PHYLOGENY OF THE NEUROPHYSINS: COMPLETE AMINO ACID SEQUENCE OF HORSE MSEL-NEUROPHYSIN

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1. Introduction

Neurophysins, the neurohypophysial hormone-binding proteins [1], can be classified in two types, MSEL-neurophysins and VLDV-neurophysins, according to the amino acid residues in positions 2, 3, 6 and 7 (ref. [2]). A recent review has been devoted to the molecular and cellular aspects of neurophysins, particularly to their interactions with neurohypophysial hormones [3].

The complete amino acid sequences of sheep [4,5], ox [4,5] and pig [5–7] MSEL-neurophysins have been previously published. A homologous protein has now been purified from horse posterior pituitary glands and the amino acid sequence has been determined. The comparison between species belonging to the Artiodactyla (sheep, ox, pig) and Perissodactyla (horse) orders shows that substitutions in this neurophysin family have occurred almost exclusively in the C-terminal part of the polypeptide chain.

2. Materials and methods

Purification of horse MSEL-neurophysin has been carried out under the conditions described for ox and sheep MSEL-neurophysins through isolation of the neurohypophysial hormone–neurophysin complex by fractionated salt precipitation [8], separation of 'crude' neurophysins by molecular sieving and chromatography on diethylaminoethyl-Sephadex A-50 [9,10]. The material appears homogeneous when subjected to polyacrylamide disc electrophoresis at pH 9.5 [11].

MSEL-neurophysin is oxidized by performic acid, split either by trypsin and or by *Staphylococcus aureus* protease ('staphylopeptidase') [12] and resulting peptides are separated by peptide mapping under conditions previously described [13]. Peptides are analyzed and partial or complete amino acid sequences were determined by manual Edman procedure [14]. On the other hand, the purified protein was reduced by dithiothreitol, alkylated with iodoacetamide [15], and subjected to automated degradation according to Edman and Begg [16] in a SOCOSI model P 110 sequencer. Phenylthiohydantoin amino acids were identified by thin-layer chromatography [17]

3. Results and discussion

Tryptic peptides (T_1 to T_8, fig.1) were recognized on the map by comparison with ox, sheep or pig tryptic maps previously determined [5]. The amino acid compositions and N-terminal sequences confirmed the homology. T_2, T_3, T_4, T_5 and T_6 are identical with those of ox MSEL-neurophysin. T_1 was present as two truncated forms T'_1 and T''_1 with 3 and 6 residues missing at the N-terminal end. These truncated forms were also observed for the other mammalian MSEL-neurophysins. T_7 showed two substitutions when compared with the ox homologous peptide, and T_8 one substitution. The alignment of tryptic peptides was determined through the sequencer (T_1 to T_3) or with overlapping staphylopeptidasic peptides (T_3 to T_8) (fig.1).

Staphylopeptidasic peptides (St_1 to St_6) were purified and analyzed in the same way. These overlapping peptides were partially or completely sequenced (fig.1); they proved the alignment of the tryptic peptides deduced by homology. Cleavage

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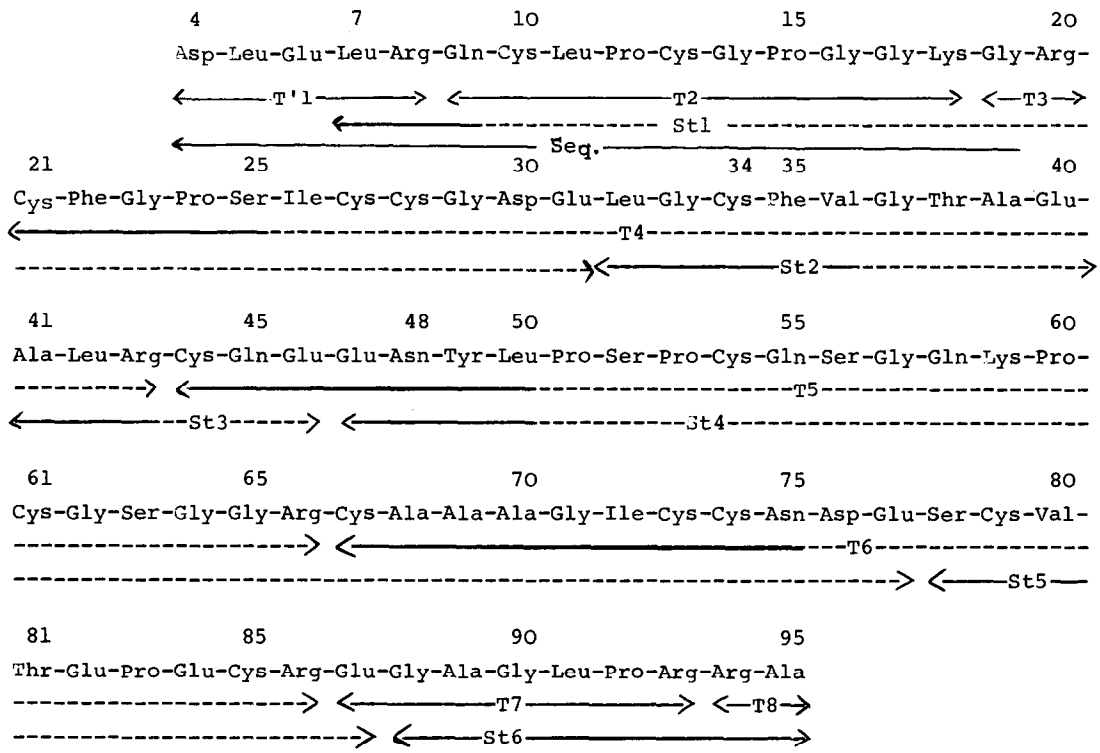

Fig.1. Alignment of tryptic (T) and *Staphylococcus* protease (St) peptides of horse MSEL-neurophysin. Solid line: amino acid sequence determined by manual Edman degradation. Dashed line: sequence deduced from amino acid composition and homology with sheep peptides. Seq: Sequence determined by automated Edman degradation. T'_1 referred to a putative truncated form of T_1, homologue of the sheep octapeptide T_1.

specificity of staphylopeptidase at the level of the glutamic acid carboxyl groups [12] could be confirmed (fig.1).

Figure 2 shows the amino acid sequences of sheep, ox, pig and horse MSEL-neurophysins. Horse neurophysin characterized in this work belongs to the MSEL-family because of the presence of aspartic acid and leucine in the positions 6 and 7 of the 95-residue frame typical for MSEL-neurophysins [5]. As found for the other MSEL-neurophysins, the 3-residue N-terminal deletion is likely to be the result of a degradative action. In the MSEL-family, substitutions appear mainly located in the C-terminal portion of the polypeptide chain. In position 48, an asparagine residue is found as in the ox or the pig, so that in species investigated, up to now, this position was only substituted in the sheep (isoleucine instead of asparagine) [4,5]. When compared with the sheep or the ox MSEL-neurophysins which are nearly identical, substitutions occur in positions 89 (alanine in place of isoleucine or valine), 91 (leucine in place of phenylalanine) and 95 (alanine in place of valine). It is of interest to note that Ala-89 and Ala-95 were previously found in pig MSEL-neurophysin [5–7].

Up to now complete amino acid sequences of four MSEL-neurophysins have been published, but in dog [18] and whale (unpublished results), this type of neurophysin has been recognized by the N-terminal sequence and it will probably be found in most mammals.

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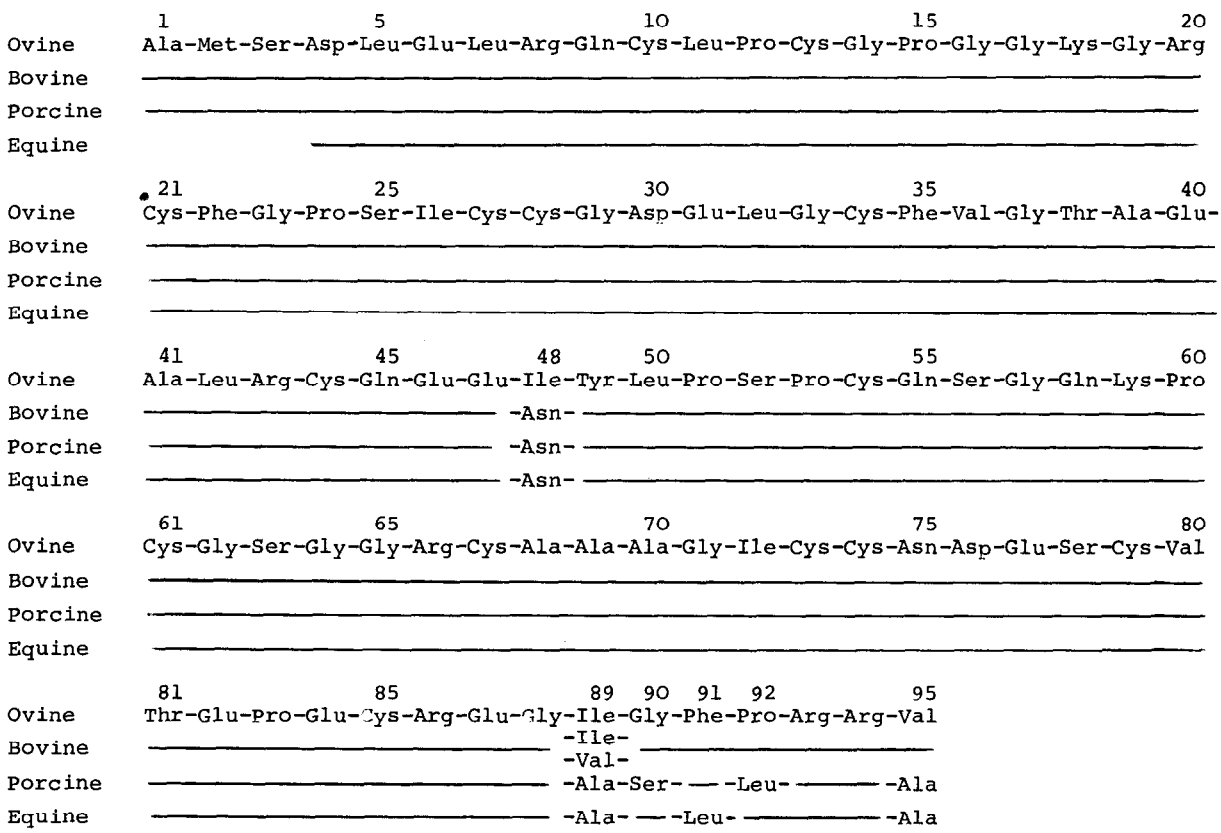

Fig.2. Comparison of sheep, ox, pig and horse MSEL-neurophysins. Sequences identical with that of ovine neurophysin are shown as solid lines.

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