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Molecular Cloning and Sequence Analysis of the cDNAs for Pituitary Glycoprotein Hormone α Subunits from Two Species of Synbranchiformes, *Monopterus albus* and *Ophisternon bengalense*

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Abstract

No information is available regarding the cDNA nucleotide and protein sequences of pituitary glycoprotein hormone α subunits (PGH α) from fish of the order Synbranchiformes. For better understanding of phylogenetic diversity and evolution of PGH α in fish, we have cloned cDNAs for PGH α subunits from swamp eels, *Monopterus albus* and *Ophisternon bengalense*, two members of the order Synbranchiformes, suborder Synbranchoidei, family Synbranchidae. The PGH α subunit cDNA was cloned by reverse transcription and polymerase chain reaction amplification from total pituitary RNA. The full length PGH α cDNA was obtained using 5′- and 3′- rapid amplification of cDNA ends (RACE). The PGH α of these two species possessed 354 bp of coding region, which encoding a protein of 117 amino acids consisting of a putative signal peptide of 23 amino acids and a mature peptide of 94 amino acids. The amino acid sequence identity of PGH α between the two species is 93.8%. All 10 cysteine residues, forming 5 disulfide linkage, and 2 putative N-linked glycosylation sites are conserved in the PGH α subunits of the two species. Three proline residues, presumably responsible for changing the backbone directions of the protein structure, are conserved as well. Phylogenetic analysis of PGH α subunits based on their amino acid sequences revealed that the percent identities of the swamp eels are highest (92.5%) with fishes of Perciformes, intermediate with Pleuronectiformes (82.5%) and Cyprinodontiformes (72.2%), and lowest with Salmoniformes, Cypriniformes, Siluriformes, Anguilliformes, Acipenseriformes and Ceratodontiformes (56.7%–65.1%).

Introduction

The pituitary luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH) are members of glycoprotein family. Each hormone is a heterodimer molecule, consisting of α and β subunits. The α subunit is common to all glycoprotein hormones and its main role is to confer biological action through the signal transduction pathway; whereas β subunit is specific to each hormone and determines the hormonal activity and species specificity (Pierce and Parsons 1981; Ryan et al. 1987). The α and β subunits are initially synthesized as separate glycoproteins from different genes, but are associated in cytoplasm by non-covalent binding to form bioactive dimmer molecules (Naylor et al. 1983; Gharib et al. 1990).

Fish is the largest class in vertebrates, consisting of more than 24 600 living species with valid scientific descriptions in 482 families and 57 orders (Nelson 1994). The pituitary glycoprotein hormone α (PGH α) that have been cloned or chemically sequenced are about 31 species from 9 orders (see references indicated in Table 1). These studies demonstrated that the homologies of amino acid sequences of the PGH α subunits are much greater for species from the same

orders than from different orders, although 10 cysteine residues, forming 5 disulfide linkage, and 2 putative N-linked glycosylation sites are conserved. The variation of amino acid sequences of PGH α subunits among different orders of fishes depends on their phylogenetic relationships. For example, the amino acid sequence identity between Perciformes (N=7) and Pleuronectiformes (N=2) is averaged 82.5%, while between Perciformes and Anguilliformes (N=3) is 59.1%.

For better understanding of the phylogenetic diversity and evolution of the pituitary α subunit, more studies on species representing different orders are needed. To our knowledge, no information has been available in cDNA nucleotides and deduced amino acid sequences of the PGH α from species of the order Synbranchiformes. The members of Synbranchiformes, inhabit mainly in tropical and subtropical freshwater/swamp, thus deriving its common name as swamp eels (Gosline 1983; Travers 1984a, b). The species of this order possess common characters such as: eel-like elongate body without pelvic fins; regressed pectoral fins (totally disappeared in Synbranchoidei); the gill opening confined to lower half of head; enlarged ectopterygoid; reduced or absent mesopterygoid; nonprotrusible premaxillary without ascending process; ribs and swim bladder absent (Johnson and Peterson 1993; Nelson 1994). We therefore have cloned the PGH α cDNA from two swamp eels, *Monopterus albus* Zouiev (Berg 1949) and *Ophisternon bengalense* McClelland (Rosen and Greenwood 1976), of the order Synbranchiformes. We report here the cDNA nucleotides and deduced amino acid sequences of their PGH α subunits.

Materials and methods

Animals

Two species of swamp eels, *Monopterus albus* and *Ophisternon bengalense*, were obtained from a local dealer in Taipei, Taiwan. *Monopterus albus*, total length of 30–50 cm from both Taiwan and southern China, was investigated separately for comparison. *Ophisternon bengalense*, total length of 50–70 cm, was imported from Bengal. The animals were stunned by ice, and the pituitary glands were removed and immediately frozen in liquid nitrogen. Samples were then stored at -120 °C until analysis.

Designs of oligonucleotides

Oligonucleotides, used as PCR primers for amplification of the PGH α subunit cDNA in two swamp eels are listed below and shown in Figure 1. The sense primer (SP: 5′→3′) and antisense primer (ASP: 3′→5′) were designed from the conserved region of PGH α of yellow-fin porgy and striped bass deposited in the GenBank.

Primer 1. SP for PGH α subunit: 5′-CACGATGGGCT CAGTGAAATC-3′.
Primer 2. ASP for PGH α subunit: 5′-GCAGTGGCA-GTCTGTGTGGTT-3′.
Primer 3. Gene specific primer (GSP) of PGH α subunit of *Monopterus albus* for 3′RACE: 5′-GCACAGCTATGAGACAGAGG-3′.
Primer 4. PGH α subunit GSP of *Ophisternon bengalense* for 3′-RACE: 5′-GCTTCTCCAGAGCGTA CCCAACGCC-3′.
Primer 5. PGH α subunit GSP of the two swamp eels for 5′-RACE: 5′-TGAAAATAGCAGGTGCTGCAG TG-3′.
Primer 6. PGH α subunit GSP of *Monopterus albus* for 5′-RACE: 5′-TTAGCAACACAGCATGTTGCCTC-3′.
Primer 7. PGH α subunit GSP of *Ophisternon bengalense* for 5′-RACE: 5′-CACCTCTGTCTCGTAGC TGTGC-3′.
Primer 8. Adapter primer (AP) for 3′-RACE: 5′-GGCCACGCGTCGACTAGTACTTTTTTTTTTTT TTTT-3′.
Primer 9. Abridged universal amplification primer (AUAP) for 3′-RACE: 5′-GGCCACGCGTCGACTA GTAC-3′.
Primer 10. Abridged anchor primer (AAP) for 5′-RACE: 5′-GGCCACGCGTCGACTAGTACGGG I IGGGI IGGG I IG-3′, where I is the base inosine.

Total RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from pituitary glands using the total RNA miniprep system kit (Viogene, Sunnyval, CA, USA). The concentration and purity of the extracted RNA were measured at A260nm/A280nm (Kontron Spectrophotometer, UVIKON 810). cDNA was synthesized from 1 μg total RNA with oligo- d $(T)_{18}$ primer (100 ng) by employing the first strand cDNA synthesis kit (Stratagen, CA, USA). Reverse transcription was performed with moloney murine leukemia virus reverse transcriptase (MMLV-RT) (Stratagene) for 15 min at 37 °C. PCR

Table 1. Fish species used in sequence analysis of glycoprotein α subunits

Taxa name	Abbreviation	Accession No.	Reference
Synbranchiformes			
Monopterus albus	albus swamp eel	AF502395	This study
Ophisternon bengalense	Bengalense swamp eel	AF502394	This study
Perciformes			
Thunnus obesus	tuna	P37204	Okada et al. 1994
Dicentrarchus labrax	European sea bass	AF269157	Mateos et al., unpublished
Acanthopagrus latus	yellowfin porgy	M94038	Tsai and Chen 1993, unpublished
Chrysophrys major	red seabream	AB028211	Gen et al. 2000
Sparus aurata	gilthead seabream	AF300425	Meiri et al. 2000, unpublished
Morone saxatilis	striped bass	L35071	Hassin et al. 1995
Tilapia mossambica	tilapia	AF303087	Gur et al. 2000, unpublished
Pleuronectiformes			
Hippoglossus hippoglossus	Atlantic halibut	AJ417770	Weltzien et al., unpublished
Paralichtys olivaceus	Bastard halibut	AF268692	Lee and Kim, unpublished
Cyprinodontiformes			
Fundulus heteroclitus	killifish	U12923	Lin et al. 1994, unpublished
Salmoniformes			
Oncorhynchus tshawytsha	chinook salmon	S77059	Suzuki et al. 1995
Oncorhynchus masou $\alpha 1$	masu salmon	S69273	Gen et al. 1993
Oncorhynchus masou $\alpha 2$	masu salmon	S69274	Gen et al. 1993
Oncorhynchus keta $\alpha 1$	chum salmon	M27652	Kitahara et al. 1988
Oncorhynchus keta $\alpha 2$	chum salmon	M27653	Kitahara et al. 1988
Oncorhynchus kisutch $\alpha 1$	coho salmon	–	Dickey and Swanson 2000
Oncorhynchus kisutch $\alpha 2$	coho salmon	–	Dickey and Swanson 2000
Oncorhynchus mykiss	rainbow trout	AB050834	Morita et al. 2000, unpublished
Cypriniformes			
Ctenopharyngodon idella	grass carp	X61050	Huang 1991, unpublished
Hypophthalmichthys molitrix	silver carp	P37037	Chang et al. 1990
Cyprinus carpio $\alpha 1$	common carp	M37379	Chang et al. 1988
Cyprinus carpio $\alpha 2$	common carp	M37380	Chang et al. 1988
Carassius auratus $\alpha 1$	goldfish	D86551	Kobayashi et al. 1997
Carassius auratus $\alpha 2$	goldfish	D86552	Kobayashi et al. 1997
Siluriformes			
Clarias gariepinus	African catfish	X97760	Rebers et al. 1997
Ictalurus punctatus	channel catfish	AF112190	Liu et al. 1997
Anguilliformes			
Muraenesox cinereus	pike eel	P12836	Liu et al. 1989
Anguilla japonica	Japanese eel	–	Nagae et al. 1996
Anguilla anguilla	European eel	X61038	Quérat et al. 1990
Acipenseriformes			
Acipenser baerii	Siberian sturgeon	AJ310342	Quérat et al. 2000
Ceratodontiformes			
Neoceratodus forsteri	Australian lungfish	AB050093	Arai et al. 1998

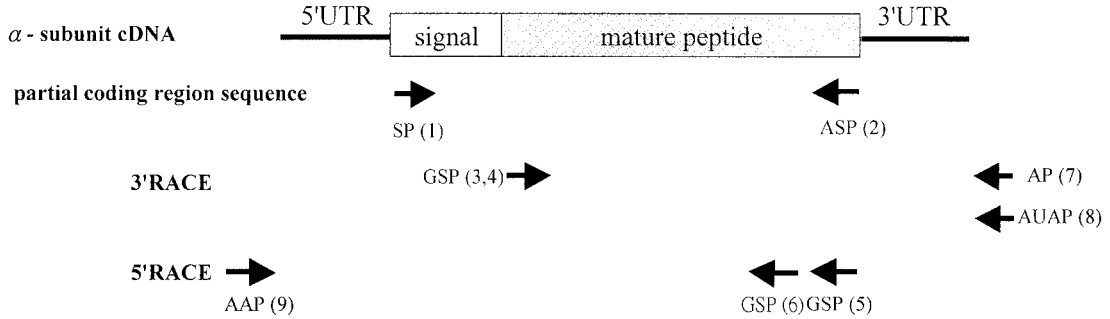

Figure 1. Procedures of RT-PCR sequencing of PGH α subunit cDNAs from pituitary glands of *Monopterus albus* and *Ophisternon bengalense*. Numbers in the parentheses indicate the corresponding oligonucleotides listed in Materials and methods; open box indicates the signal peptide; gray box indicates the mature protein.

was performed with primers 1 and 2 in 50 μl final volume containing 1 × reaction buffer, 1.5 mM $MgCl_2$, 200 μM dNTP, 100 ng of each primer, and 2.5 U Taq DNA polymerase (Gibco BRL, MD, USA). After an initial 2 min denaturing step at 94 °C, 35 cycles of amplification were performed using a cycle profile of 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min. Elongation was extended to 8 min at 72 °C after the last cycle. PCR products were then sequenced by a local company (Mission Biotech, Taipei, Taiwan).

Rapid Amplification of cDNA ends (RACE)

3′-RACE was performed using the 3′-RACE kit (Gibco BRL, MD, USA) according to the manufacturer's instructions. Briefly, 1 μg of the pituitary total RNA was reverse- transcribed using 10 pmol adapter primer 8 (AP) by 200 U of Superscript II reverse transcriptase (Gibco BRL), followed by PCR between the primer pairs (primers 3 and 9 for PGH α cDNA of *Monopterus albus*, primers 4 and 9 for *Ophisternon bengalense*). PCR procedures were described as above.

5′-RACE was performed using the 5′-RACE kit (Gibco BRL) according to the manufacturer's instructions. Briefly, 1 μg of the pituitary total RNA was reverse- transcribed by 200 U of Superscript II reverse transcriptase (Gibco BRL) with first GSP (primer 5). The cDNA was column purified and then oligo-dC tailed using terminal deoxynucleotidyl transferase (Gibco BRL). PCR was performed using the Abridged anchor primer 10 (AAP) and the second GSP (*Monopterus albus*: primer 6; *Ophisternon bengalense*: primer 7). PCR procedures were the same as described above.

Phylogenetic analysis

The two PGH α subunit cDNA sequences of the swamp eels obtained in this study, together with other 31 PGH α subunit mature peptide sequences from 9 fish orders, retrieved from the GenBank/EMBL or published papers, were included for phylogenetic analysis (Table 1). The mature peptides sequences of PGH α subunit were aligned using the Clustal W (Thompson et al. 1994) network service of the European Bioinformatics Institute of EMBL. The phylogenetic tree of PGH α subunits was constructed by Neighbor-Joining (NJ) method, and rooted with an Australian lungfish as the outgroup using the computer program MEGA 2.1 (Kumar et al. 2001). The phylogenetic distance was measured by Poisson correction model (Kimura and Ohta 1980). The robustness of the phylogenetic tree was tested by bootstrap of 1000 replicates.

Results

Partial PGH α subunits cDNAs were amplified from pituitary glands of two swamp eels by RT-PCR using the primers 1 and 2. This resulted in a single band in 1.5% agarose gel and agreed with the amino acid sequences of other PGH α subunits. The complete cDNA sequences were determined using 3′-RACE and 5′-RACE. The PGH α subunit cDNA of *Monopterus albus* was 642 bp in total length, including 64 bp of the 5′-untranslated region (5′UTR), 354 bp of the coding region, and 206 bp of the 3′-untranslated region (3′UTR) followed by a 18 bp poly (A^+) tail. A polyadenylation signal, ATTAAA, was appeared 12 bp upstream from the poly (A^+) tail. The coding region encoded a 117 amino acids peptide, which containing

Table 2. Percentage identities of mature PGH α mature peptides among fish orders

	1	2	3	4	5	6	7	8	9	10
1 Synbranchiformes (2)*	93.8	92.5	82.5	72.2	61.9	65.1	63.9	59.1	60.9	56.7
2 Perciformes (7)		92.6	81.6	73.2	62.4	66.2	64.7	59.0	60.8	57.4
3 Pleuronectiformes (2)			84.5	69.1	61.2	61.5	60.8	58.6	61.4	55.2
4 Cyprinodontiformes (1)				100	56.9	58.6	58.8	53.6	55.7	52.6
5 Salmoniformes (8)					84.6	71.5	68.5	64.0	65.6	64.1
6 Cypriniformes (6)						96.1	89.9	74.4	72.9	73.0
7 Siluriformes (2)							96.9	73.2	70.1	71.1
8 Anguilliformes (3)								93.8	75.3	71.5
9 Acipenseriformes (1)									100	71.1
10 Ceratodontiformes (1)										100

*The number in parenthesis represents species numbers of each order.

a putative signal peptide of 23 amino acids and a mature protein of 94 amino acids (Figure 2a). The PGH α subunits cDNA of *Monopterus albus* from Taiwan or southern China were identical.

For *Ophisternon bengalense*, the PGH α subunit cDNA was 674 bp long, including 78 bp of the 5′UTR, 354 bp of the coding region, and 229 bp of the 3′UTR followed by a 13 bp poly (A^+) tail. A polyadenylation signal, ATTAAA, was 19 bp upstream from the poly (A^+) tail. The coding region also encoded a peptide of 117 amino acids, which containing a putative signal peptide of 23 amino acids and a mature peptide of 94 amino acids (Figure 2b).

Although the numbers of amino acids in both signal and mature peptides between *Monopterus albus* and *Ophisternon bengalense* are identical, some differences exist in their amino acid sequences (Figure 2 and Figure 3). The sequence identities of signal and mature peptides of PGH α subunits between the two swamp eels are 83% and 94%, respectively.

Discussion

cDNA nucleotides and deduced amino acids of PGH α subunits of the swamp eels

In the present study we have cloned full length cDNAs for PGH α subunits from two species of the order Synbranchiformes, *Monopterus albus* and *Ophisternon bengalense*. The mature peptides of PGH α subunits of these two species from Synbranchiformes exhibit high similarity with those of other fishes in respect to peptide size and sequence identity. Ten cysteine residues, forming 5 disulfide bonds, two putative N- glycosylation sites, and three proline residues responsible for changing the backbone directions of the protein structure (Irving and David 1999), are conserved in the PGH α subunits of these two species of Synbranchiformes.

The nucleotide and peptide sequences of the PGH α subunits are identical for *Monopterus albus* collecting from either Taiwan or southern China. According to the geographic history, Taiwan Island was formed as a result of the collision of the Luzon volcanic arc with the southeastern passive margin of China during the late Miocene (around 10 to 3 million years ago) (Chai 1972; Chen et al. 1985). Taiwan used to be connected with China mainland between 25 000 and 18 000 years ago because of occurrence of strong ice age, which resulted in the decrease of sea level of 120 m (Chai 1972). The geographic connection status lasted for several thousand years; presumably, the albus swamp eels could migrate from China mainland to Taiwan. After the end of ice age, the sea level rose again which resulting in the separation of Taiwan from China mainland by a strait. Since the swamp eels are freshwater fishes, the populations in the both sides, as separated by Taiwan Strait, are impossible to undertake gene flow thereafter. Thus, the totally identical cDNA nucleotide and peptide sequences of the PGH α subunits in *Monopterus albus* either from Taiwan or China implies that the isolation time is not long enough for the PGH α subunit to go through further evolution.

Sequence identities and variations of PGH α subunits among fish orders

Amino acid sequence alignments of 33 PGH α subunits from 28 species of fishes, representing 10 fish

Monopterus albus

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CAATATAAAGGAGTCCTGTAGACAGGCAAGGAAGGAGAAACTTTCTCTCAGTATGATAAC  60
AACAATGGGTTCAGTGAAGTCAGCTGGAGTGTCTCTTCTTCTGTTGTCTTTTCTTCTATA 120
     M  G  S  V  K  S  A  G  V  S  L  L  L  L  S  F  L  L  Y  -5
CGTAGCTGATTCTTACCCCAACATCGATCTGGCAAATATCGGCTGTGAGGAATGCACACT 180
  V  A  D  S  Y  P  N  I  D  L  A  N  I  G  C  E  E  C  T  L  16
CAGAAAGAACACTGTTTTCTCAAGGGATCGTCCGGTTTACCAGTGCATGGGCTGTTGCTT 240
  R  K  N  T  V  F  S  R  D  R  P  V  Y  Q  C  M  G  C  C  F  36
CTCCAGAGCGTACCCAACACCTCTCAAGGCCATGAAGACAATGCCGATCCCAAAGAACAT 300
  S  R  A  Y  P  T  P  L  K  A  M  K  T  M  P  I  P  K  N  I  56
CACCTCAGAGGCAACATGCTGTGTTGCTAAGCACAGCTATGAGACAGAGGTGGCCGGCAT 360
  T  S  E  A  T  C  C  V  A  K  H  S  Y  E  T  E  V  A  G  I  76
AAGGGTGAGAAACCATACAGACTGCCACTGCAGCACCTGCTATTTTCATAAGATATGACA 420
  R  V  R  N  H  T  D  C  H  C  S  T  C  Y  F  H  K  I  *     94
GATGGGAACTGCAGACCAGTTTGTAGCCCCCAGCTTGCCAACCAGCTGTGTTTCTTTTAA 480
TATGCAAAAGCTATTCTCTGTTTTAAAATGTAGCCTATGTTCTTATGTTGCCAGATAATA 540
TTTTTGTAGCAATTCCTACACTGTTTCCTTGATAAATGTGTAAGAGTAACTGTAGATTGA 600
AAAGCAATTAAAATGTGCACCAAGAAAAAAAAAAAAAAAAAA                    642
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Ophisternon bengalense

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AGACAATATAAAGAAGTCCGGTAGAGAGGCACAATGAAGGAGAAACTTTCTCTCGACATG  60
ATAACCGCTAAAGTGACAATGGGCTCGGCAAAATCTGCTGGACTGTCTCTTCTTATGTTG 120
                   M  G  S  A  K  S  A  G  L  S  L  L  M  L  -10
TCCTTTCTTCTCTACATAGCTGATTCTTACCCCAACATTGACTTGTCAAACACGGGCTGT 180
 S  F  L  L  Y  I  A  D  S  Y  P  N  I  D  L  S  N  T  G  C   11
GAGGAATGCACACTCCGAAAGAACAATGTTTTCTCAAGAGATCGTCCGATTTTCCAGTGC 240
 E  E  C  T  L  R  K  N  N  V  F  S  R  D  R  P  I  F  Q  C   31
ATGGGCTGTTGCTTCTCCAGAGCGTACCCAACGCCCCTCAAGGCCATGAAGACCATGACG 300
 M  G  C  C  F  S  R  A  Y  P  T  P  L  K  A  M  K  T  M  T   51
ATCCCAAAGAACATCACCTCCGAGGCAACATGCTGTGTCGCTAAGCACAGCTACGAGACA 360
 I  P  K  N  I  T  S  E  A  T  C  C  V  A  K  H  S  Y  E  T   71
GAGGTGGCCGGCATAAGGGTGAGAAACCACACAGACTGCCACTGCAGCACCTGCTATTTT 420
 E  V  A  G  I  R  V  R  N  H  T  D  C  H  C  S  T  C  Y  F   91
CATAAGATATGACAGATGGGAACTGGAGGCCATTCTGCACCTCTCAGCTTGGCAACAAAT 480
 H  K  I  *                                                    94
CGTGTTTCTTTTAATATGCACAGGCTATTCTCTGTTTTAAAATTTATGTTCTTGTGTTGC 540
CAGATAATATTTTTGTAGGGATTCTGTGTGTGTTGTGTAGTTTGTCTACATTGTTCTCTT 600
GATAGATGTGTAAGAGTAACTGTAGATTGAAAAGCAATTAAAATGTGTACCCAGATACTG 660
CAAAAAAAAAAAAA                                               674
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Figure 2. Nucleotide and deduced amino acid sequences of the cDNAs encoding the PGH α subunits of the *Monopterus albus* (a) and *Ophisternon bengalense* (b). The first amino acid (Y) of the mature peptide is shown by arrowheads (▲). The nucleotide sequences corresponding to the polyadenylation signal are underlined.

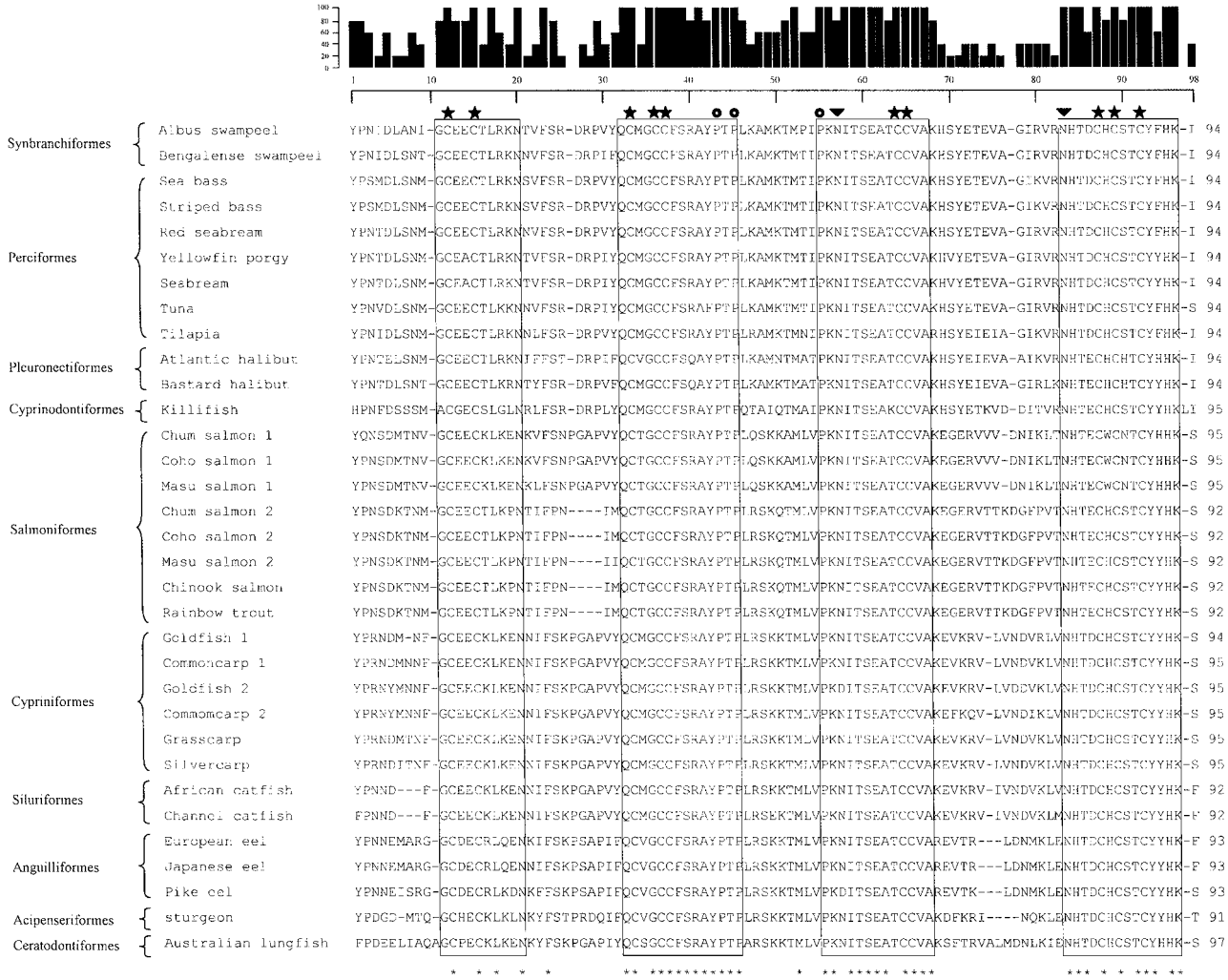

Figure 3. Alignment of the PGH α mature peptides of fishes. The sources and references of these sequences are given in Table 1. The 10 conserved cysteine residuals are indicated with stars (★), the two putative N-linked glycosylation sites are indicated by arrowheads (▼), three conserved prolines are indicated with circles (○), and gaps are marked by dashes (–). The 4 conserved amino acid regions are boxed. The identity degrees of individual amino acids in each positions are shown above the sequence alignments.

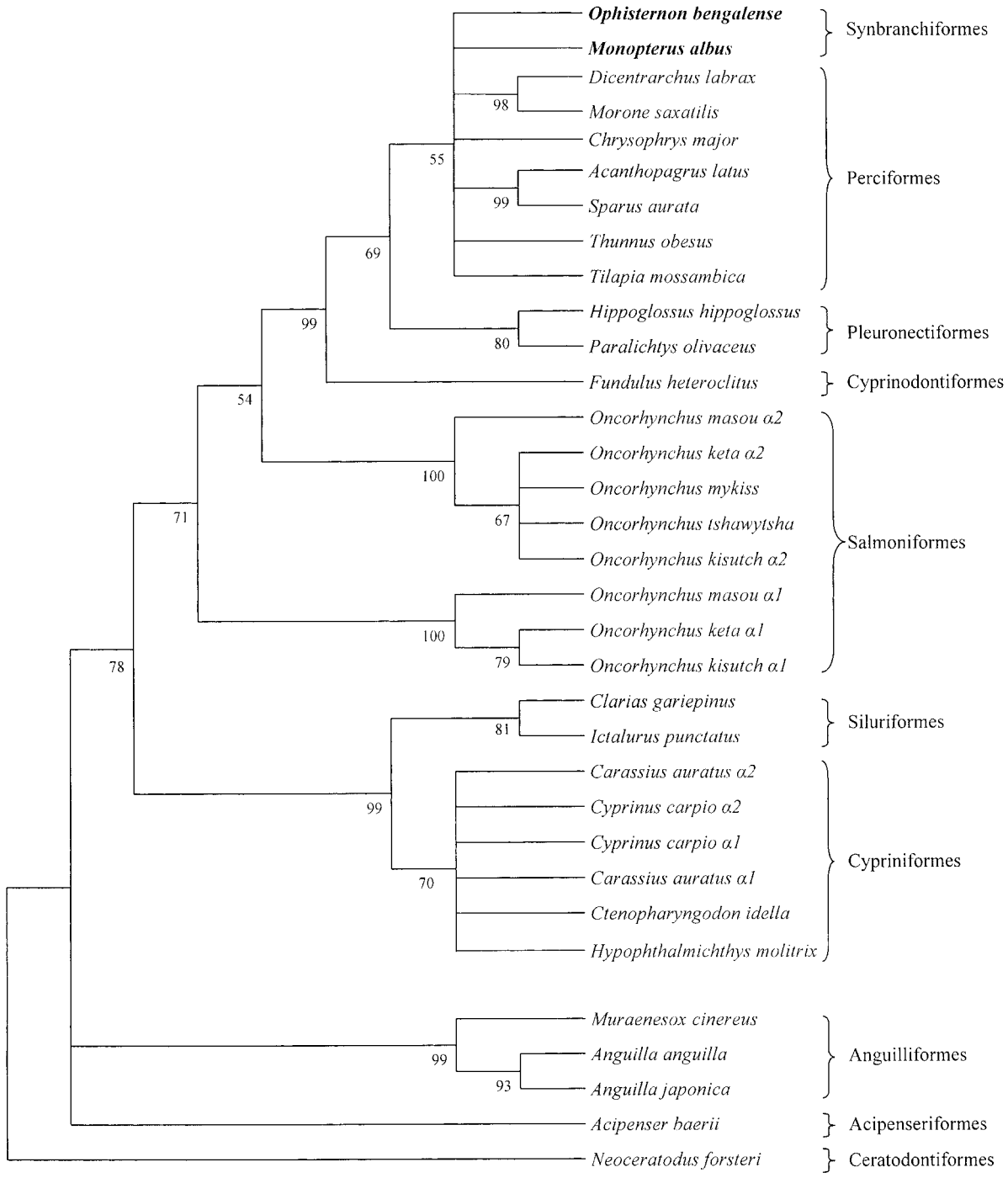

Figure 4. A consensus phylogenetic tree of the mature peptides of PGH α subunits from 10 fish orders basing on their amino acid identities. The tree was constructed with Neighbor-Joining method with Australian lungfish as the outgroup species. The numbers indicate the bootstrap robustness from 1000 replicates.

orders, are presented in Figure 3. The numbers of amino acid residues of PGH α subunits of fishes so far identified range from 91 to 97, with sturgeon, order Acipenseriformes, being the shortest and Australian lungfish, order Ceratodontiformes, being the longest. Teleosts from the orders Synbranchiformes, Perciformes, and Pleuronectiformes have PGH α subunits of 94 amino acids.

The more conserved regions in amino acid sequences of fish PGH α subunits are at positions of 11–20, 32–45, 55–67, and 83–96 (boxes in Figure 3). These conserved regions include 10 cysteine residues, 2 putative asparagine (N) – linked glycosylation sites, and 3 proline residues. It is interesting to note that there are 39 amino acids conserved in fish PGH α subunits so far identified. The less conserved regions of fish PGH α subunits are at positions of 3–10 and 25–31. The least conserved region is the amino acid residues at position of 69–82. In this region, sequence identities are higher among the orders of Synbranchiformes, Perciformes, Pleuronectiformes, and Cyprinodontiformes, and between the orders of Cypriniformes and Siluriformes (Figure 3).

Phylogenetic relationships of PGH α subunits among fish orders

The percentage identities of PGH α mature peptides of the two species from Synbranchiformes in comparison with other members of different orders of fishes are shown in Table 2. Highest identity is found with members of Perciformes (92.5%, n=14), least with Acipenseriformes (60.9%, n=2), Anguilliformes (59.1%, n=6), and Ceratodontiformes (56.7%, n=2), and intermediate with Pleuronectiformes (82.5%, n=4) and Cyprinodontiformes (72.2%, n=2). A consensus phylogenetic tree of the mature peptide of PGH α subunits constructed by NJ method with Australian lungfish as the outgroup is shown in Figure 4. Such data consist of 33 PGH α mature peptide sequences from 28 fish species of 10 fish orders. The topology shows that the two swamp eels of Synbranchiformes are grouped together with fishes of Perciformes.

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