Fish & Shellfish Immunology (2008) **24**, 18–25

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journal homepage: www.elsevier.com/locate/fsi

The vitronectin gene in rainbow trout: Cloning, expression and phylogenetic analysis

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Received 18 April 2007; revised 19 June 2007; accepted 1 July 2007
Available online 20 July 2007

KEYWORDS
Evolution;
Comparative immunology;
Cloning;
Gene organization;
mRNA expression;
Rainbow trout;
Vitronectin

Abstract Vitronectin is a major cell adhesion glycoprotein that is found in plasma and the extracellular matrix. Vitronectin consists of an N-terminal somatomedin B domain and two hemopexin-like domains and controls functions including cell adhesion, migration, haemostasis and immune defence. In order to study the molecular evolution of the complement lytic pathway regulation, we have cloned and characterized the vitronectin gene from rainbow trout (*Oncorhynchus mykiss*). The deduced amino acid sequence of trout vitronectin exhibits 45%, 46%, 47% and 63% identity with human, chicken, Xenopus and zebrafish orthologs, respectively. The domain architecture of the trout vitronectin, consisting of a somatomedin B domain and two hemopexin-like domains, resembles that of mammalian vitronectins. Analysis of partial genomic clones shows that trout vitronectin gene exhibits the same exon–intron organization profile as the human ortholog gene. The trout vitronectin gene is probably present as a single copy in the trout genome, showing a differential expression pattern among tissues investigated.

Introduction

Vitronectin (VN), also known as complement S protein and serum spreading factor, is a 75-kDa multifunctional adhesion glycoprotein that is present abundantly in plasma (200–500 μg ml^{-1}) and the extracellular matrix [1,2]. The majority of plasma VN, like other plasma proteins, originates from the liver in mammals [3]. VN not only regulates adhesive events, but also controls a number of these proteolytic enzyme cascades, including the complement, coagulation, and fibrinolytic systems [2,4].

VN is anchored to the extracellular matrix via collagen or proteoglycan binding and promotes cell attachment, spreading, and migration through specific interactions of its single Arg–Gly–Asp (RGD) sequence with cellular integrins [5,6]. In addition to its function in cell adhesion and migration, VN interacts with several critical proteins that regulate thrombosis and fibrinolysis, protecting, for example, thrombin from rapid heparin dependent inactivation by antithrombin III, possibly because it acts as a heparin scavenger [7–9]. Plasma VN also functions in the complement cascade to

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doi:10.1016/j.fsi.2007.07.007

prevent innocent bystander cytolysis by binding fluid phase C5b-7 and forming soluble, noncytolytic complexes [10,11].

The human VN gene, 5.3 kilobases in size, consists of eight exons and seven introns [12]. Its intron positions and their phase type are compared to those in the hemopexin gene which shares amino acid sequences homologies with tansin and VN. Intron 1 and 2 are located at the boundaries of the somatomedin B domain (SO) of VN gene, whereas introns 3—7 occur at a conserved glycine residue within repeating peptide segments. Comparison of the VN gene structure with that of hemopexin can be used to formulate a model for their evolution [12].

The mature human VN contains 459 amino acid residues and is composed of a number of functionally unique domains [6,13]. The VN NH_2- terminus (residues 1—44) contains the Cys-rich SO domain. The high affinity type 1 plasminogen activator inhibitor (PAI-1) binding site has been localized at the VN SO domain [14]. This domain contains eight Cys residues arranged in four disulphide bonds (Cys^5—Cys^9, Cys^{19}—Cys^{21}, Cys^{25}—Cys^{31} and Cys^{32}—Cys^{39}), suggesting that it is folded into a compact structure that is stabilized by the disulphide bonds [6,15,16]. All eight Cys residues are strictly conserved in the VN molecules isolated from a number of other species, including rabbit [17], mouse [3], porcine [18] and chicken [19], as well as in other SO-like proteins, such as plasma cell membrane glycoprotein PC-1 [20] and autotoxin [21].

The single RGD sequence of human VN is located immediately COOH-terminal to the SO domain in the so-called connecting region, a region that also contains a putative collagen-binding site [22]. Cell surface vitronectin receptor, mainly integrin $\alpha v\beta 3$, interacts with VN through this tripeptide [23]. The two most COOH-terminal domains of VN have three and four tandem repeat sequences (HX), respectively, with weak homology to sequences of hemopexin [24]. The second HX domain contains the positively charged heparin-binding segment (residues 348—370) [25].

At present, although sequences of hypothetical proteins from Xenopus, tetraodon and zebrafish that are deposited in GenBank (accession numbers AAH89081, CAG08847 and AAH55570 respectively) seem to be the VN orthologs, this is the first report of characterization of a VN gene in low vertebrates. To gain further insight into the evolution of this molecule, we report here the primary sequence, the mRNA expression, the phylogenetic analysis and a partial genomic organization of the VN gene from rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Cloning of trout vitronectin

RNA isolation and cDNA library construction

Total liver cDNA was prepared from RNA extracted from liver as previously described [26].

Trout vitronectin probe isolation

Degenerated oligonucleotides were designed based on conserved regions of known vitronectin orthologs: sense TVNF1 21-mer: 5′-(T/C)TNAA(A/G)AA(C/T)GGN AG(C/T)AT(A/C/T) TA(C/T)-3′ based on LKNGSIY amino acids and anti-sense TVNR1 21mer: 5′-NCC(C/T)TTNA(G/A)(A/G)AA(A/G)TANGT (C/T)TT-3′ based on KTYFLKG amino acids corresponding to amino acids 165—171 and 216—222 of chicken vitronectin (GenBank accession number:Y11030), respectively (the symbol N represents the mixture of nucleotides: A, T, C and G). These primers were subsequently applied in a RT—PCR reaction, using as template total trout liver RNA. One cycle was conducted at 50 °C for 30 min. Thirty cycles were conducted, using a PCR thermocycler under the following program: 95 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. The PCR product of the expected size (170 nt) was gel-purified (QIAquick, Qiagen), ligated into the T/A cloning vector pGEM-T easy (Promega) at 4 °C overnight and transformed into *Escherichia coli* DH5a subcloning competent cells (Invitrogen). Positive clones were selected and plasmid DNA was extracted (mini-prep kit, Roche).

Screening of a trout liver cDNA library

2×10^5 λgt11 recombinant phages of a trout liver cDNA library were screened under high stringency conditions (65 °C) using an α-^{32}P labeled cDNA probe corresponding to the 171 nt product described above. The probe was labeled using the random primed DNA labeling kit (Boehringer Mannheim). Positive plaques were picked up and cultured, the recombinant phage DNA corresponding to the longest clone in size was isolated, and the insert cDNA was subcloned into pGEM-T easy vector and sequenced.

Nucleotide sequencing of trout vitronectin

Definition of primary structure of trout vitronectin was performed by dideoxy-chain termination method using the DNA Sequencer Long Read IR 4200 (Li-Cor). All sequences were determined at least twice for both strands.

Isolation of a partial trout vitronectin genomic clone

Two specific oligonoucleotides were designed based on the full-length vitronectin cDNA sequence: sense TVITF2 21-mer: 5′-ACCTACTGACTCTGGATTTGT-3′ and anti-sense TVITR2 21mer: 5′-GGCATCAAATGTGTCACCGCG-3′ corresponding to positions 13—33 and 313—333 of trout vitronectin cDNA. These primers were subsequently applied in a PCR reaction, using as template trout genomic DNA. Thirty cycles were conducted, using a PCR thermocycler under the following program: 95 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. A single PCR product was obtained (~850 nt) that was gel-purified (QIAquick, Qiagen), ligated into the T/A cloning vector pGEM-T easy (Promega) at 4 °C overnight and transformed into *E. coli* DH5a subcloning competent cells (Invitrogen). Positive clones were selected and plasmid DNA was extracted as previously. The inserted genomic DNA was sequenced and subsequently aligned with trout vitronectin cDNA sequence.