EVOLUTION OF NEUROPHYSIN PROTEINS: THE PARTIAL SEQUENCE OF HUMAN NEUROPHYSIN-I

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1. Introduction

Neurophysins are a group of proteins found throughout the hypothalamoneurohypophyseal system (e.g., [1–4]) which are considered to function as carrier substances for the neurohypophyseal hormones during transport from the hypothalamus and storage within the posterior pituitary [5]. Most mammalian species have two major neurophysins [6]. The complete amino acid sequence of one of the bovine neurophysins has been reported [7] and its covalent structure has been determined by the localization of all of its disulfide bonds [8]. In addition, the sequence of one of the porcine proteins has been described [9], and the sequence of the N-terminal 50 amino acid residues of the second bovine neurophysin has also been reported [10] and a tentative assignment has been made through residue position 75 (Walter, Schlesinger, Breslow, Kehoe and Capra, unpublished). Foss et al. have recently described the isolation and amino acid sequence of the N-terminal 18 residues of a human neurophysin [11].

This report concerns the further purification of human neurophysin I and the determination of its N-terminal sequence through residue 54. The results are discussed in relationship to the evolution of this group of proteins.

2. Materials and methods

Human neurophysin I (HNP-I), isolated from acetone-dried pituitary powder by the method of Cheng and Friesen [12] was further purified by preparative continuous polyacrylamide gel electrophoresis (PAGE) at pH 8.7 [13]. Preparative PAGE was performed after pre-electrophoresis for 90 min at 10°C on an LKB Uniphor column electrophoresis system using 10% polyacrylamide gels (10% acrylamide, 0.31% Bis) of bed height 7.5 cm. The buffer used was 25 mM Tris–glycine (pH 8.7) and the protein (17 mg/ml) was applied in a solution of reduced conductivity obtained with a one-fifth dilution of buffer containing 30% sucrose [14]. Electrophoresis was carried out at 15 mA (660–700 V) and the elution rate was 0.2 ml/cm^2 gel surface/min. Protein in the eluate was monitored at 280 nm on an LKB 8303A Uvicord II absorptiometer. Acid hydrolyses were carried out on samples of purified HNP-I for 24, 48 and 72-hr periods and the hydrolysates were analyzed for ninhydrin-active components according to Moore [15]. The values given for HNP-I are those obtained after extrapolation to zero hydrolysis time.

Another sample of the purified protein (20 mg) was reduced with 2-mercaptoethanol (0.7 mmole) for 4 hr and S-alkylated with ^{14}C-labeled iodoacetamide (1.44 mmoles; 0.17 mCik/mmole) in the presence of 8 M urea in 0.6 M Tris–HCl buffer at pH 8.6; the product

was dialyzed against 0.2 M acetic acid in a model 52 Amicon ultrafiltration cell fitted with a UM-2 filter [16]. The extent of S-alkylation was examined by amino acid analysis.

Automated amino acid sequence analysis of the S-alkylated protein was performed on an updated Beckman model 890A Sequencer utilizing a DMAA buffer system [17–19]. All PTH amino acids were analyzed by gas chromatography [20] and thin layer chromatography [21], and ^{14}C was counted in a Packard scintillation spectrometer Model 3003.

3. Results

3.1. *Neurophysin*

One major absorbance peak at 280 nm was obtained during electrophoresis and eluate associated with the center portion of this peak was pooled, dialyzed against 0.2 M acetic acid and lyophilized to yield 60% of the original protein applied. The amino acid composition of this HNP-I preparation is compared in table 1 with results obtained previously by Cheng and Friesen [12], Foss et al. [11] and North et al. [16]. There is good agreement, particularly between the results of this study and that of Foss et al., with possibly the exception of the relative amounts of prolines.

3.2. *Preparation and sequence determination of S-alkylated neurophysin*

Purified neurophysin (20 mg) was converted to its ^{14}C-labeled S-alkylated derivative. The analysis of the PTH derivative from each step from the sequencer is listed in table 2. The combination of techniques utilized unambiguously identifies all the amino acid residues. The identification of the first 54 steps occurred during a single run on the sequencer using a load of 10 mg. The sequence is displayed in fig. 1 and compared to the N-terminal 54 residues of the other neurophysins sequenced to that position.

Table 1
Amino acid composition of human neurophysin

Amino acid	(a)	(b)	(c)	(d)
Lys	3	3	3	4
His	1	1	1	1
Arg	4	5	4	5
Asp	7	8	7	7
Thr	2	2	2	2
Ser	4	5	4	5
Glu	10	11	10	11
Pro	9	9	7	6
Gly	12	15	12	12
Ala	9	12	9	9
Cys*tine*	7*	7	6.5**	6.5**
Val	3	4	3	4
Met	0	0	0	0
Ile	1	1	1	1
Leu	7	8	7	7
Tyr	1	1	1	1
Phe	3	3	3	3
NH_3	7 (minimum)			
Mol. wt.	9257	10333	8983	9100

(a) Results of present study with unmodified protein: values obtained by setting lysine equal to three residues per molecule; mol. wt. from amino acid analysis. For experimental detail see text.
(b) Data reported by Cheng and Friesen [12], obtained by setting histidine equal to one residue per molecule; mol. wt. from amino acid analysis.
(c) Data reported by Foss et al. [11] obtained by setting aspartic acid equal to seven residues per molecule; mol. wt. from amino acid analysis.
(d) Data reported by North et al. [16] based on mol. wt. determined by sedimentation equilibrium.
* Determined as carboxymethyl-cysteine with a sample of S-alkylated HNP-I.
** Determined as cysteic acid.

4. Discussion

Previous structural studies on the bovine and porcine neurophysins established a remarkable degree of homology among these proteins from the same Order of mammals (Artiodactyls), with over 90% sequence identity among the three proteins studied. The present study shows that a neurophysin isolated from a separate Order (Primates) also bears a striking homology to the bovine and porcine structures with 89% of its sequence being identical to the same part of the sequence of bovine neurophysin I (fig. 1).

The results presented in this study were accomplished on a single run on the protein sequencer and again demonstrate the significant amount of structural data which can be obtained in a relatively short time using only limited amounts of pure material. The sequence

Table 2*

Position	G.C.	Sil. G.C.	TLC	cpm	Sequence
1	A		A	22	ALA
2	A		A	24	ALA
3	T/P		P	25	PRO
4			D	22	ASP
5	L/I	L	L/I	21	LEU
6		D	D	19	ASP
7	V		V	31	VAL
8			R	23	ARG
9		K	K	24	LYS
10	S/C		C	4029	CYS
11	L/I	L	L/I	249	LEU
12	T/P		P	89	PRO
13	S/C		C	3794	CYS
14	G		G	145	GLY
15	T/P		P	34	PRO
16	G		G	23	GLY
17	G		G	26	GLY
18		K	K	28	LYS
19	G		G	41	GLY
20			R	36	ARG
21	S/C		C	2987	CYS
22	F	F	F	243	PHE
23	G		G	131	GLY
24	T/P		P	54	PRO
25		N	N	43	ASN
26	L/I	I	L/I	32	ILE
27	S/C		C	2458	CYS

Table 2 (continued)

Position	G.C.	Sil. G.C.	TLC	cpm	Sequence
28	S/C		C	2491	CYS
29	A		A	325	ALA
30		E	E	210	GLU
31		E	E	145	GLU
32	L/I	L	L/I	85	LEU
33	G		G	53	GLY
34	S/C		C	1869	CYS
35	F	F	F	369	PHE
36	V		V	201	VAL
37	G		G	142	GLY
38	T/P		T	65	THR
39	A		A	63	ALA
40		E	E	64	GLU
41	A		A	59	ALA
42	L/I	L	L/I	59	LEU
43			R	57	ARG
44	S/C		C	974	CYS
45		Q	Q	354	GLN
46		E	E	210	GLU
47		E	E	145	GLU
48		N	N	100	ASN
49	Y	Y	Y	109	TYR
50	L/I	L	L/I	103	LEU
51	T/P		P	99	PRO
52	S/C		S	87	SER
53	T/P		P	88	PRO
54	S/C		C	587	CYS

* Positional analysis of the PTH derivatives from each step of the sequencer run. The single letter code (IUPAC-IUB Commission on Biochemical Nomenclature, Biochemistry 7, 2703, 1968) is used in the first three columns. In some systems certain amino acids cannot be resolved and both are reported. This problem is generally resolved in another system. G.C., gas chromatography of PTH amino acid; Sil. G.C., gas chromatography of silylated PTH amino acid; TLC, thin layer chromatography; cpm, ^{14}C counts per minute

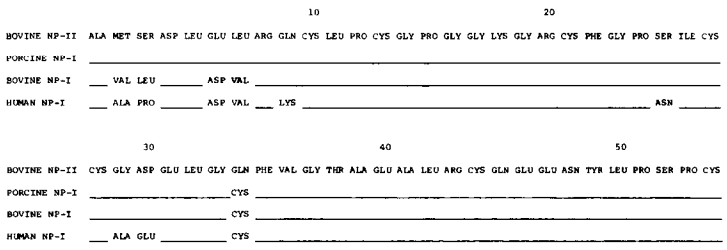

Fig. 1. The amino acid sequence of the N-terminal 54 residues of four neurophysins. All are compared to bovine neurophysin II. A line indicates identity with the bovine neurophysin II sequence. The sequence Cys–Cys–Gly (residues 27–29) of porcine neurophysin I was originally reported to be Cys–Gly–Cys [9] but has been recently corrected [22].

differs in one position (#17) from that reported previously by Foss et al. [11], in a study done by manual Edman degradations.

The extensive homology between bovine neurophysin II (BNP-II) and porcine neurophysin I (PNP-I) is illustrated in fig. 1. The only difference in the N-terminal 54 residues of these two proteins is the Gln-Cys interchange at position 34. Bovine neurophysin I (BNP-I) varies considerably from BNP-II and PNP-I with positions 2, 3, 6 and 7 different from both, but with position 34 identical to PNP-I. The homology between these three proteins and the sequenced portion of HNP-I is evident from fig. 1. The largest difference is between HNP-I and BNP-I (90%). Among the five positions in which variations have been found to date in the N-terminal 54 residues of the bovine and porcine neurophysins (residues 2, 3, 6, 7, 34), HNP-I is identical to BNP-I in three (residues 6, 7, 34) while positions 2 and 3 differ from the other three proteins. In addition, HNP-I differs in four additional positions which have not been found to vary in the other sequenced neurophysins (residues 9, 25, 29, 30). The structural preservation in the central portion of the molecule is evident between positions 35-54. In this 20 amino acid residue stretch all four neurophysins have identical primary structures. We have previously postulated that the central region of the molecule may play an important role in the binding between the neurophysins and the posterior pituitary hormones, oxytocin and vasopressin [10].

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References

[1] Van Dyke, H. B., Chow, B. F., Greep, R. O. and Rothen, A. (1942) J. Pharmacol. Exptl. Ther. 74, 190–209.
[2] Chauvet, J., Lenci, M. T. and Acher, R. (1960) Biochim. Biophys. Acta 38, 266–272.
[3] Zimmerman, E. A., Hsu, K. C., Robinson, A. G., Carmel, P. W., Frantz, A. G. and Tannenbaum, M. (1973) Endocrinology 92, 931–940.
[4] Livett, B. G., Uttenthal, L. O. and Hope, D. B. (1971) Philos. Trans. Roy. Soc. London B 261, 371–378.
[5] Sawyer, W. H. (1961) Pharmacol. Rev. 13, 225–277.
[6] Walter, R. and Breslow, E. (1974) in: Research Methods in Neurochemistry (Marks, N. and Rodnight, R., eds.), Vol. 2, pp. 247–279, Plenum Press, New York.
[7] Walter, R., Schlesinger, D., Schwartz, I. L. and Capra, J. D. (1971) Biochem. Biophys. Res. Commun. 44, 293–298.
[8] Schlesinger, D. H., Frangione, B. and Walter, R. (1972) Proc. Natl. Acad. Sci. U.S. 69, 3350–3354.
[9] Wuu, T. C., Crumm, S. and Saffran, M. (1971) J. Biol. Chem. 246, 6043–6063.
[10] Capra, J. D., Kehoe, J. M., Kotelchuck, D., Walter, R. and Breslow, E. (1972) Proc. Natl. Acad. Sci. U.S. 69, 431–434.
[11] Foss, I., Sletten, K. and Trygstad, O. (1973) FEBS Letters 30, 151–156.
[12] Cheng, K. W. and Friesen, H. G. (1972) J. Clin. Endocrinol. 34, 165–176.
[13] Righetti, P. and Secchi, C. (1972) J. Chromatogr. 72, 165–175.
[14] Hjertén, S., Jerstedt, S. and Tiselius, A. (1965) Anal. Biochem. 11, 219–223.
[15] Moore, S. (1972) in: Chemistry and Biology of Peptides (Meienhofer, J., ed.), pp. 629–653, Ann Arbor Science Publ. Co., Ann Arbor, Michigan.
[16] North, W. G., Heber, R., Hawker, R. W. and Zerner, B. (1974) Biochim. Biophys. Acta, in press.
[17] Edman, P. and Begg, G. (1967) Eur. J. Biochem. 1, 80–91.
[18] Capra, J. D. and Kunkel, H. G. (1970) Proc. Natl. Acad. Sci. U.S. 67, 87–92.
[19] Capra, J. D. and Kehoe, J. M. (1974) Proc. Natl. Acad. Sci. U.S. 71, 845–848.
[20] Pisano, J. J. and Bronzert, T. J. (1969) J. Biol. Chem. 244, 5597–5607.
[21] Summers, M. R., Smythers, G. W. and Oroszlan, S. (1973) Anal. Biochem. 53, 624–628.
[22] Dayhoff, M. O. (1973): Atlas of Protein Sequence and Structure, Vol. 5, Suppl. I, Natl. Biomed. Res. Foundn., Silverspring, Md.