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Cloning of stanniocalcin (STC) cDNAs of divergent teleost species: Monomeric STC supports monophyly of the ancient teleosts, the osteoglossomorphs

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Abstract

Molecular cloning of teleost stanniocalcin (STC) cDNAs was undertaken in two species of order Osteoglossiformes of subdivision Osteoglossomorpha and one species of each of orders Cypriniformes and Perciformes within the subdivision Euteleostei. The elephant-nose (*Gnathonemus petersii*) and the butterflyfish (*Pantadon buchholzi*) are basal teleosts in different osteoglossiforme suborders yet their 218 amino acid (aa) mature hormones, from prehormones of 249 and 251 aa, respectively, have only 10 cysteine residues. A substitution for cysteine at the intermonomeric disulfide linkage site, implies that their STCs exist as monomeric peptides, as is the case with STC from another osteoglossormorph, arawana [Amemiya, Y., Marra, L.E., Reyhani, N., Youson, J.H., 2002. Stanniocalcin from an ancient teleost: a monomeric form of the hormone and a possible extracorpuscular distribution. Mol. Cell. Endocrinol. 188, 141–150]. The STC cDNA of the generalized teleost and cyprinid, the white sucker (*Catostomus commersoni*), encodes a prehormone of 249 aa with a signal peptide of 31 aa and a mature protein of 218 aa that possesses 11 cysteine residues. The latter feature is consistent with a previous analysis that white sucker mature STC is a glycosylated, homodimeric peptide [Amemiya, Y., Marra, L.E., Reyhani, N., Youson, J.H., 2002. Stanniocalcin from an ancient teleost: a monomeric form of the hormone and a possible extracorpuscular distribution. Mol. Cell. Endocrinol. 188, 141–150]. An open reading frame of the STC cDNA of the derived teleost and perciforme, the smallmouth bass (*Micropterus dolomieui*), encodes a prehormone of 255 aa with a signal peptide of 33 aa and a mature protein of 222 aa. The position of the 11 cysteines in smallmouth bass STC suggests that it exists as a homodimeric peptide. A phylogenetic analysis, using the new STC-1 amino acid sequences and those in the gene data base provided strong support for monophyly of the Osteoglossomorpha and indicated, with positioning of white sucker and smallmouth bass, that this molecule has some utility as a taxonomic marker. This analysis also suggested that two STC-1 gene sequences exist in multiple fish genomes, and that they may be a product of the fish-specific genome duplication. The mutation in the osteoglossomorph STC likely occurred after the appearance of the first teleosts and before movement of the tectonic plates.

Keywords: Stanniocalcin; cDNA cloning; Monomer; Homodimer; Teleost; Phylogeny; Osteoglossiforme monophyly

1. Introduction

Stanniocalcin (STC) is a hormone, first described in Neopterygii of the actinopterygian (ray-finned) fishes and most recently in mammals (Wagner, 1994; Chang et al., 1995; Wagner et al., 1995). In fishes, STC is a product of the kidney-associated, corpuscles of Stannius (CS) but expression of the STC gene occurs within several extracorpuscular tissues of a number of species (McCudden et al., 2001; Amemiya et al., 2002; Amemiya and Youson, 2004). In mammals, there are no corpuscles of Stannius but STC mRNA expression is present in many tissues and organs (Chang et al., 1995, 1996; Wagner et al., 1995).

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In most of the ray-finned fishes, and in all mammals, the principal STC (or STC-1) is a glycosylated, homodimeric protein. Usually there are 11 cysteine residues located in the same position in a preSTC of approximately 250 aa of fish and mammals (Hulova and Kawauchi, 1999). The first 10 cysteines are involved in establishing intramonomeric disulfide linkages whereas the cysteine at position 169 serves as the site of disulfide linkage of the two monomers. The exceptions to this rule seem to be rodent and human STC-2 (with serine replacing cysteine) and STC from arawana, a basal teleost from the order Osteoglossiformes (Chang and Reddel, 1998; Amemiya et al., 2002). In the latter case, arginine replaces cysteine at position 169; Western blot analysis under nonreducing and reducing conditions confirmed the monomeric nature of arawana STC (Amemiya et al., 2002). Since the bonytongues, the osteoglossomorphs, are considered among the most basal (closest to the ancestral line) teleosts, this finding of a mutation in STC was interpreted as potentially representing an ancestral condition. The question arose as to the nature of the STC molecule in extant non-teleost, actinopterygian fishes that have some connection with ancestors from which the modern teleosts were believed to have been derived. However, an examination of the primary structure of STC in both the bowfin (order Amiiformes) and the gar (order Semiontiformes) revealed STCs that were glycosylated homodimeric proteins with 87% amino acid sequence identity with one another and only 65% with STC from arawana (Amemiya and Youson, 2004). These data suggested that STC-1 among neopterygian fishes has been highly conserved during their evolution and that monomeric STC in arawana is an anomaly.

Order Osteoglossiformes is comprised of a number of strictly, freshwater species that are present in either Africa, Southeast Asia, Northern Australia or South America (Nelson, 1994) and have an ancient history. The osteoglossomorphs are interesting from the point of view that they may have all been originally connected to one another in the Late Jurassic before their radiation and distribution following continental movements (Li and Wilson, 1996). Despite the fact that fossil evidence implies some common origins of various subgroups with this order, there are still some questions about monophyly among the two Suborders, the Osteoglossoidei and the Notopteroidei. Our recent evidence from cDNAs and deduced amino acid analyses of preproinsulin and preprosomatostatins from osteoglossomorph species provides support for monophyly within the order (Al-Mahrouki et al., 2001; Youson et al., in press).

The present study was undertaken to see: (1) whether monomeric STC of arawana is an anomaly or whether it is characteristic of osteoglossomorphs; (2) whether STC structure has value in the question of osteglossomorph monophyly or in any phylogenetic analysis. This study was carried out through the cloning of STC cDNAs of the butterflyfish (*Pantodon buchholzi*), representing, like arawana, a member of suborder Osteoglossoidei, and of the elephantnose (*Gnathonemus petersii*), a member of suborder Notopteroidei. In addition, to expand the view of STC structure among teleosts, and in particular to search for other examples of a monomeric molecule, we provide the full length STC cDNAs of the white sucker (*Catostomus commersoni*), a cyprinid and a general teleost, and of the smallmouth bass (*Micropterus dolomieui*), a representative of the more derived teleosts, the Perciformes.

2. Materials and methods

2.1. Materials

Pantodon buchholzi (butterflyfish), and *G. petersii* (elephantnose) were purchased from a local commercial aquarium in Toronto. *C. commersoni* (white sucker) and *M. dolomieui* (smallmouth bass) were captured from the Lake Ontario watershed. The live fishes were sacrificed in their holding water using an overdose of 0.05% (W/V) tricaine methanesulfonate (MS-222). The corpuscles of Stannius (CS) were quickly excised, immediately frozen in liquid nitrogen, and then were stored at −80 °C. Total RNA was extracted from the frozen tissues with TRIzol Reagent (Gibco). All procedures were carried out in accordance with the manufacturer's manuals. The yields of RNA were estimated spectrophotometrically by absorbance at 260 nm (A_{260}) and the purity was determined from the ration of A_{260}/A_{280}.

2.2. PCR amplification

2.2.1. 3′-Partial cDNA clone

Single-strand cDNA was reverse transcribed from 1 μg of total RNA at 42 °C for 60 min using a SMART RACE cDNA Amplification Kit (Clonetech) and Moloney murine leukemia virus reverse transcriptase. A STC universal sense primer (STC-A: 5′-TGCCTGGAGAACTCCAC CTGCGACACNGAYGGNATG-3′) was designed on the basis of conserved amino acid sequences (CLENSTCDTDGM) among the known fish STCs. The primers were designed using the CODEHOP program (www.blocks.fhcrc.org/blocks/help/CODEHOP) and were synthesized by Sigma Genosys (Oakville, Canada). Using STC-A and an anchor primer provided in the kit, 3′ region of the STC cDNAs were amplified with 1 μl of the cDNA from individual species, 25 pmol of each primers, and 2.5 U of HotStarTaq DNA polymerase (Qiagen) in 50 μl volume of *Taq* reaction buffer by PCR. The reactions were carried out after denaturation at 94 °C for 2 min for 30 cycles (1 min at 94 °C, 30 s at 50 °C, 1 min 30 s at 72 °C) and a final extension for 5 min at 72 °C. The sample was rapidly cooled to 4 °C and analyzed on an agarose gel.

2.2.2. 5′-Partial cDNA clone

Based on the nucleotide sequence of the 3′ region of individual species STC cDNA, the gene specific antisense primers (butterflyfish STC: 5′-TGTTAGGGAA GGTACTGGGCAGCTGG-3′; elephantnose STC: 5′-CTCTGCAACAGTGTGCTGTAATGCC TGTTG-3′; white sucker STC: 5′-CCACGTCCCCAATGGCCTCAGGATTGG-3′ and smallmouth bass STC: 5′-CACCTCAGCGATCATCCTCTGGAAGG-3′) were synthesized. These primers ("STC-B" in Fig. 1) and anchor primer provided in the kit were used to amplify full-length clones of the cDNAs. All PCR conditions were the same as those described above.

2.2.3. Full-length cDNA clone

Gene specific sense primers ("STC-C" in Fig. 1) designed from the 5′-partial cDNA clones of individual species (butterflyfish STC: 5′-GACACGCTTCACCTA TAGACAGGTGGA-3′; elephantnose STC: 5′-GGACATCAAACGGAAACCACAGGAAGC-3′; white sucker STC: 5′-GCTCAGACAAGCAGCAGAATCAGC-3′ and smallmouth bass STC: 5′-ACAGGCAGCAGGA CCAGCAGGAGGAC-3′) and anchor primer provided in the kit were used to amplify full length of the cDNAs.