Identifying the SPARC binding sites on collagen I and procollagen I by atomic force microscopy

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Statement of Purpose: SPARC (secreted protein acidic and rich in cysteine) is a matricellular protein associated with the extracellular matrix (ECM). It has been found that the production of collagen I is a requisite for the association of SPARC with ECM, and studies with SPARC-null mice indicate that SPARC plays a role in modifying the structure of collagen fibers. Characterization of the SPARC-binding sites on collagen I and procollagen I will provide useful information for further understanding of the functional implications of their interactions [1].

Methods: To form SPARC-collagen I or SPARC-procollagen I complexes, collagen I or procollagen I (50 µg/ml) was incubated with SPARC at a 1:4 molar ratio in Tris-buffered saline with 1mM $CaCl_2$, overnight at 4 ºC. Each sample was diluted 200 times before deposition to achieve an optimum coverage for the purpose of visualizing individual collagen I or procollagen I molecules with bound SPARC in AFM images. 20 µL of the diluted sample was deposited onto freshly cleaved mica substrates (Asheville-Schoonmaker Mica Co., Newport News, VA) immediately after dilution. After 5 minutes, the mica substrate was thoroughly rinsed with DI water to remove loosely bound molecules a nd salts, followed by drying with N_2. All AFM images were acquired using a multimode Nanoscope IV (Digital Instruments, Santa Barbara, CA) equipped with a 10 µm E scanner and operated in tapping mode. The height profiles of collagen I, procollagen I, as well as SPARC-collagen I and SPARC-procollagen I complexes, were obtained as a function of the lengths of collagen I or procollagen I by manually tracking on AFM images using the software WSxM 4.0 (Nanotec Electronica, Madrid, Spain). The length distributions of collagen I and procollagen I were also mapped from their AFM images. Only those collagen I and procollagen I molecules within a range of the most probable lengths were used to determine the SPARC binding sites.

Results / Discussion: The TM-AFM technique was applied to obtain the height and length profiles of collagen I, procollagen I and SPARC adsorbed onto mica and to visualize SPARC-collagen I and SPARC-procollagen I complexes (Figure 1). The distances of bound SPARC to the C- terminus of procollagen I and to the closest end of collagen I were measured, and their histograms were constructed. Only those collagen I and procollagen I molecules with the most probable lengths were used to obtain the binding profile of SPARC. There is a broad distribution of SPARC binding sites on both collagen I and procollagen I, with a most preferred SPARC binding region located 87.5-125 nm from the C-terminus of procollagen I. The binding of the highly-negatively charged SPARC to this highly positively-charged region could be electrostatic. Excellent agreement between the distributions of SPARC binding sites on collagen I and procollagen I ensures the reliability of the approach and the results. The overlap of the most preferred SPARC binding region with the binding sites of other ligands, as well as the fibrillogenesis inhibition and collagenase cleavage site of collagen I, provides insight for the functional implications of the interactions between SPARC and collagen I.

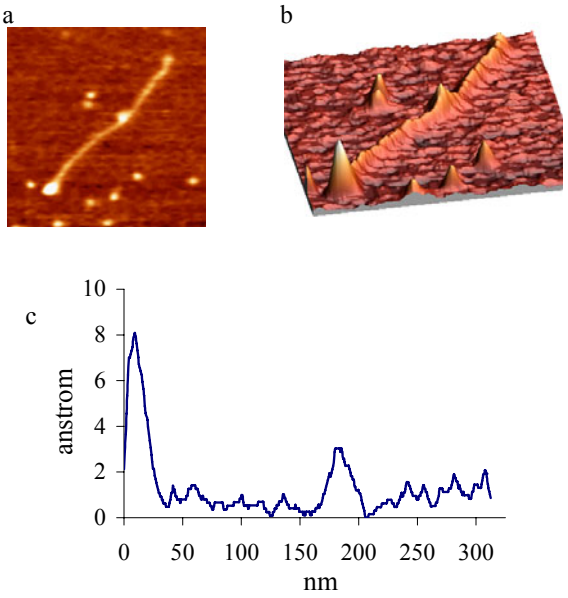

Figure 1. A selected region from AFM image showing the SPARC-procollagen I complex. (b) and (c) are the side-view and height/length profile of the procollagen I molecule shown in (a), respectively.

Conlusions: There is a broad distribution of SPARC binding sites on procollagen I with the most preferred binding region located about 1/3 from the C-terminus. Characterization of the SPARC-binding sites on collagen I and procollagen I provides useful information for further understanding of the functional implications of their interactions.

References:

1. Brekken, R. A.; Sage, E. H. Matrix Biol. 2001, 19, 815-827.