Molecular cloning of the regulator of G signaling (RGS) protein from the head kidney cells of common carp *Cyprinus carpio* L.

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INTRODUCTION

Many extracellular stimuli rely on heterotrimeric guanine nucleotide regulatory proteins (G proteins), and their associated signaling pathways to exert their effects at target tissues. Circulating non-steroid hormones (e.g. adrenaline), neurotransmitters, cytokines and various sensory stimuli activate specific G-protein-coupled receptors (GPCRs) at the cell surface.¹⁾ Regulators of G protein signaling (RGS) proteins are a family of highly diverse, multi functional signaling proteins that share a conserved domain.¹⁾ Presently, RGS molecules have not been cloned in fish and this is the first report.

MATERIALS AND METHODS

Common carp (mean weight=100 g) was obtained from Sunaso fisheries farm from Miyazaki, Japan. The fish were acclimated in an aerated fresh water tank at 20 °C and fed for two weeks. Head kidney (HK) cells of 12 fish were treated with 5 µg of Concanavalin A (Con A; Wako) and 10 µg/ml Lipopolysaccharide (LPS; Difco) for 1, 4, 8, 12, 24 and 48 h in RPMI 1640 (Nissui) medium supplemented with 5% carp serum and 1% Streptomycin/Penicillin (Gibco). These cells were pooled in ISOGEN (Nippon Gene) for RNA isolation. Total RNA was isolated, according to the manufacturers protocol, from 0.2 g of HK cells stimulated with LPS and Con A. Poly(A) RNA was purified from total RNA using a quick miniprep micro mRNA kit (Amersham Pharmacia Biotech) and cDNA was synthesized using cDNA synthesis kit (Gibco BRL) according to manufacturer's instructions. The library was constructed in pSPORT cloning vector.

Plasmid DNA was extracted by alkaline lysis method by Sambrook *et al.*²⁾ The cDNA clones were subjected to pre-sequencing reaction using thermosequenase (Amersham) with T7 or Sp6 (Nisshinbo) primers and sequenced on an automated

DNA sequencer LIC-4200 (Li-Cor). The sequences were compared with sequences in the database using blast algorithm.³⁾ Assignment of putative identities was set to a minimum p value of 10⁻⁵. Phylogenetic analysis was done using PAUP program⁴⁾ and by distance analysis by parsimony method. Sequences used in this paper are under the accession numbers, human RGS 2 P41220; rat RGS3, P49796; mouse RGS5, O08850; rat RGS 8, P49804, human RGS 13, O14921 and H68, AU183410.

RESULTS

The cDNA library of 10⁵ cfu/ml clones was constructed from the polyadenylated fraction of mRNA from common carp head kidney cells stimulated by LPS and Con A. Out of 200 clones 129 (64.5%) revealed homologies with nucleic acid and/or amino acid sequences, whereas the remaining 71 (35.5%) clones did not show any significant homology to the sequences in the databases. The detailed catalogue of genes and EST analysis from carp provided the clone for further analysis.⁵⁾

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TCGGGTATTCCTCGG
GTGACCCACGGCTCCGCAAAAGCAGCCACATATCTGCACCAAA
AAAGTTATTAGGATGCCAATATTTTATCATCACTGAAATAAGAAC
M P T I L S S T E I R N 12
ATGAACAAGGAGGATAAACAGTCAGTCTCAGGAGACTAGACAGTGG
M N K E D K F V S P R R L D K W 28
CGGTTTCGTGCGCTGTTGACAGACAAATTGAACCTTAGTGAGACTCTG
R F R R L L T D K L N P S E T L 44
CTGTGTCCAGTCATTGGAAAACCTTCTTAGATCCGAATATGGCATG
L W S Q S L E N L L R S R Y G M 60
GCAACATTCCACACCTTCTCAAATCTGAGTTTCAAGGATGAGAACATT
A T F H T F L K S H P S D E N I 76
GAATTTTGGCTGTATGCGAAGACTTCAAAAAAATCAGGAGTCTCTCC
E F N L V C E D F K X I R S S 92
AGACTATCTCAAGGCGCAAGAGATATTCGAGCAITACATCAAGGCA
R L S S R A K K I F E N Y I K A 108
GAAGCTCCCAAGAGATAAATCATCGACCGTCAAGAGAGACCTTATC
E A P K S I N I D H V T R D L I 124
AAGCAGAACTCCAGCTCCCAAGAGAGTGTGTTTCTGATGAAGCTCAG
K Q N V Q A P T R V C F D H A Q 140
AGGATGTTCTACGGCTTATGGAGAGGAGTCTCTTCCAGGTTTCTT
R I V Y G L M E R D S F P F L 156
CGCTGGACATGTACAGATCATCTCTGGAACTAGTCTCACAACGGATT
R S D N T R S L L E S V S Q R I 172
AAGGTCGAGAACATCTTGAATGAAGGACTTTAGGACTCCCAACACAT
X V * 174
CTGTGGCACTGCTTTGAGCAATGACAGATGCGCGTGGAGTAATCAG
GCTTTGTGGAGACTGAGGCTGGACTTGGGTTGCACTGGACATTGTGA
TCCAAAAAAGAGCTTAATAAGCTATTGTTTATATGATCTGTTATTACA
CTGCTCTATTGTTCCCTGGCTGTGGGATATACAAGACCAATGA

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Fig. 1. Nucleotide and inferred amino acid sequence of RGS cDNA clone H68. Amino acids comprising RGS domain is shown in bold oblique alphabets.

The DNA sequence of common carp RGS protein was found to consist of 1052 nucleotides, encoding 174 amino acid residues (Fig.1). The RGS reported for vertebrates consists of a RGS domain (residues 49 to 165).

CarpRGS	SIENLLRSKYOMATFHTFLKSEFSDNIEFWLVCEDFKKIRS	45
RGS3	...K...VH...L.V.QA...RT...E...L...A...VK	45
RGS2	APDE...A...L.A.RA...CE...A...TK	45
RGS13	F...MAT...PVYAAY...M.H...Q...MA...TY...A	46
RGS8	FDV...SH...V.A.RA...T...E...L...A...EP...T	45
RGS5	...DK...QNS...P...KS...E...L...VA...NY...K	45
CarpRGS	-SSRLSRRAKIFEHYIKAEAPKEINIDHVTDLIKQNVQAP	92
RGS3	-Q.KMA.K...AE...AIQ.C...V.L.SY...EHT.D.L.SV	92
RGS2	-PQK...K.R...YTDF.EK...PQ.KT...A...I.EA	92
RGS13	RW...I...LYKI...QPQS.R...SS...T.IR.I.E	92
RGS8	-TAK.VTK.HR...EFVDVQ...R.V...FQ...ATRK.M.E	92
RGS5	-PIKMAEK...Q.Y.EF.QT...V...F.KDITMK.LVE	92
Identity(%)		
CarpRGS	TRVCFDRAQRIYVGLMERDSYPRFLRSDMYRSL	117
RGS3	..G...L...KRIFG...K...DL.LD.I	117 (59.8)
RGS2	.SG..TT..KR..S...NN...E.EF.QD.C	117 (58.9)
RGS13	.ET..E...K...MH...K.E...QK..	117 (55.5)
RGS8	SLT...Q...GK.HS...K...K.LD..	117 (54.7)
RGS5	SPRS..L...KRI.A...K.L...V...EF.KE.I	117 (52.9)

Fig.2. An alignment of RGS domain of various mammalian RGS molecules with carp homologue. Dashes (-) are introduced for optimal alignment & identical residues are indicated as dots(...).

Fig.3. Phylogenetic tree of RGS with carp to other mammalian RGS regulators.

The domain in common carp RGS is well conserved compared to other RGS proteins. Common carp RGS was found to be homologous to previously reported RGS of vertebrates in both DNA and amino acid sequence analysis. The homology of carp RGS to other vertebrates ranged between 47 to 59.8%.

DISCUSSION

This paper describes the first report of cloning an RGS homologue in teleost fish. The identification and cloning was based on a search in Genbank. This was based on the alignment analysis in this study (data not shown). Alignment of the H68 carp clone with mammalian RGS domains revealed that all the conserved regions are intact in the RGS homologue

of carp (Fig.2). RGS domains have a wide range of amino acid identity (25-85%). Typically RGS is known to have a 120 amino acid domain and flanked by highly variable arms on both the sides.⁶ Mitogenic stimulation appear to induce many RGS proteins, BL34/1R20 (RGS1) expression is specific to B lymphocytes and is induced by mitogenic stimulation.⁷ Similarly, 211 residue protein (RGS2), was isolated because of the expression of its corresponding mRNA in human blood lymphocytes is induced by Con A in combination with cyclohexamide.⁸

Phylogenetic tree drawn using PAUP method revealed that the carp RGS had vast differences compared to that of its mammalian counterparts (Fig.3). The members of this family have a broad range of biological roles, not only in feed back regulation of G protein function but also in the coordination of the activities of G protein regulated signaling systems with other related pathways. RGS proteins were discovered as negative regulators of G protein signaling in *Saccharomyces cerevisiae* and *Caenorhabditis elegans*.¹ Further studies have to be done on their activity and localization.

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