APPLICATION OF SINGLE MOLECULE FORCE SPECTROSCOPY TO THE STUDY OF PROTEOGLYCAN STRUCTURE-FUNCTION RELATIONSHIP

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Proteoglycans are a diverse group of macromolecules composed of one or more glycosaminoglycan chains covalently bound to a protein core. The biological roles assigned to proteoglycans are highly diversified, ranging from relatively straightforward mechanical functions to effects on more dynamic processes such as cell adhesion and motility, to complex and still poorly understood roles in cell differentiation and development. The efforts aimed to a better understanding of proteoglycan structure and function are often hampered by their large molecular size. Marine sponge proteoglycans are relatively small and can be extracted in considerable amounts while preserving their biological activity [1]. We have applied atomic force microscopy imaging and single molecule force spectroscopy (SMFS) to the study of proteoglycan structure-function relationships using sponge proteoglycans as model [2]. Sponge cells associate in a species-specific process through multivalent self-interactions of extracellular bifunctional proteoglycan molecules [3]. A molecular dissection experimental strategy has allowed us to track the individual self-binding units down to a 200-kDa glycan (g200), using a SMFS approach that highlights the potential of this technique in its applications to the emerging area of glycomics, the study of cellular carbohydrate components such as glycans. Glycan structures have immense structural diversity, ubiquitous distribution, and are associated with the cell surface, as required of cell recognition molecules [4]. g200-g200 interactions are characterized by relatively weak forces which, when multimerized, can be easily potentiated by orders of magnitude, representing a highly versatile form of cell recognition and adhesion. The multiplicity of binding sites confers a high degree of modulability as required in most biological interactions, in contrast to the higher stability of a single, strong bond. Our force spectroscopy data reveal surprisingly high forces and selectivity of intermolecular adhesion domains. Fitting to a worm-like chain model of the individual peaks in proteoglycan force-extension curves indicates that there is an elastic component involved with a persistence length of ca. 20 nm. This elastic behavior likely results from the molecular stretching of the protein to which g200 is covalently linked that precedes the dissociation of individual glycan-glycan interactions. This modular elongation mechanism, be it intra- or intermolecular, has been proposed to be a general strategy for conveying toughness to natural fibers and adhesives.

References:

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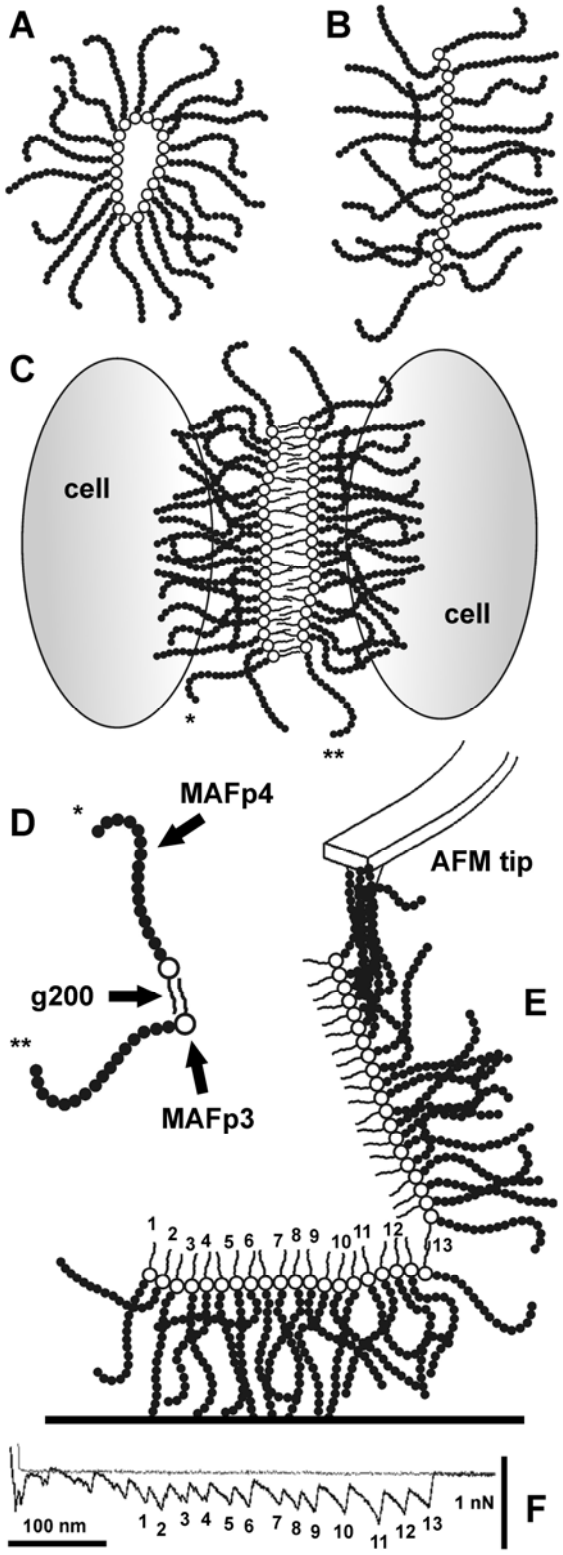

Figure 1. SMFS study of carbohydrate-carbohydrate interactions in proteoglycans. E, Scheme of a SMFS experiment to study the adhesive interactions between two adhesion proteoglycan molecules. For clarity, both molecules are represented linearized. F, Typical SMFS approach-retract curve.

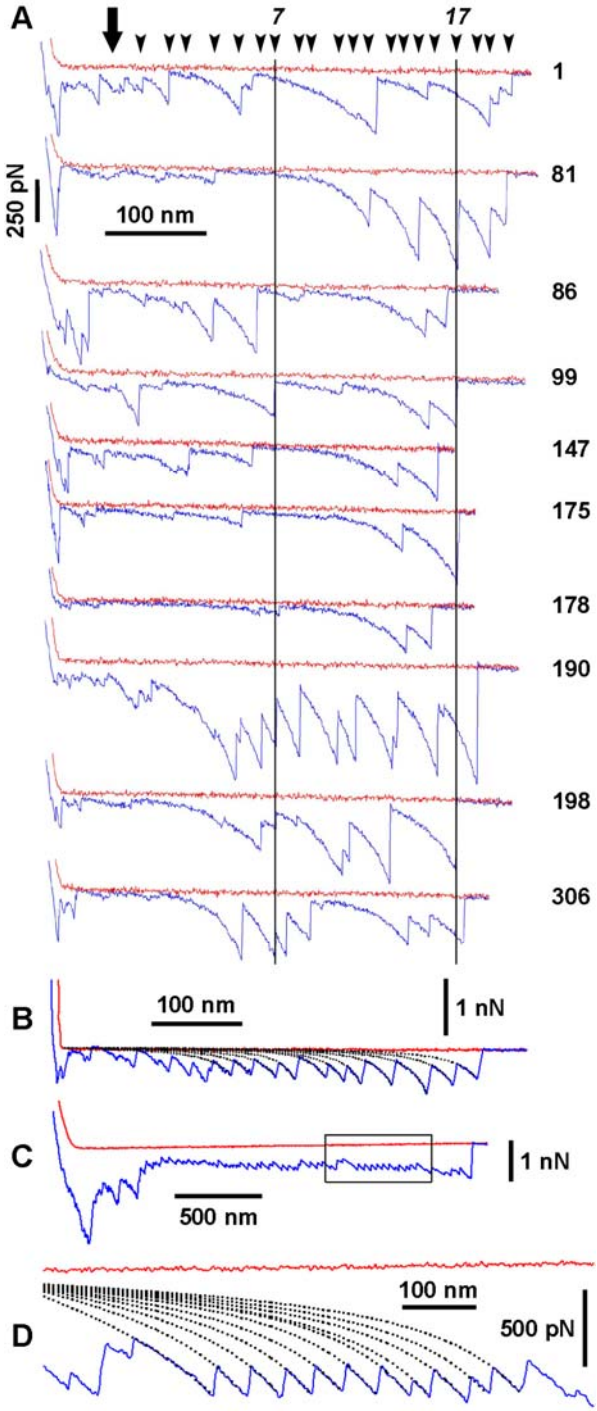

Figure 2. SMFS analysis of carbohydrate-based proteoglycan adhesion. A, Alignment of 10 force-extension curves selected from several hundred consecutive approach-retract cycles in a single SMFS experiment. B, Worm-like chain (WLC) fit of a proteoglycan force-extension curve in a typical single-molecule interaction. C, Force-extension curve corresponding to a concatamer of several molecules. D, Blow-up of the retraction curve section boxed in C, showing the WLC fit.