

**DIVERSITY OF THE MOLECULAR STRUCTURE OF
STANNIOCALCIN IN THE ACTINOPTERYGIANS**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

For some time, stanniocalcin (STC) has been described as a homodimeric glycoprotein that is involved in calcium and phosphate regulation. This hormone was first discovered within small kidney-associated endocrine glands, referred to as the corpuscles of Stannius (CS), that occur only in certain rayfined fishes (Actinopterygii), the holostean and teleostean fishes. Recently, it was revealed that several mammalian tissues also contain STC and that STC mRNA expression is also present in many tissues and organs. Similar to the function in fish, mammalian STC plays a role in mineral metabolism. Primary structures of fish STC have been determined only in basal and generalized teleosts, such as several salmonid species and the Australian eel. Knowledge of the primary structure of STC in both more ancient and more derived actinopterygians would help in our understanding of the evolution of this molecule. At the same time, sequence comparison of STC could assist in our view of the taxonomic relationship of the various groups of fishes. The present report describes recent data that are part of a larger investigation into the molecular evolution of STC among the actinopterygians.

The bowfin (*Amia calva*) is one of two extant holostean species with ancient ancestral links to modern-day teleosts. The bowfin provides us with the opportunity to study STC structure in the most ancient extant species to possess CS. The CS of the bowfin are abundant (several hundred) and scattered throughout much of the kidney, while in the teleostean fishes, the number of CS is low (2-5) and localized (Youson et al., 1976). Osteoglossiformes (bonytongues) is among the most ancient of the teleostean orders. The Osteoglossiformes are believed to have developed in a common

geographical region prior to the separation of the tectonic plates (Li and Wilson, 1996). Currently, the geographical distribution of the species is very diverse. Several osteoglossiforme species of interest to the present study are the elephantnose (*Gnathonemus petersii*) and the butterflyfish (*Pantodon buchholzi*) from Africa, and the silver arawana (*Osteoglossum bicirrhosum*) from South America. The relationship of species within the Osteoglossiformes and the connections of this order to other teleosts are not completely clear (Li and Wilson, 1996). An examination of the STC of these species allows for an evolutionary and comparative analysis of the taxonomic relationships of the individual Osteoglossiformes and with other teleosts. In the present study, we cloned STC cDNAs and compared deduced amino acid sequences from bowfin (holostean) and three species of Osteoglossiformes, elephantnose, butterflyfish and arawana, to obtain further insight into the molecular evolution of the STC gene.

Salmonid and Australian eel STCs and mammalian STC-1 have eleven cysteine residues located in an identical position. High numbers of cysteines provide greater potential for intra- and inter-monomeric disulfide linkages. For example, in chum salmon, five intra-monomeric disulfide linkages exist. One cysteine, which is located closest to the C-terminus of the protein, is unpaired and is involved in the formation of stanniocalcin homodimers (Hulova et al., 1999). The present study revealed that bowfin STC consists of eleven cysteine residues like that of other vertebrate STCs. The bowfin STC shows the highest amino acid sequence identity with the Australian eel (69%), compared to the coho salmon (65%), Osteoglossiformes (59-61%), and mouse STC-1 (56%). Since bowfins are the most ancient of vertebrates known to possess CS and STC, these data suggest that the structure of STC has been well conserved throughout vertebrate evolution. However, the STCs from the three Osteoglossiformes contain only ten cysteine residues because one cysteine is replaced by either arginine or histidine (Fig.1).

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      bowfin: 151 SRLGPMSMLFQLLQSKPCPASSASSTSSA 190
Australian eel: 151 ---E---GV-----T-AC-P-A-GG-GPV 190
      coho salmon: 151 A----D-ET-----N-HC-QG-NQGPN-- 190
      mouse-1: 151 EKI--N-AS--HI--TDHCAQTHPRADFNR 190
silver arawana: 151 A-----VA---K----SSRSG-A-QASNND 190
      elephantnose: 149 -----VA---K----S-RS-PDS-QNKVE 188
      butterflyfish: 151 -----AL--K----S-HT-P-DPALLG- 190

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Fig.1. Sequence comparison of a common C-terminal region in STCs of various fishes and the mouse. Note the cysteine (C) that is critical for the homodimeric molecular STC (box) is substituted (in bold)

type) by either arginine (R) or histidine (H) in the these Osteoglossiforme species. An hyphen (-) represents an identical amino acid to bowfin STC.

The cysteine substitution occurs at the site of inter-monomeric disulfide linkage. Western blot analysis of arawana CS extract with anti-salmon STC revealed a single 21 kDa band under non-reducing conditions, and a single band of 25 kDa under reducing conditions. These data indicate that arawana STC exists as a monomeric peptide but the nature of the STC peptide from the other Osteoglossiformes needs to be confirmed. The STC moleculars from the osteoglossiforme species that have been studied to date seem to be unique among known vertebrate STCs. It remains to be determined whether the monomeric form of STC as describe herein introduces any significant change in known biological activity of CS-generated peptides. Furthermore, in order to obtain further insight into the molecular evolution of STC in actinopterygians, it is important to characterize STC genes from the gar (holostean, order Semionotiformes), and other species of Osteoglossiformes such as mooneye or goldeye from North America.

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References

- Hulova, I. and Kawauchi, H. 1999. Assignment of disulfide linkages in chum salmon stanniocalcin. *Biochem. Biophys. Res. Commun.* 257: 295-299.
- Li, G-Q. and Wilson, M. V. H. 1996. Phylogeny of Osteoglossomorpha. In: *Interrelationships of fishes* (eds. Stiassny, M. L. J., Parenti, L. R. and Johnson, G. D). Academic Press, San Diego, pp163-173.
- Youson J. H., Butler D. G. and Chan, A. T. C. 1976. Identification and distribution of the adrenocortical homolog, chromaffin tissue and the corpuscles of Stannius in *Amia calva* L. *Gen. Comp. Endocrinol.* 29:198-211.

