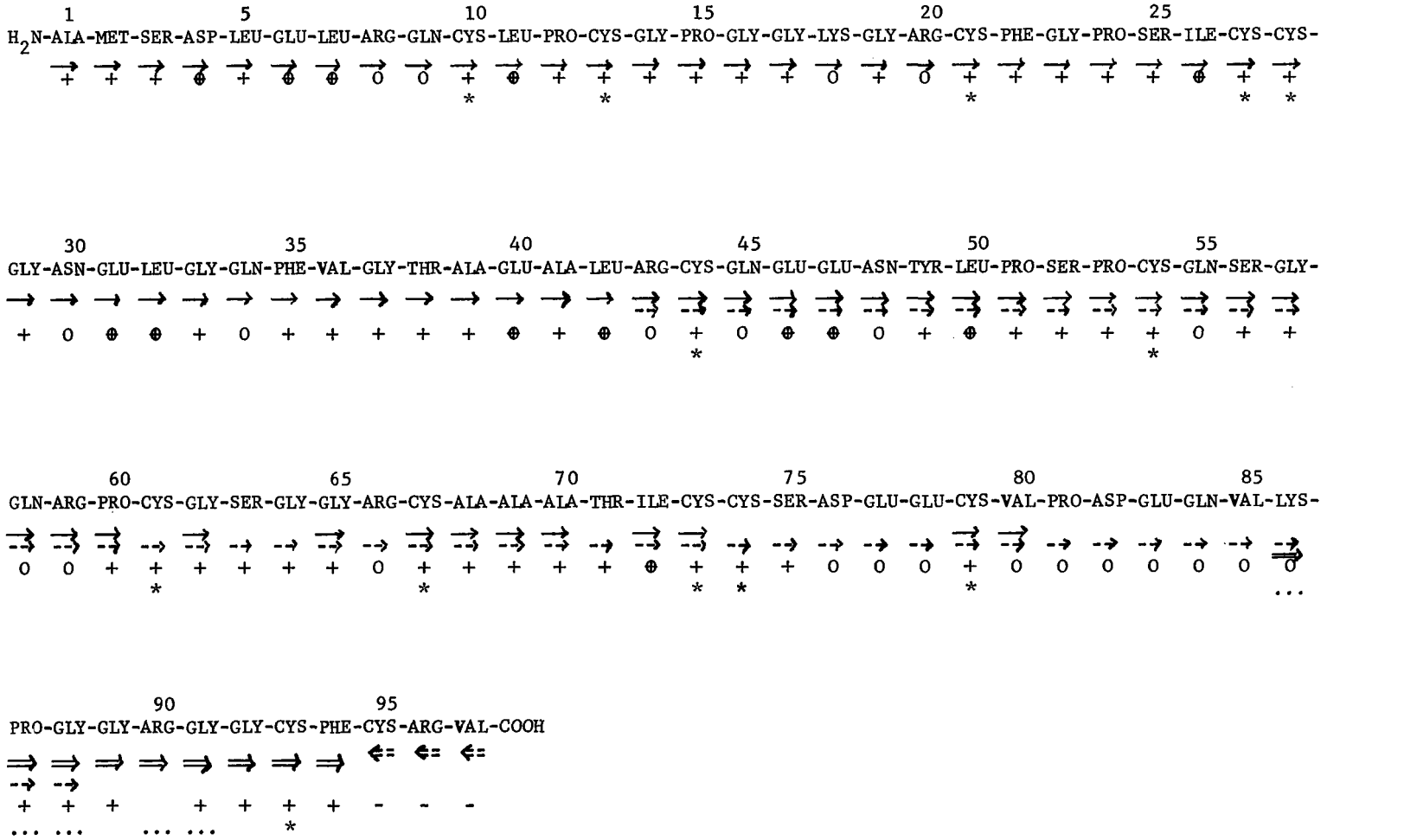

Fig. 1 Whenever possible four criteria of identification of each resulting phenylthiohydantoin amino acid (PTHAA) were applied during sequence analysis: gas and thin layer chromatography, ^{14}C determination of PTHAAs, and amino acid analysis of the acid hydrolysates of PTHAAs. Best means of identification is indicated: gas chromatography of PTHAA (+), gas chromatography of silylated PTHAA (⊕), thin layer chromatography (O), ^{14}C-counting (*) and amino acid analysis (—)[in case of C_5 subtractive method (...)].

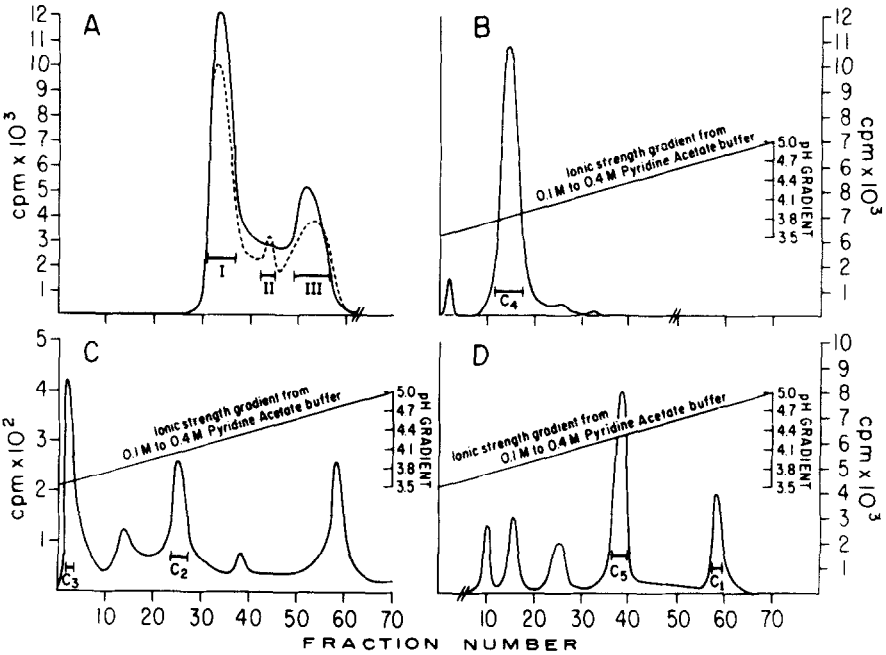

Fig. 2 A. The chymotryptic digest of ^{14}C-NPII was applied to a Sephadex G-25 column (1.0 x 160 cm) and eluted with 0.1N formic acid, resulting in the elution pattern depicted in panel A (solid line represents ^{14}C-counts and dashed line optical density of eluent fractions). Three fractions, representing high (I), intermediate (II), and low molecular weight peptides (III) were obtained as indicated. B. Fraction I was lyophilized, dissolved in starting buffer 0.1N pyridine acetate (pH 3.5) and applied to a column (0.9 x 22 cm) packed with PA-35 Beckman Custom Research ion-exchange resin. Peptide material, eluted with a linear pH and ionic strength gradient, resulted in ^{14}C-NPII fragment C_4. C. Similarly, fraction II was subjected to ion-exchange chromatography yielding peptides C_2 and C_3. D. Analogously, fraction III gave the nonapeptides C_1 and C_5 upon ion-exchange chromatography.

carboxypeptidases A and B. Amino acid compositions of the remaining three chymotryptic fragments, of the isolated tryptic fragment and of the fragments isolated from cyanogen bromide treatment (18), support the proposed amino acid sequence of bovine neurophysin II.

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