Molecular Analysis of Complement Regulatory Protein-Like cDNA Composed of 12 Tandem SCRs from the Japanese Flounder

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We cloned and analyzed a complement regulatory protein-like cDNA termed HCRF-2 from a liver cDNA library of the Japanese flounder Paralichthys olivaceus. The cDNA was 3101 bp in length and the predicted translation product of 737 amino acids contains a hydrophobic signal sequence followed by a stretch of 12 short consensus repeats (SCRs). These SCRs were closely related to those of the previously described SBP1 of complement regulatory plasma protein of barred sand bass. SCR domains Nos. 1 to 12 of HCRF-2 resembled domains Nos. 1 to 10 of SBP1. Recently, we reported a complement regulatory protein-like cDNA designated HCRF that contained 7 SCRs. SCR domains Nos. 2 to 7 of HCRF resembled domains Nos. 12 to 17 of SBP1, but SCR domains of HCRF did not resemble those of HCRF-2. In these comparisons, schematic models of HCRF, HCRF-2 and SBP1 clearly showed their close relationship, indicating that these cDNAs originated from the same ancestral gene.

Key words: complement, complement regulatory protein, short consensus repeat (SCR), Japanese flounder

Human complement regulatory proteins, factor H, C4b-binding protein (C4bp), membrane cofactor protein (MCP), decay accelerating factor (DAF) and complement receptor type 1 (CR1) are composed of structural related units known as short consensus repeats (SCRs) (Kristensen et al., 1987; Reid and Day, 1989; Kinoshita, 1991), and interact with C3b and/or C4b (Kristensen et al., 1987). Each SCR covers a stretch of approximately 60 amino acids with a framework of four completely conserved cysteine residues along with highly conserved residues for phenylalanine, glycine, tryptophan, tyrosine and proline (Kristensen et al., 1987; Reid and Day, 1989; Kinoshita, 1991). Furthermore, the DNAs coding for these complement regulatory proteins are located and closely linked within the regulators of the complement activation (RCA) gene on the arm of human chromosome 1, band q3.2 (Lublin et al., 1987; Farries and Atkinson, 1991).

In bony fishes, there are several reports of the cloning of the cDNAs of complement regulatory proteins. Dahmen et al. (1994) and Zipfel et al. (1996) cloned and analyzed two complement regulatory proteins from the barred sand bass Paralabrax neblifer, namely SBP1 and SBCFR-1. Recently, we isolated a complement regulatory protein-like cDNA from the Japanese flounder Paralichthys olivaceus, which we termed HCRF (Katagiri et al., 1998). The SCR domain structures of HCRF were closely related to those of SBP1 and SBCFR-1.

In this paper, we describe the isolation of a complement regulatory protein-like cDNA (HCRF-2) composed of 12 tandem SCRs and the structural relationships among HCRF-2, HCRF and SBP1.

Materials and Methods

cDNA library, sequencing and genomic DNA

A liver cDNA library of the Japanese flounder was prepared, and cloned cDNAs were sequenced as described previously (Inoue et al., 1997). The nucleotide sequences obtained were analyzed using GENETYX-MAC Ver. 8.0 software (SDC software Development). Genomic DNA was isolated from the blood cells of the Japanese flounder by the method of Strauss (1989).

Probes

A survey of the genes expressed in the liver of the Japanese flounder was performed and three hundred and...
thirty randomly selected individual cDNA clones were determined (Inoue et al., 1997). In a preliminary experiment, 396 bp of a clone, designated LG7(8), was sequenced (DDBJ, EMBL and GenBank accession no. C23363). This sequence was found to show extremely high homology (68%) with complement regulatory plasma protein cDNA of barred sand bass (SBP1: DDBJ, EMBL and GenBank accession no. L21703) as indicated by a BLAST program database search. Consequently, we used LG7(8) as a probe in cDNA library screening and Southern blot analysis. The cDNA clone LC11(5) (DDBJ, EMBL and GenBank accession no. C23193) that coded for a part of HCRF (DDBJ, EMBL and GenBank accession no. AB003803) described in Inoue et al. (1997) and Katagiri et al. (1998) was also used as a probe in Southern blot analysis.

cDNA screening and Southern blot analysis

The DNA fragments LC11(5) and LG7(8) were purified from the agarose gel and labeled with \( \alpha \)-\(^{32}\)P-dCTP using the Random Primer DNA Labeling Kit (Takara). The cDNA library screening was performed in a mixture of 5x SSPE (standard sodium phosphate), 5x Denhart’s solution and 0.5% SDS at 65°C. After hybridization for 14 to 18 h, the filter was washed at a final stringency of 0.1% SSPE and 0.1% SDS at 65°C.

Ten µg of genomic DNA, digested by EcoRI, was separated by electrophoresis in an agarose gel and transferred to a Hybond-N+ (Amersham) membrane. Consequently, Southern blot hybridization was performed according to the conditions described above.

Results

A complement regulatory protein-like probe

The sequence of LG7(8) was similar to SBP1, having 68% nucleotide identities over a region of 396 bp, as revealed using BLAST program database searching. Consequently, we used LG7(8) as a probe to screen the cDNA library. A comparison of the nucleotide sequences of LG7(8) and HCRF revealed no homology. We concluded that LG7(8) coded for a complement regulatory protein of a different type of HCRF.

Fig. 1. Nucleotide and deduced amino acid sequences of HCRF-2. The signal sequence, as predicted by PSORT (http://psort.nibb.ac.jp), is underlined. The asterisk indicates the position of the stop codon. The mark \( \alpha \) at position 632 indicates the start of LG7(8). SCR boundaries are shown as “\( \alpha \)”, which are based on the alignment with HCRF, HCRF-2 and SBP1.
Complement regulatory protein-like cDNA

DNA and deduced amino acid analyses

Screening of the Japanese flounder liver cDNA library with LG7(8) yielded 5 positive clones. One of these positive clones, designated HCRF-2, was subcloned and the nucleotide sequence was determined. The nucleotide length of HCRF-2 was 3101 bp. The nucleotide sequence of HCRF-2 showed complete identity with that of LG7(8) over a region of 396 bp beginning at base 632 (Fig. 1). The size of the coding region of HCRF-2 was 2211 nucleotides, encoding 737 amino acid residues.

The first 22 residues of the amino acid polypeptide represent a hydrophobic leader sequence, indicating a signal peptide of a secreted protein. HCRF-2 was organized into repetitive elements (SCRs) which are found in a number of complement regulatory proteins (Figs. 1 and 2). Alignment of these elements showed

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**Table 1.** Homology comparison of the amino acid sequences of SCR domains of flounder (HCRF-2) with those of HCRF, sand bass cofactor protein 1 (SBP1), sand bass cofactor related protein (SBCFR-1), human factor H and human C4bp

<table>
<thead>
<tr>
<th>Flounder</th>
<th>Sand bass</th>
<th>SBCFR-1</th>
<th>Factor H</th>
<th>C4bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCRF-2 (12 SCRs)</td>
<td>SBP1 (17 SCRs)</td>
<td>SBCFR-1 (3 SCRs)</td>
<td>Factor H (20 SCRs)</td>
<td>C4bp (8 SCRs)</td>
</tr>
<tr>
<td>SCR 1</td>
<td>61%</td>
<td>SCR 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCR 2</td>
<td>38%</td>
<td>49%</td>
<td>31%</td>
<td>31%</td>
</tr>
<tr>
<td>SCR 3</td>
<td>31%</td>
<td>66%</td>
<td>32%</td>
<td>31%</td>
</tr>
<tr>
<td>SCR 4</td>
<td>52%</td>
<td>52%</td>
<td>30%</td>
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<tr>
<td>SCR 5</td>
<td>52%</td>
<td>52%</td>
<td>30%</td>
<td>31%</td>
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<tr>
<td>SCR 6</td>
<td>51%</td>
<td>45%</td>
<td>45%</td>
<td>45%</td>
</tr>
<tr>
<td>SCR 7</td>
<td>38%</td>
<td>SCR 7</td>
<td>36%</td>
<td>32%</td>
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<tr>
<td>SCR 8</td>
<td>38%</td>
<td>33%</td>
<td>33%</td>
<td>36%</td>
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<tr>
<td>SCR 9</td>
<td>36%</td>
<td>36%</td>
<td>36%</td>
<td>36%</td>
</tr>
<tr>
<td>SCR 10</td>
<td>55%</td>
<td>SCR 7</td>
<td>31%</td>
<td>SCR 8</td>
</tr>
<tr>
<td>SCR 11</td>
<td>42%</td>
<td>32%</td>
<td>32%</td>
<td>SCR 9</td>
</tr>
<tr>
<td>SCR 12</td>
<td>52%</td>
<td>31%</td>
<td>SCR 10</td>
<td>36%</td>
</tr>
</tbody>
</table>

Only amino acid identities exceeding 30% are listed in the Table.
HCRF-2 to be organized into 12 SCRs (Fig. 2). The amino acid sequences in most of the SCRs (Cys, Pro, Phe or Tyr, Cys, Gly, Cys, Gly, Trp, Pro and Cys) were typically aligned.

**Homology comparison of SCRs**

Table 1 shows homology comparisons of the amino acid sequences of the SCR domains of the Japanese flounder (HCRF-2) with those of HCRF, SBP1, SBCFR-1, human factor H and C4bp. In the case of HCRF-2 and SBP1, the highest amino acid identities of SCRs 1 to 7 and 10 to 12 of HCRF-2 were recorded with SCRs 1 to 7 and 8 to 10 of SBP1 (Table 1). The highest amino acid identities of SCRs 8 and 9 of HCRF-2 were also found with SCR 7 of SBP1.

No such alignments could be discerned between HCRF-2 and HCRF, SBCFR-1, factor H or C4bp.

**Southern blot analysis**

Figure 3 shows the Southern blot analysis. Two signals around 4 kbp and one signal around 12 kbp were confirmed using probes LC11(5) and LG7(8), respectively.

**Discussion**

We identified and analyzed a complement regulatory protein-like cDNA composed of 12 tandem SCRs, designated HCRF-2, from the Japanese flounder. HCRF-2 has a signal sequence of 22 amino acids, and the C-terminus region of SCR 12 does not have a transmembrane domain, cytoplasmic domains or any known homologous domains to those found in non-secreted proteins such as CR1, MCP and DAF (Reid and Day, 1989). These findings strongly suggest that HCRF-2 is a secreted protein.

Recently, we reported that a complement regulatory protein-like cDNA of Japanese flounder, designated HCRF, was composed of 7 SCRs (Katagiri et al., 1998), and indicated the structural relationship between the putative proteins HCRF and SBP1 following their alignment. SCR domains Nos. 2 to 7 of HCRF closely resembled domains Nos. 12 to 17 of SBP1. Similarly, SCR domains Nos. 1 to 12 of HCRF-2 closely resembled domains Nos. 1 to 10 of SBP1 (Table 1), but did not resemble the SCR domains of HCRF. The close homologies of HCRF-2 and HCRF to separate sequential regions of SBP1 suggest that they might be linked, with SCR12 of HCRF-2 preceding SCR 1 of HCRF. From these analyses, we propose the schematic comparison of HCRF, HCRF-2 and SBP1 (Fig. 4). These data strongly suggest that these three genes originated from the same ancestral gene.

For human factor H, three different mRNA species (4.3, 1.8 and 1.4 kb) are abundantly expressed in the liver (Schwaeble et al., 1987). Estaller et al. (1991) cloned and analyzed two cDNAs derived from the 4.3 kb and 1.8 kb mRNAs. The sequence analysis of the 1.8 kb mRNA showed that this species has a very high identity to the 5' portion of the 4.3 kb mRNA. A partial analysis of the factor H gene indicates that these 1.8 kb and 4.3 kb mRNAs may arise by alternative splicing from a single structural gene. We analyzed whether HCRF and HCRF-2 are derived from a single gene by alternative splicing. To do this, we conducted a Southern blot hybridization of HCRF and HCRF-2 with EcoRI-digested genomic DNA (Fig. 3). The result demonstrated that HCRF and HCRF-2 have different loci.

There are many types of complement regulatory protein members in the higher vertebrates (Kristensen et al., 1987; Reid and Day, 1989; Kinoshita, 1991), but there have been few studies on these proteins in bony fishes. The preceding results indicate that HCRF and HCRF-2 should be powerful tools for examining the
evolution of complement regulatory proteins in the vertebrates.

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References


