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Molecular cloning and amino acid sequence of human enkephalinase (neutral endopeptidase)

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We have isolated a cDNA clone encoding human enkephalinase (neutral endopeptidase, EC 3.4.24.11) in a λgt10 library from human placenta, and present the complete 742 amino acid sequence of human enkephalinase. The human enzyme displays a high homology with rat and rabbit enkephalinase. Like the rat and rabbit enzyme, human enkephalinase contains a single N-terminal transmembrane region and is likely to be inserted through cell membranes with the majority of protein, including its carboxy-terminus, located extracellularly.

Enkephalinase; Neutral endopeptidase; Metallo peptidase; cDNA cloning; (Human)

1. INTRODUCTION

Enkephalinase (neutral endopeptidase, EC 3.4.24.11) is a membrane-bound zinc-metallo peptidase which cleaves the Gly^3-Phe^4 amide bond of the opioid peptides, enkephalins, both in vitro and in vivo [1-3]. Thus, acetorphan, a parenterally active enkephalinase inhibitor, was shown to display analgesic properties in humans [4,5]. Enkephalinase was initially identified in rodent brain, but it was soon realized that it is also present in many peripheral organs. In particular, its activity is highest in the kidney, where it was shown to be identical to an insulin B-chain degrading enzyme [6-8] identified several years before [9], the so-called neutral endopeptidase. We have recently cloned enkephalinase from both rat brain and kidney [10], further demonstrating the co-identity of the brain and kidney enzymes. In addition, enkephalinase has also been recently cloned from rabbit kidney [11].

Enkephalinase activity has been detected in several human tissues including brain [12], neutrophils [13,14], kidney [15] and placenta [16]. We now report the isolation and characterization of a full-length cDNA clone encoding human enkephalinase, constructed from placenta mRNA, and present a comparison of the human, rat and rabbit enzymes.

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2. MATERIALS AND METHODS

A partial rat enkephalinase cDNA clone was used to screen a human placenta cDNA library constructed from polyadenylated mRNA, in λgt10. This library had been previously used to clone the insulin receptor [17]. The 466 base pair *Eco*RI-*Bgl*II cDNA fragment of the rat clone λK3 [10] was labelled by random priming, and used to screen in duplicate 1.6×10^6 human placental cDNA clones. Hybridization was carried out at 37°C in 2 × SSC, 20% formamide, and filters were washed at 37°C in 0.5 × SSC, 0.1% SDS. Inserts of λ

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phage were subcloned in an M13 derivative and sequenced by the chain-termination method [18].

3. RESULTS AND DISCUSSION

Using a fragment of rat enkephalinase cDNA clone as a probe, we identified three positive clones in the human placenta library we screened. The largest clone, λH7, was sequenced. Its insert, 3181 base pairs in length, contains an open reading frame starting at an ATG initiation codon at base 21, with a TGA stop codon at base 2247 (fig.1). This reading frame encodes a 742 amino acid long polypeptide. One of the three cDNA clones we identified differs from clone λH7, with an A in place of a G at base 1413 (fig.1). An A base would change the corresponding codon from an Ala to a

Fig.1. Nucleotide and deduced amino acid sequence of human enkephalinase. The 8 amino acid stop-transfer sequence PKPKKKQR is indicated by a black bar, and the putative 23 amino acid transmembrane spanning domain is indicated by an open bar. Six potential *N*-linked glycosylation sites are shown by hatched bars. A potential poly(A) addition signal AATAAA is underlined.

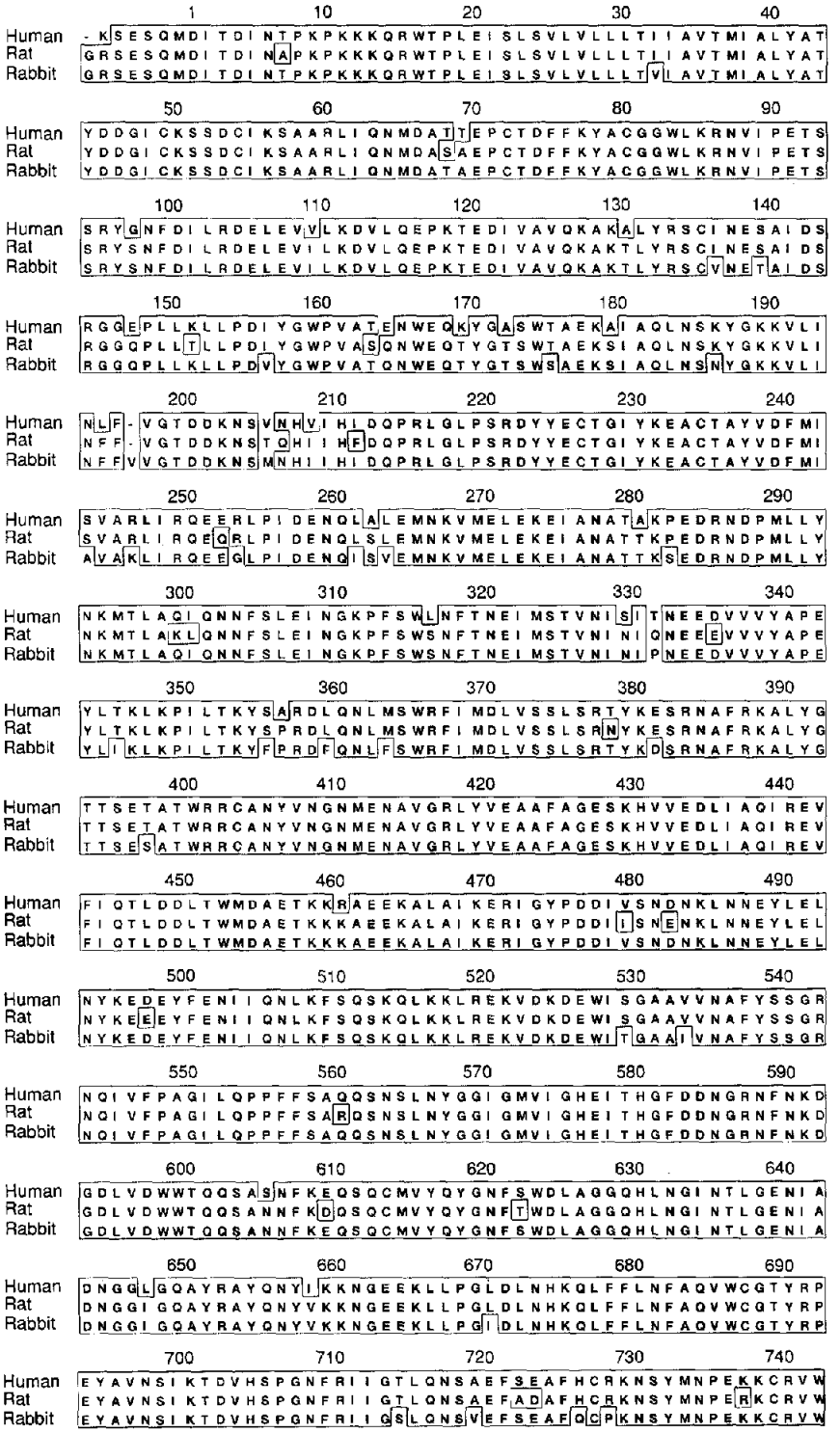

Fig.2. Comparison of the amino acid sequences of human, rat and rabbit enkephalinase. The amino acid sequences of human, rat and rabbit enkephalinase are shown in this order. Numbering of amino acids refers to the human and rat sequences.

Thr. Since both the rat and rabbit enkephalinases contain an Ala in this position (Ala^{465}, fig.2), the presence in this clone of an A is probably due to an error of reverse transcriptase of the mRNA.

The rat enkephalinase gene contains two potential ATG initiation codons, and amino-terminal protein sequence analysis has shown that the second is used [10]. Clone λH7 does not extend far enough to the 5′-end of the message to include the first ATG. Since neither of the other two clones we identified contained any additional 5′-sequence, we do not know if, like the rat, the human enkephalinase gene contains a first initiation codon that would be 4 bases upstream from the 5′-end of clone λH7. The amino-terminal sequence of human enkephalinase has not been established, but, by analogy with the rat protein, we propose that the ATG at base 21 initiates the translation of human enkephalinase, with an aspartyl residue at its amino-terminus. We have therefore numbered the amino acid sequence of human enkephalinase starting from this aspartyl residue. However, the first ATG of the rabbit enkephalinase gene is used for initiation [11], therefore this assignment must remain tentative.

Human enkephalinase displays a high degree of homology with the rat enzyme. Of the total of 742 amino acids, 700 (94%) are conserved and, of the 42 differences, only 6 are non-conservative changes (fig.2). The rabbit enzyme also shows high homology to rat and human enkephalinase (93%) but, while no insertions are needed to optimally align the rat and human sequences, the rabbit enzyme contains an additional amino acid, a valine residue after Phe^{196} (fig.2).

One other important difference between human, rat and rabbit enkephalinase concerns potential *N*-linked glycosylation sites. Both rat and human enkephalinase contain six potential glycosylation sites (Asn-X-Ser/Thr), while the rabbit enzyme has five. The site missing in the rabbit, involving Asn^{203}, is also missing in the human enzyme, but in the latter there is a sixth site, at Asn^{327} (fig.1). The significance of these differences is not clear. However, the fact that rat and human enkephalinase have been shown to be identical with respect to a number of properties, including optimal pH, sensitivity to inhibitors and kinetic parameters for the hydrolysis of a number of substrates [12,13,15,16], suggests that these differences do not affect the enzymatic activity of the enzyme.

Several important domains of enkephalinase are fully conserved between rat, rabbit and human proteins. The highly charged, conformationally restrained fragment PKPKKKQR (residues 8–15), which we proposed to serve as a stop transfer sequence [10], the hydrophobic region which follows this fragment (residues 21–43), which is likely to be a transmembrane spanning domain, as well as two fragments (residues 568–580 and 627–635), which have been proposed to serve as binding sites for the zinc atom of this metallo peptidase [11] are conserved in the three species. In addition, all cysteine residues present in the rat protein, which may be involved in disulfide bridges, are also found in the human and rabbit enzyme. These results suggest that enkephalinase is highly conserved among mammalian species.

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