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Molecular cloning, structural analysis and expression of complement component Bf/C2 genes in the nurse shark, *Ginglymostoma cirratum* ☆

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Abstract

Factor B and C2 are serine proteases that provide the catalytic subunits of C3 and C5 convertases of the alternative (AP) and classical (CP) complement pathways. Two Bf/C2 cDNAs, GcBf/C2-1 and -2 (previously referred to as nsBf/C2-A and nsBf/C2-B), were isolated from the nurse shark, *Ginglymostoma cirratum*. GcBf/C2-1 and -2 are 3364 and 3082 bp in length and encode a leader peptide, three CCPs, one VWFA, the serine protease domain and have a putative factor D/C1s/MASP cleavage site. Southern blots show that there might be up to two Bf/C2-like genes for each of the two GcBf/C2 isoforms. GcBf/C2-1 and -2 are constitutively expressed, albeit at different levels, in all nine tissues examined. Expression in erythrocytes is a novel finding. Structural analysis has revealed that the localization of glycosylation sites in the SP domain of both putative proteins indicates that the molecular organization of the shark molecules is more like C2 than factor B. Phylogenetic analysis indicates that GcBf/C2-1 and -2 and TrscBf of *Triakis scyllia* (another shark species) originated from a common ancestor and share a remote ancestor with Bf and C2 of mammals and bony fish.

Keywords: Shark complement; Factor B; Complement C2; Gene cloning; Evolution; Phylogenetics; *Ginglymostoma cirratum*

Abbreviations and definitions: AP, alternative pathway; C, complement; C1, C2, …, C9, complement components 1 through 9; CP, classical pathway; CCP, complement control protein; E, sheep erythrocyte; EA, antibody-sensitized E; EAC1-7, EA reacted sequentially with the first seven mammalian C components; $DGVB^{2+}$, isotonic dextrose–gelatin–Veronal buffer containing divalent calcium (0.00015 M) and magnesium (0.0005 M); EDTA, ethylenediaminetetraacetate; functionally pure, not contaminated with another C component; intermediate complexes, sensitized E reacted sequentially with various C components, each conferring reactivity for the next component

☆ The nucleotide data reported in this paper have been submitted to the DDBJ, EMBL, and the GenBank nucleotide sequence databases with the following accession number(s): DQ005639 for GcBf/C2-1; DQ007342 for GcBf/C2-2. The accession numbers for amino-acid sequences cited in this paper are as follows: bovine Bf, NP_001035616; bovine C2, NP_001029664; chymotrypsin, BC015118; carp Bf/C2-A, AB007004; carp Bf/C2-B, AB007005; carp Bf/C2-A2, AB021177; carp Bf/C2-A3, AB047361; human Bf, X72875; human C2, NM_000063; lamprey Bf, D13568; medaka Bf, D84063; mouse Bf, NM_008198; mouse C2, NM_013484; pig C2, AAR20890; rat Bf, AAH87089; rat C2, AAH70923; sea urchin Bf, AF059284; trout Bf1, AF089861; trout Bf2, AF089860; Trsc Bf (*Triakis scyllia*), AB049450; xenopus Bf, D29796; xenopus C2, ABB85337.

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1. Introduction

In mammals, the C3 convertase activity associated with the activation of the classical (CP) and alternative (AP) complement pathways resides in two highly specific serine proteases, C2, and factor B, respectively. These two proteins have similar function: specific limited cleavage of the C3 molecule to release two biologically active fragments C3a and C3b [1]. In addition, factor B and C2 also serve as the catalytic moiety of the C5 convertases. A common structural feature shared by these two enzyme complexes is the presence of a serine protease domain containing a triad of active amino-acid residues located in human Bf at His^{526}, Asp^{576}, Ser^{699}, and in C2 at H^{507}, D^{561}, S^{679} [1–4]. Factor B and C2 are modular proteins containing structural motifs which they share with related, but functionally different, serine proteases of the complement system [5]. The two molecules have identical modular structures: each contains three complement control protein (CCP) modules at the N-terminal, one Von Willebrand factor A (VWFA) domain and a serine protease domain at the C-terminal [6,7]. It is believed that the CCP and VWFA structural modules of C2 and factor B contain binding sites for C4b and C3b, respectively [6–9].

The genes encoding factor B and C2 are believed to have evolved from a common ancestor by gene duplication [10]. The point of this duplication can only be determined when sufficient phylogenetic data from a variety of organisms has been obtained. In the nurse shark (*Ginglymostoma cirratum*), the presence of two proteins, analogous to mammalian factor B and C2, has been assumed from functional studies which indicate the presence of two distinct activation pathways, analogous to the CP and AP complement pathways of mammals [11,12, authors' unpublished data]. Cloning of factor B and/or C2 genes from human and other vertebrate species [13–17] has been carried out. Bf/C2 cDNA clones have been isolated from several teleost species [18–22], we report here the cloning, structural characterization, and expression of two distinct Bf/C2 cDNAs from an elasmobranch, the nurse shark (*G. cirratum*).

Bf/C2-like clones from several lower vertebrates generally show the same level of sequence similarity to both C2 and factor B of mammals; consequently, comparison of cDNA sequence alone fails to definitively distinguish between Bf and C2. In the absence of corresponding functional and/or genomic data on the two shark clones reported here, we present an in-depth analysis of the deduced protein structure to determine whether, based on specific structural features, we can predict the putative functional role of fB/C2-1 and -2 as either fB or C2.

2. Materials and methods

2.1. Materials

Restriction enzymes, PCR Supermix High Fidelity, $Oligo(dT)_{12–18}$ primer, TOPO Cloning Kit, TriZol reagent, and Superscript II Reverse Transcriptase were purchased from Invitrogen/Life Technologies (Carlsbad, CA, USA). Big-Dye Terminator Cycle Sequencing Kit (V.2.0) was obtained from PE Biosystems (Foster city, CA, USA). Wizard PlusSV Minipreps DNA Purification System was purchased from Promega (Madison, WI, USA). PCR digoxygenine (DIG) Probe Synthesis Kit was obtained from Boehringer Mannheim (Indianapolis, IN, USA). Hybond N^+ nylon membrane was purchased from Amersham Biosciences (Piscataway, NJ, USA). 5′/3′-RACE PCR Kit was obtained from Clontech (Palo Alto, CA, USA). Lumiphos Plus was purchased from Whatman Biosciences (MA, USA).

2.2. Animal

A 2 kg female nurse shark (*G. cirratum*) was obtained from the Keys Marine Laboratory (Long Key, Florida Keys, FL). The animal was transported to Florida International University (FIU) in a 124 litre tank in aerated seawater. It was anesthetized in 3-aminobenzoic acid ethyl ester, bled from the caudal vein and then sacrificed for meticulous dissection of tissues while avoiding cross contamination. All dissected tissues were immediately frozen in liquid nitrogen and stored at −80 °C until used.

2.3. Isolation of peripheral blood cells

Whole anticoagulated (3.8% trisodium citrate) blood was withdrawn from the caudal vein and diluted with an equal volume of shark-RPMI (RPMI with 0.35 M urea, 0.25 M NaCl). Eight milliliters of diluted blood was layered onto the surface of 6 ml Ficoll–Paque gradient mixture [23] and centrifuged at $435 \times g$ for 30 min at 15 °C. The separated layer of leukocytes was carefully removed by aspiration and transferred to a sterile 15 ml tube.