Expression of two proopiomelanocortin genes in the pituitary gland of *Xenopus laevis*: complete structures of the two preprohormones

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ABSTRACT

A number of cDNA clones corresponding to *Xenopus* POMC mRNA was isolated from a cDNA library constructed from *Xenopus* pituitary polyadenylated RNA. Characterization of the cDNA inserts revealed two groups of structurally different proopiomelanocortin mRNAs, indicating that two proopiomelanocortin genes are expressed to virtually the same level in *Xenopus* pituitary glands. From the mRNA structures the complete amino acid sequences of the two *Xenopus* preproopiomelanocortins could be deduced. Comparison with proopiomelanocortin mRNA and protein sequences from other species shows regions of high homology (including the portion of the prohormone located N-terminally of γ -melanophore-stimulating hormone) and regions of extremely low homology (including the signal sequence).

INTRODUCTION

Proopiomelanocortin (POMC) is the precursor protein for a number of peptide hormones and neuropeptides that possess very diverse biological activities, e.g. melanophore-stimulating hormones (MSHs), the steroidogenic hormone adrenocorticotropin (ACTH) and the opiate peptide β -endorphin. Molecular cloning and sequencing of cDNAs or genomic DNA fragments have revealed the complete primary structures of some mammalian POMC molecules (1-4) and partial POMC structures for the salmon (5) and toad (6). Several observations have indicated that more than one form of POMC may occur in one species and even in one tissue (7-9). From biosynthetic studies with pituitary cells it appeared that in rat and mouse the multiple forms differ mainly in the degree of glycosylation rather than in their primary structures (10-11). Although in these two species the existence of two POMC genes has been reported (12-13), there is no evidence that both genes are transcribed. In fact, in mouse one of the two genes has been shown to be a pseudogene (13,14) and a recent study indicates that only one POMC gene is actually present in rat (15). For the salmon it was concluded from amino acid sequence analyses of POMC-derived peptides that two sequentially different POMC proteins should occur in this species (16-18). A similar con-

clusion has been made for the toad on the basis of biosynthetic studies (19). No conclusive evidence for the existence of two transcriptionally active genes coding for POMC or any other opioid peptide precursor has, however, been presented.

We now report the expression of two POMC genes in the pituitary gland of the South-African clawed toad, Xenopus laevis. From the nucleotide sequences of cDNA clones isolated from a pituitary cDNA library the complete amino acid sequences of two structurally different Xenopus POMC molecules could be deduced. A comparative analysis shows the presence of conserved and, thus, functionally significant regions within POMC.

MATERIALS AND METHODS

Screening of cDNA library

A cDNA library was constructed from Xenopus pituitary poly(A⁺)RNA as described (6). Using a synthetic oligomer corresponding to part of the α -MSH sequence as probe, hybridization-positive clone pXPL was isolated and characterized (6). In order to isolate more Xenopus POMC cDNA clones and 5'-extended cDNA clones of pXPL, a 186-bp BstN1-fragment of pXPL, covering the 5' end of pXPL, was isolated and used to rescreen the cDNA library. A total of 36 hybridization-positive cDNA clones was isolated and characterized by restriction mapping and partial or complete nucleotide sequence analysis. Among these was clone pXP20 (size of insert approximately 1150bp) which appeared to contain the nearly full-length nucleotide sequence of a Xenopus POMC gene transcript (transcript B) different from that coded for by pXPL. In addition, it was found that a 5'-extended clone of pXPL (clone pXP123, 430bp) which covered the 5' region of POMC gene transcript A was among the 36 clones. Despite further extensive screening of the library no 5'-extended clone of pXP20 could be isolated. It was, therefore, decided to screen a λ J1 Xenopus laevis genomic library (gift of Dr. I. Dawid, National Institutes of Health, Bethesda, Maryland, USA) with pXPL as hybridization probe and the exons containing the 5' end of POMC gene transcript B were identified with pXP123.

DNA sequence analysis

DNA sequences were determined with the dideoxysequencing method (20) after subcloning of the proper DNA fragments in M13 mp10/11 RF. More than 95% of the sequences presented was read from both strands and confirmed using different clones.

RESULTS AND DISCUSSION

From a cDNA library constructed from Xenopus pituitary mRNA 36 cDNA clones corresponding to Xenopus POMC mRNA were isolated. Upon partial or complete sequence analysis of eight of these cDNA clones and detailed restriction mapping of the remaining ones, it became clear that these clones could be classified into two groups, reflecting the existence of two different POMC mRNAs, designated POMC mRNA-A and mRNA-B. Of the 36 hybridization-positive cDNA clones, 17 (47%) corresponded to POMC mRNA-A and the remaining 19 (53%) were related to POMC mRNA-B. The nucleotide sequences of Xenopus POMC mRNA-A and -B, together with the deduced amino acid sequences of Xenopus POMC-A and -B are presented in Figure 1. The nucleotide sequence homology between the two transcripts is 91% and the amino acid sequence homology between the two proteins is 92%. Since Northern blot analysis showed one species of Xenopus POMC mRNA (6), the size difference between POMC mRNA-A and -B is not detectable on Northern blots. Although each of the two Xenopus POMC mRNAs contains two polyadenylation signals (AAUAAA, Figure 1), it appears that only one of these is used. The 3'-untranslated regions (354 and 360 nucleotides) of the Xenopus transcripts are larger than in mammals (100-172 nucleotides) but smaller than in salmon (898 nucleotides).

The Xenopus POMC-A and -B proteins consist of 259 and 260 amino acids, respectively, which is slightly less than human, bovine and porcine POMC (265-267 amino acids) but more than mouse and rat POMC (235 amino acids). The calculated molecular weights of Xenopus POMC-A and -B are 29844 and 29883, respectively. On the basis of comparison with mammalian POMCs (1-4), the signal sequence cleavage site can be tentatively assigned between residues 25 and 26 of the Xenopus preprohormones. Moreover, this cleavage site fulfills the (-3,-1) rule for signal peptidase recognition sites (21). Thus, the hydrophobic regions of 25 amino acids at the NH₂-terminus of the Xenopus precursor proteins likely comprise the signal peptides, giving rise to calculated molecular weights for the mature forms of Xenopus POMC-A and -B of 27014 and 27224, respectively. Each of the toad POMCs contains a potential site for asparagine-linked N-glycosylation (Asn-X-Ser/Thr; 22), namely Asn-residue 90, located within the γ -MSH region (Figure 1). Previous *in vitro* biosynthetic studies with neurointermediate lobes revealed that indeed both prohormones are glycosylated at this site (19). Gel electrophoretic analysis gave molecular weights of 38200 and 37000 daltons for these glycosylated Xenopus POMC molecules. A single potential glycosylation

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site, located in the γ -MSH region, is a characteristic of most mammalian POMC structures with the exception of mouse and rat which have an additional glycosylation site in the ACTH region (4,12). It is, thus, not surprising that we were unable to detect in Xenopus neurointermediate lobes newly synthesized glycosylated 13K ACTH, as found in mouse and rat pituitary cells (8,11). In the presence of tunicamycin, an antibiotic which blocks asparagine-linked glycosylation (23), the Xenopus tissue synthesizes non-glycosylated prohormones with apparent molecular weights of 35000 and 34200 (19), in contrast to the calculated molecular weights of 27014 and 27224. Such a difference between calculated and experimentally determined molecular weights has been previously noted for mammalian POMCs (1) and may be due to the relatively large number of charged amino acids in the POMC protein, resulting in an overestimation of the molecular weight by electrophoretic analysis (24).

The structural organizations of Xenopus POMC-A and -B are remarkably similar to those of their mammalian counterparts (1-4). As in mammals, the bioactive peptides in the toad prohormones are flanked on both sides by pairs of basic amino acids which may function as recognition sites for specific proteolytic cleavage enzymes. The fact that proteolytic cleavage of both Xenopus precursors yields α -MSH with an N-terminal alanine, and not, as in mammals, with an N-terminal serine, indicates that an N-terminally diacetylated form of this hormone can not occur in Xenopus. Such a diacetylated form of α -MSH has been found in most species examined (25-29), including an amphibian species (30). It is interesting to note that a recent study revealed that serine and alanine are in over 72% of the cases found as N-terminal amino acid of naturally occurring N-terminally acetylated peptides (31).

The availability of the complete nucleotide and amino acid sequences of the Xenopus POMC structures allows a comparative analysis of toad, mammalian and salmon POMC mRNAs and proteins. From the results of such an analysis, summarized in Table 1, the general picture arises that the portions of the molecules corresponding to the bioactive peptides are well conserved during vertebrate evolution. The regions between these portions (J1, J2 and J3 in Table 1), the 5' and 3' untranslated regions (5'UT and 3'UT, respectively), and the signal sequences (Pre), however, show a high rate of divergence, except for the J1-regions between the presequences and the sequences corresponding to γ -MSH. The degree of conservation of the J1-region is remarkable and could indicate a functional role for this frag-

TABLE 1

Amino acid (nucleotide) sequence homology between regions within a number of vertebrate proopiomelanocortins.

Species	Region within proopiomelanocortin												
	5'UT	Pre	J1	Y-MSH	J2	α -MSH	CLIP	ACTH	J3	β -MSH	β -end	β -LPH	3'UT
Xenopus/Human	(43)	23(36)	63(64)	58(60)	21(26)	93(81)	68(70)	77(74)	19(28)	78(67)	81(73)	56(53)	(<30)
Xenopus/Mouse	(33)	23(33)	60(58)	46(46)	12(20)	93(81)	64(67)	74(71)	15(20)	65(65)	87(74)	57(53)	(<30)
Xenopus/Salmon	-	-	-	-	-	75(75)	42(54)	57(62)	6(22)	65(67)	45(50)	35(43)	(<30)
Human/Mouse	(67)	69(82)	90(83)	81(78)	23(31)	100(100)	91(88)	95(91)	28(32)	78(85)	94(92)	64(66)	(<40)
Human/Salmon	-	-	-	-	-	81(77)	46(53)	62(63)	3(33)	67(69)	42(56)	34(51)	(<30)
Mouse/Salmon	-	-	-	-	-	81(77)	46(51)	62(63)	3(16)	53(71)	42(61)	30(45)	(<30)

The human, mouse and salmon sequences have been taken from references 2, 4 and 5, respectively. The Xenopus sequences correspond to POMC gene transcript A; similar percentages as those listed were found with the sequences corresponding to transcript B. Gaps were introduced to maximize homology. 5'(3')UT: 5'(3') untranslated region; Pre: signal sequence; J: spacer regions between bioactive domains; CLIP: corticotropin-like intermediate lobe peptide; β -end: β -endorphin; β -LPH: β -lipotropin.

ment since, in general, the extent of sequence conservation exhibited by different regions within a protein molecule is directly related to the functional importance of each region (32). Indeed, a biological function, namely adrenal mitogenic activity, has been proposed for this fragment (33). The fact that the J2- and J3-regions of the prohormone are very poorly conserved would indicate a structural rather than a biological role for these regions; the peptide corresponding to the J2-region was named "joining" peptide and has been reported to be present in rat and human pituitary (27,34). The low degree of conservation of the prepeptides of mammalian and toad POMC molecules reinforces the general concept of high variability of signal sequences (35).

In conclusion, our cloning and characterization of cDNAs corresponding to two Xenopus POMC mRNAs shows that the biosynthesis of two POMC proteins in the pituitary gland reflects the translational products of two separate transcripts derived from two POMC genes. The occurrence of the two POMC genes might well be the consequence of a relatively recent genome duplication in the genus Xenopus, which has been postulated on the basis of chromosomal studies (36,37). From the ratio between POMC gene transcripts A and B it can be concluded that the level of transcription of the two Xenopus POMC genes is virtually the same. The presence of two POMC genes which are transcriptionally active in the pituitary gland of Xenopus laevis could be advantageous to the animal since in the pars intermedia of the pituitary gland proteolytic cleavage of POMC yields α -MSH, a hormone with an important physiological function in Xenopus, namely mediating the process of background adaptation by causing dispersion of pigment granules in dermal melanophores.

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