Comp. Biochem. Physiol. Vol. 104B, No. 4, pp. 803–810, 1993
Printed in Great Britain

0305-0491/93 $6.00 + 0.00

MOLECULAR CLONING AND EXPRESSION OF YELLOWFIN PORGY (*ACANTHOPAGRUS LATUS* HOUTTUYN) GROWTH HORMONE cDNA

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(*Received* 14 *August* 1992; *accepted* 25 *September* 1992)

Abstract—1. The growth hormone cDNA of yellowfin porgy (ypGH cDNA) consisted of 915 base pairs.
2. The deduced amino acid (aa) sequence showed that the pre-GH comprised 204 residues, of which the first 17 residues formed a signal peptide.
3. Comparison of aa sequence of ypGH to seabream, tuna, rainbow trout and chum salmon showed that ypGH shared 95.1, 94.1, 65.3 and 62.4% homology with these species, respectively.
4. By expressing the ypGH cDNA in *E. coli*, a polypeptide around 23 kilodaltons (kDa) was found which was immunoreactive to GH antibody.

INTRODUCTION

Growth hormone (GH) is one of the major important polypeptide hormones produced in the anterior pituitary cells to regulate growth and metabolism in vertebrates. Since fish constitute an enormous variety of vertebrate species, comparative studies on the primary structures of teleostean GHs, the predicted secondary structures and the regulation of their expression will provide a significant insight to the understanding of the structure–function relationships, evolutionary implications and biological actions of GH.

Yellowfin porgy is a major marine aquaculture fish, especially in Asia. Taxonomically, it is classified as order Perciformes, suborder Percoidei and family Sparidae. Physiologically, it is a protandrous hermaphroditic fish (Chang and Yueh, 1990). However, the molecular biological studies of genes involved in its growth have been limited, although various fish GH genes have been studied: those of carp (Chiou *et al.*, 1990), eel (Saito *et al.*, 1988), rainbow trout (Agellon and Chen, 1986; Agellon *et al.*, 1988), salmon (Johansen *et al.*, 1989; Hew *et al.*, 1989; Sekine *et al.*, 1985, 1989; Gonzalez-Villasenor *et al.*, 1988), seabream (Momota *et al.*, 1988; Funkenstein *et al.*, 1991), tilapia (Rentier-Delrue *et al.*, 1989) and tuna (Sato *et al.*, 1988).

We here describe the molecular structure of yellowfin porgy growth hormone (ypGH) cDNA. In addition to providing the needed information on the primary structure of the ypGH cDNA, this cDNA was genetically engineered into an expression vector to confirm that ypGH cDNA encoded GH. The latter may be used for producing recombinant GH (rGH) in micro-organisms for research and other applications.

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MATERIALS AND METHODS

cDNA library construction

Total RNA was extracted from pituitary glands by following the modified phenol–chloroform–SDS method (Chen, 1980). Poly $(A)^+$-RNA was prepared on an oligo (dT)-cellulose column (Aviv and Leder, 1972) and used as a template for the synthesis of cDNA (Agellon and Chen, 1986). The cDNAs were inserted at the *Eco* RI site of lambda gt10 (Strategene, CA) and the resulting chimeric phages were amplified in *E. coli* C600 hfl^-.

Screening

The rainbow trout GH cDNA (rtGH cDNA) (Agellon and Chen, 1986) was used as a probe for isolating clones containing ypGH cDNA from the cDNA library by the plaque hybridization method (Maniatis *et al.*, 1982). Approximately 1×10^6 recombinant bacteriophage were plated on LB plates (3×10^4 pfu/plate) and then transferred to the membranes. After blotting, the membranes were hybridized to the $[^{32}P]$-labeled rtGH cDNA. Hybridization was carried out in a buffer containing 5 × Denhardt's solution (1 × Denhardt's: 0.02% Ficoll, 0.02% polyvinylpyrolidone and 0.02% bovine serum albumin), 5 × SSPE (1 × SSPE: 0.15 M NaCl, 11 mM NaH_2PO_4, 1 mM EDTA), 0.1% SDS and 100 μg/ml denatured salmon sperm DNA at 42°C for 16 hr. The membranes were washed three times (10 min each) at room temperature in a solution containing 0.1% SDS and 1 × SSPE, followed by another three washes in a solution of 0.1% SDS and

0.2 × SSPE at 60°C. Positive clones were purified for further analysis.

Nucleotide sequence determination and amino acid deduction

The GH cDNA inserts were subcloned into the *Eco*RI site of pUC18 for further restriction map analysis. Two DNA fragments generated by digesting the entire cDNA with *Cla*I were further subcloned into the phagemid, pGEM-7Zf(+), for nucleotide sequence determination (Sanger *et al.*, 1977), using synthetic oligomers 5′-GATTTAGGTGACACTATAG-3′ and 5′-TAATACGACTCACTATAGGG-3′ as sequencing primers.

The amino acid (aa) sequences of GH from other fish species were retrieved from the GeneBank and National Biomedical Research Foundation data bases for comparison. Alignment of aa sequences was carried out by introducing gaps to maximize the identity and similarity, using the Wisconsin Package.

Expression of ypGH cDNA in E. coli

The cDNA fragment encoding the mature ypGH was amplified by the polymerase chain reaction (PCR) following the standard conditions (Innis and Gelfand, 1990). PCR primers, 5′-ATATCATATG-CAGCCGATCACAGACGGCCAGCG-3′ (ATAT, *Nde*I site and ATG preceded the sense strand of +104 to +126) and 5′-ATATAAGCTTTTACTACAG-GGTGCAGTTGGC-3′ (ATAT and *Hin*dIII site preceded the antisense strand of +667 to +650 and one more stop codon, TAA) were used for synthesizing the mature region of ypGH cDNA. The PCR product was ligated into the *Sma*I site of pUC19. The resulting cDNA fragment released from the vector by cutting with *Nde*I and *Sal*I was ligated into the expression vector, pRE (Reddy *et al.*, 1989), which was digested with *Nde*I and *Sal*I. This construct was then introduced into *E. coli* MZ1. The transformants were grown in LB media under the permissive temperature (30°C) to an OD_{650} of 0.5 and then shifted to 41°C for approximately 2 hr for the induction of ypGH synthesis. Harvested cells were lysed and dissolved in a sample buffer (Laemmli, 1970) and analyzed by SDS–PAGE on a 20% polyacrylamide gel (Hames, 1990).

Western blot analysis

The protein bands on the gel were transferred to a polyvinylidene difluoride membrane (Towbin *et al.*, 1979). The resulting membrane was incubated in a blocking solution [0.5% skim milk in 50 mM Tris–HCl, 0.5 M NaCl, pH 7.5 (TBS)] at 4°C overnight, and then in a fresh blocking solution containing a rabbit antiserum raised against the natural chum salmon GH (csGH) for 1 hr at 37°C. The membrane was washed three times in a TBST solution (TBS containing 0.05% Tween 20), then incubated in the blocking solution containing goat anti-rabbit IgG conjugated with alkaline phosphatase for 1 hr at 37°C. The immunoreactive band was visualized by reacting the blot in a solution containing color-developing reagents (5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium) for 5–30 min at room temperature.

RESULTS AND DISCUSSION

Isolation and nucleotide sequence of ypGH cDNA

A cDNA library containing 1×10^6 recombinant phage clones was constructed. About 40 putative

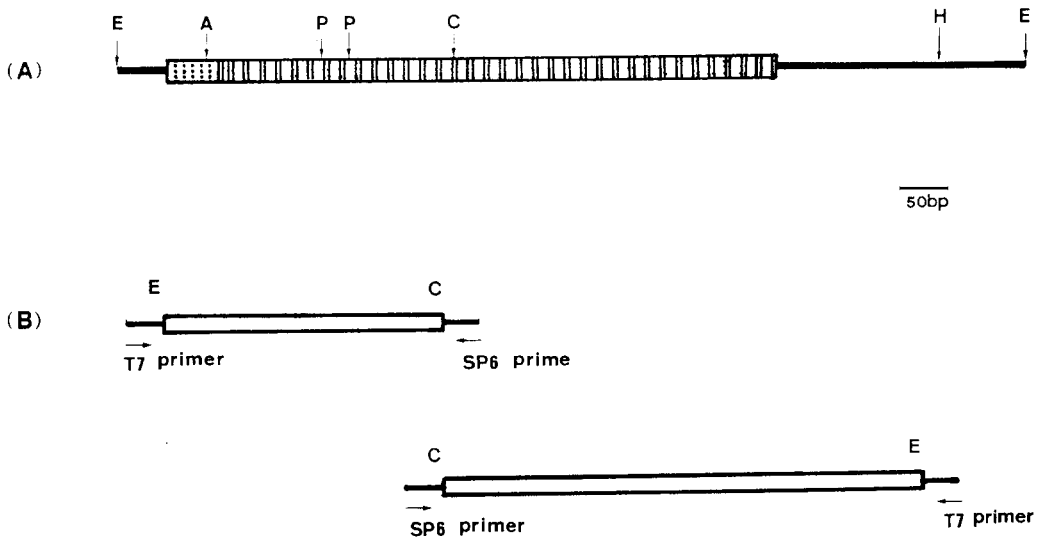

Fig. 1. A partial restriction map and sequence strategy. (A) Restriction and map of yellowfin porgy GH cDNA. The 5′-untranslated region (left thick line), the signal peptide coding region (dot box), the mature GH coding region (hatch box) and the 3′-untranslated region (right thick line) are shown. A, *Aha*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I sites. (B) Two subclones derived from ypGH cDNA clone used in nucleotide sequence determination using T7 or SP6 primers.

positive clones were obtained after screening over 2×10^6 phages. Southern blot analysis showed that one of the longest positive clones contained an insert of 915 bp. A partial restriction map is presented in Fig. 1(A).

Since there was a unique internal *Cla*I site, giving two fragments of 575 bp and 340 bp, respectively, these two DNA fragments were separately subcloned into pGEM-7Zf(+) for sequencing. The strategy of nucleotide (nt) sequence determination is shown in Fig. 1(B). As summarized in Fig. 2, the cDNA insert contained a single open reading frame of 612 nt located at nt positions 53–664, which encoded 204 aa residues. The translation initiation codon (ATG) was located at nt positions 53–55, and was preceded with 52 nt of the untranslated leader sequence. The translation termination codon TAG (nt positions 665–667) was followed by a 3′end untranslated region of 231 nt including the polyadenylation signal (AATAAA) at nt 874–879, 19 bases upstream from the beginning of the poly(A) tail.

Four tandem direct repeat sequences, 5′-GAACC(A/T)-3′, were observed in the 5′ untranslated region (nt 26–49). Although the biological significance of these direct repeat sequences is not understood, different numbers of these sequences

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   5'--AGATCAGATCTAGTCACCAGAACTTGAACCAGAACCAGAACCTGAACCAGAC  52

 53 ATG GAC AGA GTG GTG CTC ATG CTG TCG GTG CTG TCT CTG GGC  94
-17 Met Asp Arg Val Val Leu Met Leu Ser Val Leu Ser Leu Gly  -4

 95 GTC TCC TCT CAG CCG ATC ACA GAC GGC CAG CGT CTG TTC TCC 136
 -3 Val Ser Ser Gln Pro Ile Thr Asp Gly Gln Arg Leu Phe Ser  11

137 ATC GCT GTC AGC AGA GTT CAA CAC CTC CAC CTG CTC GCT CAG 178
 12 Ile Ala Val Ser Arg Val Gln His Leu His Leu Leu Ala Gln  25

179 AGA CTC TTC TCT GAC TTT GAG AGC TCT CTG CAG ACT GAG GAG 220
 26 Arg Leu Phe Ser Asp Phe Glu Ser Ser Leu Gln Thr Glu Glu  39

221 CAA CGA CAG CTC AAC AAA ATC TTC CTG CAG GAT TTC TGT AAC 262
 40 Gln Arg Gln Leu Asn Lys Ile Phe Leu Gln Asp Phe Cys Asn  53

263 TCT GAT TAC ATC ATC AGC CCC ATC GAC AAG CAC GAG ACA CAG 304
 54 Ser Asp Tyr Ile Ile Ser Pro Ile Asp Lys His Glu Thr Gln  67

305 CGC AGC TCA GTG TTG AAG CTG CTG TCT ATC TCC TAT CGA TTG 346
 68 Arg Ser Ser Val Leu Lys Leu Leu Ser Ile Ser Tyr Arg Leu  81

347 GTC GAG TCT TGG GAG TTC CCC AGT CGT TCT CTG GCT GGC GGT 388
 82 Val Glu Ser Trp Glu Phe Pro Ser Arg Ser Leu Ala Gly Gly  95

389 TCT GCT CCA AGG AAC CAG ATT TCA CCC AAA CTG TCT GAG CTG 430
 96 Ser Ala Pro Arg Asn Gln Ile Ser Pro Lys Leu Ser Glu Leu 109

431 AAG ACA GGC ATC CAT CTC CTG ATC AGG GCC AAT GAG GAT GGA 472
110 Lys Thr Gly Ile His Leu Leu Ile Arg Ala Asn Glu Asp Gly 123

473 GCA GAG CTC TTC CCT GAT AGC TCC GCC CTC CAG CTG GCT CCT 514
124 Ala Glu Leu Phe Pro Asp Ser Ser Ala Leu Gln Leu Ala Pro 137

515 TAT GGA GAC TAC TAC CAA AGT CCG GGC ACC GAC GAG TCG CTG 556
138 Tyr Gly Asp Tyr Tyr Gln Ser Pro Gly Thr Asp Glu Ser Leu 151

557 AGA CGA ACC TAC GAA CTA CTT GCC TGT TTC AAA AAA GAC ATG 598
152 Arg Arg Thr Tyr Glu Leu Leu Ala Cys Phe Lys Lys Asp Met 165

599 CAC AAG GTG GAG ACC TAC CTG ACA GTG GCA AAA TGT AGA CTC 640
166 His Lys Val Glu Thr Tyr Leu Thr Val Ala Lys Cys Arg Leu 179

641 TCT CCA GAG GCC AAC TGC ACC CTG TAG CCCCGCCTCTCTTCTGTGA 686
180 Ser Pro Glu Ala Asn Cys Thr Leu                         187
                     *   *   *
687 AACCACAGCCCCTGTTGATGATGTAATCGTGTGTTCTGAACGTCCGCCTCCACAC 741
742 TCTCTGACTCTGATAAGTAGTGTTAGCACTAGCATTAGCATTAGTTCCTGTTCAG 796
797 TGGTCGAGGATGTAATTGAAGGTAGTCTGGTGTCATGATGATGAAAGCTTTGAAC 851
852 AGGAAGTGATGTCATACTGTGAAATAAAGTCTGTGCTGTTTGCATTCAAAAAAAA 906
907 AAAAAAAAA --3'                                          915
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Fig. 2. Nucleotide (nt) sequence of yellowfin porgy GH cDNA and the predicted aa sequence of the pre-hormone. The nt was numbered beginning with the first nt at the 5′ end. The polyadenylation signal, AATAAA, is indicated by a box. The number on the second line of each row indicates the order of aa position. Pre-ypGH contains a signal peptide of 17 aa residues (aa −17 to −1) and a mature protein of 187 aa residues (aa 1 to 187). Potential *N*-glycosylation site is marked by an asterisk.

have been observed in the 5′ untranslated region of almost all GH mRNA species studied to date (Sekine *et al.*, 1985; Agellon and Chen 1986; Gonzalez-Villasenor *et al.*, 1988; Momota *et al.*, 1988; Sato *et al.*, 1988; Saito *et al.*, 1988; Funkenstein *et al.*, 1991). Since the 3′ end of the eukaryotic 18S rDNA contained a sequence of 3′-UUUGGA-5′ (Chan *et al.*, 1984), it is conceivable that the observed direct repeat sequence of 5′-GAACC(A/T)-3′ located at 5′-untranslated region of GH mRNA may be involved in the binding of the mRNA to the 40S ribosomal subunit in the formation of the translation initiation complex (Nakashima *et al.*, 1980). Furthermore, it is of interest to note that, unlike the GH mRNA of bluefin tuna, the GH mRNA of yellowfin porgy, chum salmon, tuna or humans does not contain a direct repeat sequence of 5′-CTGTAGCC-CCGCCTCTCTGATGACGT-3′ at the 3′ untranslated region (Sato *et al.*, 1988).

The nt sequence of the translated region of ypGH cDNA was compared to that of the other fish species. ypGH cDNA differed from that of the red seabream (Momota *et al.*, 1988) and tuna (Sato *et al.*, 1988) by 23 and 60 nt out of a total 612 nt, respectively. Hence, the translated region of ypGH cDNA shared a 96.2 and 90% identity at the nt sequence level with those of red seabream and tuna.

Comparison of the deduced aa sequence of ypGH with those of other fish species

The predicted aa sequence of the yp-pre-GH polypeptide is summarized in Fig. 2. Since the authentic ypGH has not been isolated, the first aa residue at the *N*-terminus of the nature GH remains to be determined. However, as shown in Fig. 2, we predicted that the first 17 aa residues might comprise the signal peptide, as evident from its high hydrophobicity, and the remaining 187 aa residues might comprise the mature GH polypeptide. The *N*-terminus aa residue of the mature ypGH may, therefore, start with glutamine which was also observed in the GHs of red seabream (Momota *et al.*, 1988) and bluefin tuna (Sato *et al.*, 1988). The first aa residue of the mature eel GH started with valine (Saito *et al.*, 1988) and that of chum salmon, coho salmon and rainbow trout GH started with isoleucine (Sekine *et al.*, 1985; Agellon and Chen, 1986; Gonzalez-Villasenor *et al.*, 1988). The number of aa residues in the signal peptide of yp-pre-GH was the same as that reported for the red seabream (Momota *et al.*, 1988) and bluefin tuna (Sato *et al.*, 1988), but two residues fewer than the signal peptide of eel (Saito *et al.*, 1988) and five residues fewer than that of chum salmon (Sekine *et al.*, 1985), rainbow trout (Agellon and Chen, 1986)

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SB    MDRVVLMLSVLSLG--VSSQPITDGQRLFSIAVSRVQHLHLLAQRLFSDF   48
RS    ..............--..................................   48
BT    ....F.L.......--........S.........................   48
RT            MTMIT--N.RGSAIEN....N.............KM.N..   39
CS    .GQ.F.LMP..LVSCFL.QGAAIEN....N.............KM.N..   50

SB    ESSLQTEEQRQLNKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRL   98
RS    .........LK.....-P...............................   97
BT    ................................................   98
RT    DGT.LPD.R........L......S.V..V......K.......H..F..   89
CS    DGT.LPD.R........L......S.V..V......K.......H..F..  100

SB    VESWEFPSRSL--AGGSAPRN--QISPKLSELKTGIHLLIRANEDGAELF  144
RS    ..........--S.......--.........M.............I.     143
BT    ..........--S.......--.....................Q..D.M.  144
RT    I....Y..QT.IISNSLMV..AN...E...D..V..N...TGSQ..VLSL  139
CS    I....Y..QT.IISNSLMVSNA....E...D..V..N...TGSQ..VLSL  150

SB    PDSSALQLAPYGDYYQSPGTDESLRRTYELLACFKKDMHKVETYLTVAKC  194
RS    ...........N....L.A.............................    193
BT    A...........N....L.A......S.....................    194
RT    D.NDSQ..P...N...NL.G.GNV..N.....................    189
CS    D.NDSQ..P...N...NL.G.GNV..N.....................    200

SB    RLSPEANCTL                                          204
RS    ..........                                          203
BT    ..........                                          204
RT    .K.L......                                          199
CS    .K.L......                                          210
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Fig. 3. Comparison of the predicted aa sequence of yp-pre-GH (SB) with that of red seabream (RS, Momota *et al.*, 1988), bluefin tuna (BT, Sato *et al.*, 1988), chum salmon (CS, Sekine *et al.*, 1985) and rainbow trout (RT, Agellon and Chen, 1986). Numbers start at the first aa residue; the identical aa residues are indicated by dots. "—", gaps created to maximize the degree of homology among all of the sequences compared.

and coho salmon (Gonzalez-Villasenor *et al.*, 1988). When the mature ypGH was compared to the GHs of other fish species, it was observed that ypGH was in the same size range as that of bluefin tuna, but one residue shorter than that of chum salmon (Sekine *et al.*, 1985), coho salmon (Gonzalez-Villasenor *et al.*, 1988), rainbow trout (Agellon and Chen, 1986), red seabream (Momota *et al.*, 1988), rat (Seeburg *et al.*, 1977), human (Martial *et al.*, 1979) and cow (Miller and Eberhardt, 1983).

Figure 3 summarizes the comparison of the aa sequence of ypGH with GH of other fish species. The ypGH shared a 95.1% homology with that of the red seabream, 94.1% with bluefin tuna, 65.3% with rainbow trout (GH-I) and 62.4% with chum salmon (GH-I). The results of aa sequence comparison are incongruous with the taxonomical classification of these fish. Yellowfin porgy, red seabream and tuna are classified as order Perciformes, whereas rainbow trout, coho salmon and chum salmon are order Salmoniformes. Moreover, yellowfin porgy and red seabream belong to suborder Percoidei and tuna belongs to suborder Scombroidei. This reflects that the nt sequence encoded for the translated region of ypGH cDNA shared a 96.2 and 90% identity at the nt sequence level with that of red seabream and tuna.

Upon closer examination of the primary structures of ypGH and GHs of other fish and mammals, two additional specific features of ypGH were also observed. A highly conserved region existed near the *C*-terminus (Leu-174 to Arg-195) of the GH molecule, which was almost identical among fish species studied to date, and shared 64% homology with GH of humans or other mammals. Results of X-ray crystallographic studies of porcine GH suggested that this highly conserved region of the molecule might be important in the formation and stabilization of GH-specific conformation (Abdel-Meguid *et al.*, 1987). Furthermore, there were four cysteine residues at locations nearly identical to those in the GHs of other fish species and mammals. Since the contribution of cysteine residues in the formation of the tertiary structure as well as the maintenance of growth-promoting activity in mammalian GH has been well established (Paladini *et al.*, 1981; Abdel-Meguid *et al.*, 1987), the cysteine found in ypGH may play a similar role.

Frequency of codon usage

The nt codon usage in yp-pre-GH is summarized in Table 1. As in the pre-GH of chum salmon (Sekine *et al.*, 1985), tuna (Sato *et al.*, 1988) and humans (Masuda *et al.*, 1988), the codons used in yp-pre-GH were rather non-random. The preferentially used codons were as described by Maruyama *et al.* (1986): CTG for leucine, GTG for valine, GGC for glycine, ATC for isoleucine, TAC for tyrosine, CAC for histidine, CAG for glutamine, AAC for asparagine, GAC for aspartic acid, GAG for glutamic acid and TTC for phenylalanine. However, there were some

Table 1. Codon usage in GH cDNA

		Species			
aa	Codon	YP	BT	CS	HU
leu	UUA	—	—	1	1
	UUG	2	2	4	—
	CUU	1	1	—	2
	CUC	9	8	6	10
	CUA	1	—	2	4
	CUG	17	20	20	15
arg	CGU	2	3	1	1
	CGC	1	1	1	4
	CGA	3	2	—	—
	CGG	—	1	2	2
	AGA	5	4	2	—
	AGG	2	2	3	5
ser	UCU	11	11	4	3
	UCC	4	4	3	7
	UCA	2	3	2	2
	UCG	2	2	—	1
	AGU	2	1	4	3
	AGC	5	8	6	5
val	GUU	1	1	—	—
	GUC	3	4	9	3
	GUA	—	—	1	—
	GUG	6	4	4	4
pro	CCU	2	—	2	—
	CCC	3	1	2	6
	CCA	2	4	2	1
	CCG	2	2	—	1
thr	ACU	1	—	2	1
	ACC	4	2	4	4
	ACA	4	4	—	5
	ACG	—	1	1	2
ala	GCU	5	7	1	3
	GCC	4	4	3	5
	GCA	2	1	2	1
	GCG	—	—	2	—
gly	GGU	1	2	1	—
	GGC	5	—	3	8
	GGA	2	5	2	—
	GGG	—	—	4	3
ile	AUU	1	—	2	2
	AUC	9	10	7	5
	AUA	—	—	2	—
tyr	UAU	2	4	—	1
	UAC	5	3	6	5
his	CAU	1	1	1	1
	CAC	4	4	4	2
gln	CAA	3	4	3	3
	CAG	9	9	9	9
asn	AAU	1	1	2	—
	AAC	4	5	13	9
lys	AAA	5	3	2	1
	AAG	4	6	10	6
asp	GAU	4	3	3	2
	GAC	7	7	10	9
glu	GAA	1	3	3	3
	GAG	12	9	6	9
cys	UGU	3	1	2	2
	UGC	1	3	3	3
phe	UUU	1	2	2	4
	UUC	7	7	7	9
trp	UGG	1	1	1	2
met	AUG	3	3	5	3

Numbers indicate the frequency with which the codons are used in the coding region of GH mRNA. aa, amino acid; YP, yellowfin porgy; BT, bluefin tuna; CS, chum salmon; HU, human.

exceptions: (i) TGT preferentially for cysteine in yp-pre-GH and TGC for pre-GH of tuna, salmon and human; (ii) TCT preferentially for serine in pre-GH of yellowfin porgy and tuna, but AGC and TCC for pre-GH of salmon and human; (iii) AGA preferentially for arginine in pre-GH of yellowfin porgy and tuna, but AGG or CGC for pre-GH of salmon and human. Although codon CGG is not used in the pre-GH of yellowfin porgy, it is preferentially used in the pre-GH of tuna, salmon and human.

Expression

In order to confirm that the ypGH cDNA clone indeed encodes the GH polypeptide, the cDNA insert was amplified by PCR and ligated into an expression vector, pRE, following the scheme outlined in Fig. 4. The resulting plasmid was introduced into *E. coli* cells, induced at 41°C and the protein products were analyzed by SDS–PAGE and by immunoprecipitation.

A protein band around 23 kDa was detected in *E. coli* harboring pREYP after heat induction for 2 hr (Fig. 5A). This protein band was absent in cells containing pRE. Immunoblot analysis showed that this protein band reacted specifically with a GH antibody (Fig. 5B). Based on the staining intensity of different protein bands on the gels, the amount of rGH was estimated to be more than 5% of the total

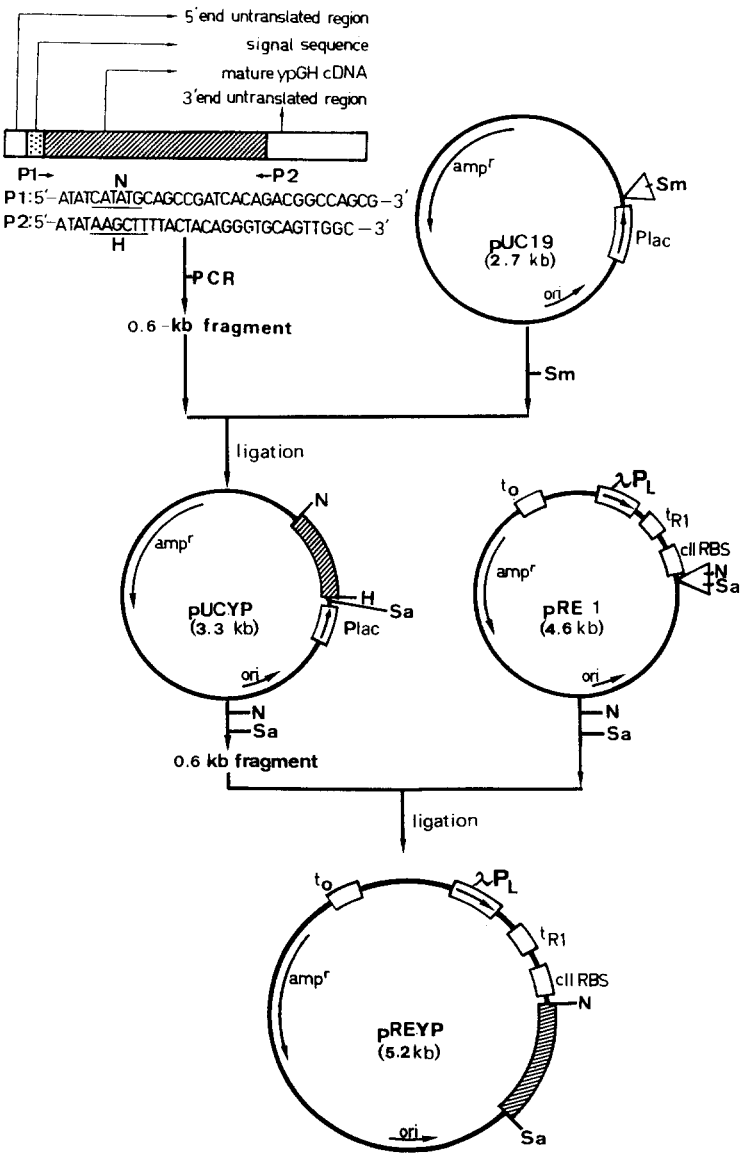

Fig. 4. Construction of the pREYP, a plasmid of 5.2 kb for the expression of ypGH cDNA. The nt sequence for the signal peptide and the mature ypGH are indicated by a stippled box and a hatched box, respectively. The empty box represents the 5′ and 3′-untranslated regions. P1 and P2 are the primers used to amplify the entire ypGH cDNA by PCR. amp, Ampicillin resistance gene; ori, origin of plasmid replication; P_L, lambda P_L promoter; P_{lac}, *lac* promoter; RBS, ribosome binding site; t_O and t_{R1}, transcription terminator. Arrow shows the transcription direction. H, *Hin*dIII; N, *Nde*I; Sa, *Sal*I; Sm, *Sma*I sites.

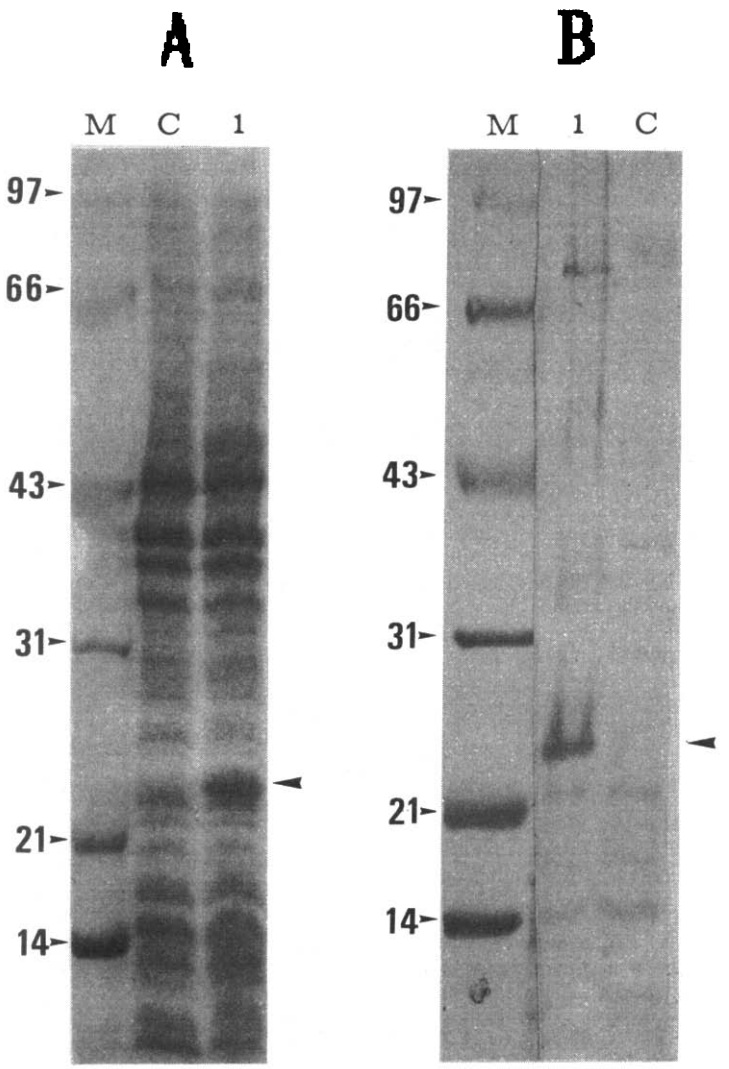

Fig. 5. Expression of ypGH in *E. coli* MZ-1 (lambda cI857 lysogen). Cells were cultured in superbroth medium with 50 μg/ml ampicillin at 30°C until the OD_{650} reached 0.5. Then, the cultivation temperature was shifted to 41°C for the induction of ypGH synthesis. Cells were harvested after 2 hr induction and their proteins were analyzed by SDS–PAGE on a 20% gel with Coomassie Blue staining (A) and by immunoblotting using polyclonal antiserum raised against the natural csGH for immunoreaction (B). Lane M: proteins molecular marker in kilodaltons (kDa); lane C: a culture containing the cloning vector alone serving as a control; lane 1: a culture harboring pREYP (with ypGH cDNA insert). The arrows indicate the biosynthetic ypGH.

bacterial proteins. Since the biosynthetic GH produced in *E. coli* cells forms insoluble inclusion bodies, they can be readily isolated and purified from the cell extract by differential centrifugation and washing. Thus this *E. coli* strain can be used for a large-scale production of biosynthetic rGH for basic research and for aquaculture applications.

Acknowledgements—We thank Miss Ling-hui Li for her technical assistance. This work was supported by grants from the National Science Council (NSC78-0209-B002-01 and NSC79-0209-B002-04), Republic of China, to HJT.

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