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Heparin/heparan sulfate controls fibrillin-1, -2 and -3 self-interactions in microfibril assembly

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ABSTRACT

Fibrillins form multifunctional microfibrils in most connective tissues. Deficiencies in fibrillin assembly can result in fibrillinopathies, such as Marfan syndrome. We demonstrate the presence of heparin/heparan sulfate binding sites in fibrillin-2 and -3. Multimerization of all three fibrillins drastically increased the apparent affinity of their interaction with heparin/heparan sulfate. Surprisingly, contrary to other reports heparin/heparan sulfate strongly inhibited homo- and heterotypic N-to-C-terminal fibrillin interactions. These data suggest that heparin/heparan sulfate controls the formation of microfibrils at the bead interaction stage.

Structured summary of protein interaction:
rFBN1-N binds to rFBN1-C by solid phase assay (View interaction)
rFBN1-N binds to rFBN2-C by solid phase assay (View interaction)
rFBN2-N binds to rFBN1-C by solid phase assay (View interaction)
rFBN2-N binds to rFBN2-C by solid phase assay (View interaction)
Fibronectin binds to rFBN2-C by solid phase assay (View interaction)
Fibronectin binds to rFBN2-N by solid phase assay (View interaction)
Fibronectin binds to rFBN1-N by solid phase assay (View interaction)
Fibronectin binds to rFBN1-C by solid phase assay (View interaction)
Fibronectin binds to rFBN3-C by solid phase assay (View interaction)

1. Introduction

Three extracellular glycoproteins, fibrillin-1, -2 and -3 constitute the fibrillin family. Each member of this family is characterized by a modular organization composed primarily of calcium-binding epidermal growth factor-like (cbEGF) domains and transforming growth factor (TGF)-β binding domains (TB) [1]. Fibrillins are the main integral components of multi-component assemblies, termed microfibrils [2]. Extracted microfibrils display a characteristic "bead-on-a-string" structure [3]. Microfibrils fulfill a number of crucial physiological functions in the cardiovascular system, bones, eyes, skin and other tissues [4]. They act as a scaffold in elastic fiber formation, as stress-bearing entities, and as reservoirs for growth factors of the TGF-β superfamily [5–7]. Deficiencies in microfibrils have devastating consequences on tissue function and integrity resulting in severe connective tissue disorders [8]. Fibrillin-1 mutations result for example in Marfan syndrome, autosomal dominant Weill–Marchesani syndrome and stiff skin syndrome, whereas fibrillin-2 mutations cause congenital contractural arachnodactyly [9–12].

Despite recent advances, the complete mechanism of fibrillin assembly into microfibrils is still poorly defined. We previously demonstrated that the recombinant C-terminal half of fibrillin-1 multimerizes in a cell-associated fashion [13]. The multimers have a characteristic bead shape with 8–12 peripheral arms, closely

Abbreviations: BSA, bovine serum albumin; cbEGF, calcium-binding epidermal growth factor-like domain; MAGP-1, microfibril-associated glycoprotein-1; TB, transforming growth factor-β binding domain; TBS, Tris-buffered saline; TBST, TBS/Tween-20

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resembling the beads in microfibrils. We also showed that multimerization of the fibrillin-1 C-terminus increases the apparent affinity to its N-terminus [13]. Elongation occurs through fibrillin N-to-C terminal interactions in a polarized manner [14,15]. Several molecules have been implicated in microfibril assembly including heparan sulfate and fibronectin [16–19].

Proteoglycans and glycosaminoglycans have been localized to microfibrils and some have been implicated in microfibril assembly. The dermatan sulfate-containing biglycan interacts with microfibrils, whereas decorin can form a ternary complex with fibrillin-1 and microfibril-associated glycoprotein-1 (MAGP-1) [20]. The heparan sulfate-containing proteoglycan perlecan in basement membranes directly interacts with fibrillin-1 and colocalizes with microfibrils at basement membrane zones with potential implications on microfibril assembly in these regions [21]. Kielty et al. have demonstrated that microfibril integrity is disrupted by treatment with chrondroitinase-4,6-sulfate lyases [22]. The most studied glycosaminoglycan in microfibril assembly is heparin/heparan sulfate. Heparan sulfate consists of repeats of sulfated disaccharide units of glucuronic acid and N-acetylglucosamine, and each unit can be modified by N- and O-sulfation as well as by uronate epimerization [23]. These modifications often occur in clusters resulting in sulfated domains. The degree of modification regulates protein interaction to heparan sulfate. For example, fibrillin-1 only interacts with highly sulfated heparan sulfate [17]. Heparan sulfate is not found as a free glycosaminoglycan in tissues. In its physiological state it is covalently linked to a number of core proteins to form various proteoglycans including cell surface located syndecans and glypicans or matrix located perlecan, agrin, collagen type XV or type XVIII [23]. Heparin is structurally very similar to heparan sulfate and is frequently used experimentally as a cost-effective substitute for heparan sulfate [23].

Fibrillin-1 interaction domains with heparin/heparan sulfate have been described in seven regions of the protein (see Fig. 1A) [17,19,24–26]. A heparin/heparan sulfate interaction site in fibrillin-2 has been localized to cbEGF7-TB3 [19]. The fibrillin-1 heparin/heparan sulfate interaction sites were shown to be specific for heparin/heparan sulfate and could not interact with other glycosaminoglycans [17]. Knowledge of heparin/heparan sulfate interactions with fibrillin-2 is still rudimentary, and it is not known if fibrillin-3 also interacts with this glycosaminoglycan. Heparin/heparan sulfate may regulate the composition of microfibrils as it inhibits in vitro the interactions of tropoelastin or MAGP-1 with

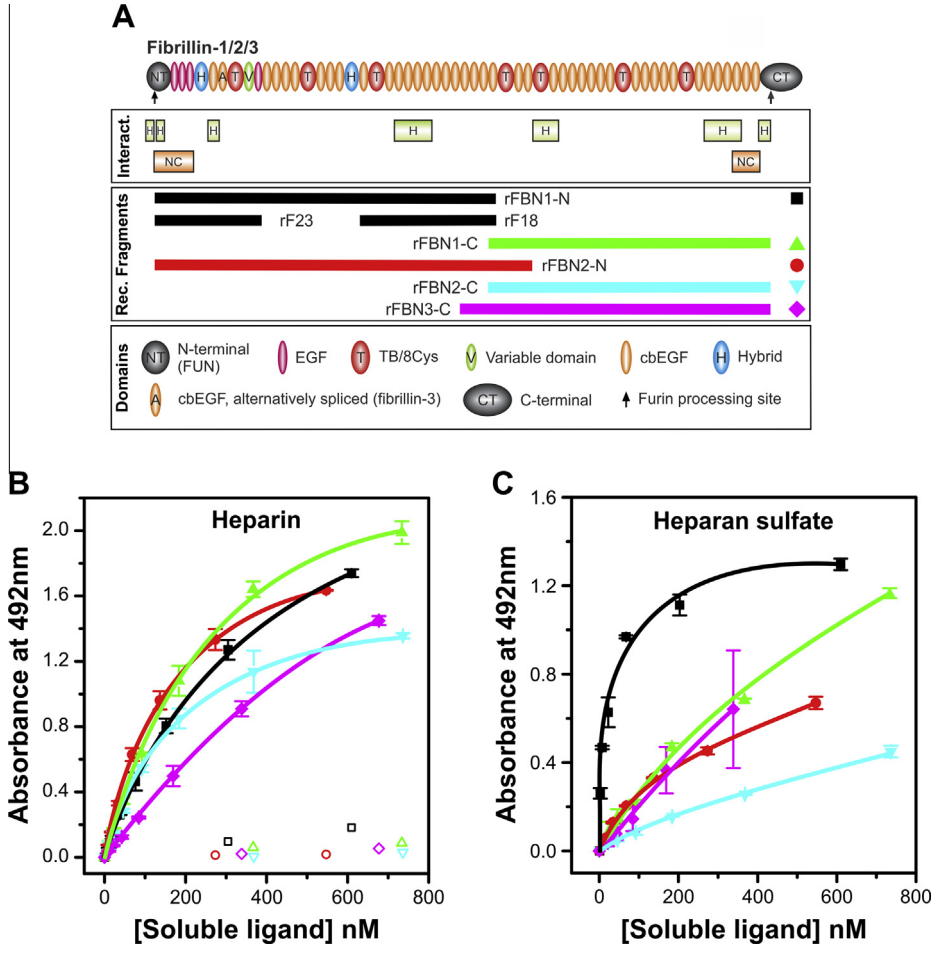

Fig. 1. Fibrillin interactions with heparin and heparan sulfate. (A) A schematic drawing of recombinant fibrillin-1, -2 and -3 is shown on top. The "Interactions" panel indicates previously identified heparin-binding sites ("H") as well as N-to-C-terminal ("NC") fibrillin interaction sites in fibrillin-1. The "Recombinant Fragments" panel illustrates the fibrillin fragments used in this study. The color and symbol of each fragment corresponds to the respective fragment binding profile in B and C. Note that the first heparin-binding site located in the N-terminal propeptide of fibrillin-1 is not included in the recombinant fragments. The "Domains" panel indicates the names of the domains corresponding to the schematic fibrillin drawing on top. (B) Shown is a representation of a typical solid phase binding assay. Heparin-BSA (closed symbols) and BSA (open symbols) used as a control, were immobilized. Serial dilutions of recombinant fibrillin fragments were added as soluble ligands at the indicated concentrations. (C) Soluble recombinant fibrillin fragments were added to immobilized heparan sulfate-BSA. For B and C, all C-terminal fragment preparations consisted of a mixture of fibrillin assembly states and were not gel-filtrated. Data sets represent means of duplicates; standard deviations are indicated. Non-specific interaction with BSA (OD_{492nm} = 0.1–0.2) was subtracted from all values.