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Short communication

Calcium-dependent self-association of the C-type lectin domain of versican

Andreas Ney[a], Patrick Booms[a], Guido Epple[b], Matthias Mörgelin[c], Gao Guo[a], Gerhard Kettelgerdes[b], Reinhard Geßner[b], Peter N. Robinson[a,*]

[a] *Institute of Medical Genetics,Charité University Hospital, Humboldt University, Augustenburger Platz 1, 13353 Berlin, Germany*
[b] *Institute of Laboratory Medicine and Pathobiochemistry, Charité University Hospital, Humboldt University, Berlin, Germany*
[c] *Department of Clinical Science, Section of Clinical and Experimental Infectious Medicine, Lund University, Sweden*

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Abstract

Versican is a large (1–2 × 10^6 Da) chondroitin-sulfate proteoglycan that can form large aggregates by means of interaction with hyaluronan and also binds to a series of other extracellular matrix proteins, chemokines and cell-surface molecules. Versican is a multifunctional molecule with roles in cell adhesion, matrix assembly, cell migration and proliferation. Characterization of the binding interactions mediated by the various domains of versican is a first step towards understanding the functions of versican and interacting molecules in the extracellular matrix. In this study we investigated a recombinant construct corresponding to the C-type lectin domain of versican and demonstrated a calcium-dependent self-association of this region by blot overlay and plasmon surface resonance assays. Electron microscopy provided further evidence of the relevance of the binding reaction by demonstrating a mixture of monomers, dimers and complex aggregates of recombinant versican C-type lectin domain. This binding reaction could contribute to the ability of versican to organize formation of the proteoglycan extracellular matrix by inducing binding of individual versican molecules or by modulating binding reactions to other matrix components.

Keywords: Versican; C-type lectin domain; Surface plasmon resonance; Extracellular matrix; Connective tissue; Electron microscopy

1. Introduction

Versican is a large (1–2 × 10^6 Da) chondroitin-sulfate proteoglycan to which 12–15 chondroitin-sulfate side chains are covalently attached, and is one of a family of extracellular proteoglycans that interact with hyaluronan including aggrecan, neurocan and brevican (Wight, 2002). These proteoglycans share a tridomain structure with an amino-terminal globular region (G1) that binds to the glycosaminoglycan hyaluronan (LeBaron, Zimmermann, & Ruoslahti, 1992), a middle region encoded by two large exons that

* Corresponding author.: Tel.: +49 30 450 569124; fax: +49 30 450 569915.
E-mail address: peter.robinson@charite.de (P.N. Robinson).

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contains the chondroitin-sulfate attachment region, and a C-terminal region (G3) with two epidermal-growth-factor-like motifs (EGF), a C-type lectin domain, and a complement regulatory protein-like module (Naso, Zimmermann, & Iozzo, 1994). Versican is found in the extracellular matrix (ECM) in a large number of tissues such as blood vessels, the central and peripheral nervous systems and skin (Bode-Lesniewska et al., 1997).

Lectins refer to non-enzymatic proteins that selectively bind to specific carbohydrate structures. The C-type (Ca^{2+}-dependent) lectins are soluble, extracellular proteins that are characterized by the presence of 18 nearly invariant residues within a ∼ 130 amino-acid domain (Drickamer, 1988). The C-type lectin domain (CLD) of versican is involved in binding to various ECM proteins including fibulin-1 (Aspberg, Adam, Kostka, Timpl, & Heinegard, 1999), fibulin-2 (Olin et al., 2001), tenascin-R (Aspberg, Binkert, & Ruoslahti, 1995; Aspberg et al., 1997), tenascin-C (Day et al., 2004) and fibrillin-1 (Isogai et al., 2002); however, the binding appears to be mediated by protein–protein interactions rather than protein–carbohydrate interactions (Aspberg et al. 1997; Aspberg et al. 1999; Olin et al., 2001). Additionally, the G3 domain of versican, which contains the CLD, forms a complex with vascular endothelial growth factor and fibronectin (Zheng et al., 2004).

Based on the tripartite domain structure of versican and the binding interactions summarized above, a major functional role of versican would appear to involve linking various components of the ECM by binding extracellular hyaluronan at its N-terminal end (LeBaron et al., 1992; Evanko, Angello, & Wight, 1999; Shibata, Fukada, Suzuki, Ogawa, & Yamashita, 2002; Matsumoto et al., 2003), and less complex sugars as well as multiple ECM proteins at its CLD at the C-terminus; the central domain provides a means of introducing glycosaminoglycan chains into the extracellular matrices where versican is expressed (reviewed in Iozzo (1998)).

In the present work we present evidence for self-interaction of the CLD of versican. We demonstrated binding by means of blot overlay assay and surface plasmon resonance investigations and showed the binding interaction was calcium-dependent. Electron microscopic molecular imaging of a solution of recombinant versican CLD revealed a mixture of monomers, dimers and larger complexes. The self-interaction of the versican CLD could modulate the biological activity of versican by adding another type of intermolecular interaction to the complex networks of interacting molecules of which versican is a part.

2. Materials and methods

2.1. Production of the versican rCLD-versican construct

A recombinant construct corresponding to the C-type lectin domain (amino acids 3164–3291; Swiss-Prot P13611) was produced ("rCLD") with the cloning primers gtat*ggcccagccggcc*caagataccgagacatgtgac (forward; nucleotides 9490–9510) and gtat*gggccc*tttctgcacgtataggtg (reverse; nucleotides 9855–9873; nucleotide numbering from start codon with sequence according to NCBI entry U16306.1). The primers contain a 4 bp dummy sequence followed by a *Sfi*I site (f) and an *Apa*I site (r) (italicized). The versican-specific sequence was previously described (Aspberg et al., 1995). Production of recombinant fragments using the eukaryotic expression vector pSecTag2 (Invitrogen Life Technologies) and HEK-293 cells and purification were performed as described (Booms, Tiecke, Rosenberg, Hagemeier, & Robinson, 2000). The construct encompasses 128 versican amino acids with a calculated molecular weight of 15 kDa (Fig. 1). Concentration of the recombinant polypeptide was determined with the BCA protein assay (Pierce) according to the manufacturer's instructions.

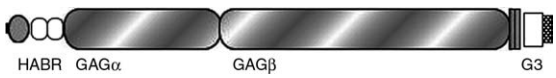

Fig. 1. Cartoon of versican (isoform V0; UniProt accession number P13611) demonstrating the location of the CLD in the protein. Versican contains an N-terminal globular domain G1, also called hyaluronan-binding region (HABR), consisting of an Ig-fold and two link-protein-like modules, followed by glycosaminoglycan attachment region alpha (GAGα) and beta (GAGβ), followed by the G3 domain. G3 consist of two epidermal growth factor-like (EGF) domains (shown here in gray), the C-type lectin domain (white rectangle), and finally the C-terminal complement regulatory protein-like module (also known as "sushi domain", and shown as a stippled rectangle). The recombinant fragment studied in the present work corresponds to the C-type lectin domain.