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An amphibian two-domain 'big' neurophysin : conformational homology with the mammalian MSEL-neurophysin/copeptin intermediate precursor shown by trypsin-Sepharose proteolysis

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A 'big' frog (*Rana esculenta*) neurophysin, encompassing sequences homologous to mammalian MSEL-neurophysin and copeptin, has been passed through a trypsin-Sepharose column in order to compare its conformation with that of the two-domain intermediate precursor isolated from guinea pig. Whereas the polypeptide possesses 8 arginine residues, only two cleavages were observed located in a putative inter-domain sequence (at Arg-94 and Arg-114). Because free vasotocin has been isolated from the frog, it is assumed that pro-vasotocin has a three-domain conformation similar to that of pro-vasopressin but processing in amphibians involves only one step rather than two steps as in mammals.

Two-domain big neurophysin; Conformation-limited proteolysis; Provasotocin-neurophysin processing; Evolution; (Amphibian neurophysin)

1. INTRODUCTION

Mammalian vasopressin is derived from a three-domain protein precursor processed into vasopressin, MSEL-neurophysin and copeptin during axonal transport from hypothalamus to neurohypophysis (review [1,2]). In mammals two types of neurophysins, MSEL-neurophysin (associated to vasopressin) and VLDV-neurophysin (associated to oxytocin) have been distinguished [1,3]. In non-mammalian tetrapods, vasopressin and oxytocin are replaced by vasotocin and mesotocin, respectively [1], and because the two types of neurophysins have been identified in birds [4,5], it has been assumed that vasotocin and mesotocin protein precursors are built in the same way as their mammalian counterparts [1]. This view has been substantiated for the Japanese toad *Bufo japonicus* by cDNA prediction [6] and for the European frog *Rana esculenta* by direct protein sequencing [7]. In particular a 'big' neurophysin encompassing sequences homologous to mammalian MSEL-neurophysin and copeptin has been identified in the frog [7]. This big neurophysin therefore resembles the intermediate neurophysin-copeptin precursor characterized in guinea pig [8,9]. On the other hand this protein displays close homology with the vasotocin-linked neurophysin of Japanese toad [6].

Whereas in mammals complete processing of the vasopressin precursor usually involves two enzymic cleavages at a dibasic and a monobasic site, respectively, in amphibians a single cleavage of the vasotocin precursor at the dibasic site gives the hormone and a fragment spanning neurophysin and copeptin. Conformation-limited proteolysis of the guinea pig intermediate precursor by immobilized trypsin has revealed cleavages only in the inter-domain region [9]. The frog big neurophysin has now been passed through a column of trypsin-Sepharose in order to compare it with the mammalian intermediate precursor and to check whether a change of conformation might ex-

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plain the lack of a second cleavage in the amphibian precursor processing.

2. MATERIALS AND METHODS

Frog big MSEL-neurophysin has been purified as previously described [7]. A column (0.3 × 25 cm) of trypsin-Sepharose 4B, prepared in order to contain 5 nmol of trypsin per ml [9], buffered with 0.05 M phosphate, pH 7.4, has been used. The passage of frog neurophysin (0.065 mg in 0.2 ml) was carried out for 3 min at room temperature (23°C). The effluent is concentrated to a small volume. Half has been used for control through high-pressure reverse-phase liquid chromatography [10]. The remainder has been subjected to microsequencing in an Applied Biosystems 470 A gas-phase microsequencer under conditions described [8,9]. Because complete sequence of the frog big neurophysin has been established [7], peptide bonds enzymatically cleaved in the protein could be located by determining new N-terminal sequences that appeared after contact with the proteolytic enzyme.

3. RESULTS AND DISCUSSION

Table 1 gives the results of the Edman degradation carried out in the sequencer on the tryptic products. Three amino acid sequences can be recognized. The first (7 residues) corresponds to the N-terminal sequence of the amphibian MSEL-neurophysin. The second sequence (7 residues) shows that cleavage has occurred at the Arg-94–Val-95 bond (fig.1). The third sequence (6 residues) reveals a cleavage at the level of the Arg-114–Leu-115 bond (fig.1). Of the 8 arginyl bonds present in amphibian neurophysin and susceptible to trypsin, only two have apparently been split by trypsin-Sepharose. These bonds are located in an inter-domain region.

Comparison of amino acid sequences between ox MSEL-neurophysin-copeptin and frog big MSEL-neurophysin does not reveal a clear variation explaining the difference in the processings. Substitution of one in the arginine pairs located in ante-penultimate position of the cleavage-specific residue has already been observed in rat [11] without modification in processing. A variation in conformation of the intermediate precursor may explain the lack of the second cleavage. Frog MSEL-neurophysin possesses two putative sites for glycosylation, one at position 100 (Asn-Met-Thr) and another at position 105 (Asn-Gly-Ser). Because the protein is bound to concanavalin A-Sepharose, at least one of them, probably Asn-105, is actually glycosylated. A change in location of the carbohydrate (in ox the carbohydrate is located 6 residues downstream the specific bond instead of 11 in frog) or in its structure might make a steric hindrance for the processing enzyme but not for trypsin. Another explanation for the lack of the second cleavage could be a difference in the specific processing enzyme or its absence.

It is of interest to note that neurophysins with apparent molecular masses of about 20 kDa have been detected in other amphibians such as *Rana pipiens* [12], *Rana temporaria* [13], *Bufo bufo* [14],

Table 1

Amino acid sequences of amphibian neurophysin fragments given by trypsin-Sepharose

Sequencing cycle	1st sequence			2nd sequence			3rd sequence		
	No.residue in the protein	Nature of the residue	pmol	No.residue in the protein	Nature of the residue	pmol	No.residue in the protein	Nature of the residue	pmol
1	1	Ser[a]	100	95	Val	358	115	Leu	300
2	2	Tyr	330	96	Ser[a]	de-tected	116	Met	180
3	3	Pro	(658)[b]	97	Pro	(658)[b]	117	His	100
4	4	Asp	(520)[b]	98	Asp	(520)[b]	118	Met	150
5	5	Thr	200	99	Gln	100	119	Ala	160
6	6	Glu	120	100	Asn	(119)[b]	120	Asn	(119)[b]
7	7	Val	250	101	Met	100			

[a] Ser derivative is unstable and value is low

[b] The same residue being present in two sequences (fig.1), the value given is cumulative

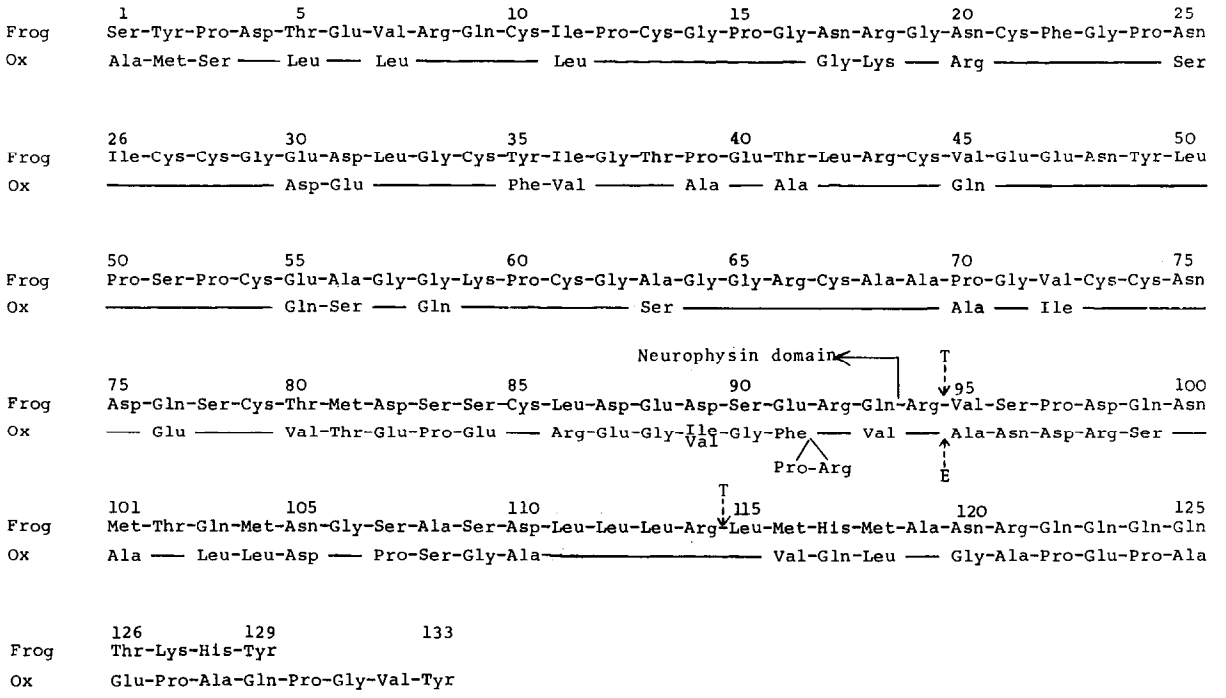

Fig.1. Comparison of amino acid sequences of amphibian big MSEL-neurophysin and bovine MSEL-neurophysin/copeptin. Frog numbering is used and a two-residue insertion (Pro-Arg) is assumed in ox protein for maximalizing homology. Residues identical to those of the frog protein are indicated by solid lines. T, trypsin-Sepharose cleavages in frog big neurophysin; E, processing endopeptidase cleavage in ox neurophysin/copeptin precursor.

Bufo ictericus [14] and *Xenopus laevis* [14,15] along with mesotocin and vasotocin [16–19]. The one-step processing therefore seems rather general in amphibians.

The transition of non-mammals to mammals is apparently accompanied not only by the replacement of vasotocin with vasopressin, which can be explained by a single mutation in the first codon of the mammalian gene [20–22], but also by the appearance of a second cleavage in the precursor, which could involve a variation in the third exon encoding the second processing site. Evolution of processing may be a composite phenomenon as is evolution of a precursor protein encoded by a three-exon gene [23].

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