Shear-Induced Adhesion in Mussel Foot Protein-1 Films

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Statement of Purpose: The need for effective biomedical glues spans a wide range of applications, from wound-healing sealants to dental implants and restorations. While most conventional adhesives lose their bridging abilities in the aqueous, saline environment of the body, nature has developed efficient solutions to this problem. Marine mussels are able to make strong, permanent attachments to nearly any available surface in their turbulent ocean habitat. The mussel uses an assembly of polyphenolic proteins rich in the amino acid 3,4-dihydroxyphenyl-alanine (DOPA), and single-molecule experiments have demonstrated high attachment strengths resulting from this moiety in isolation[1]. The largest of these proteins, mussel foot protein-1 (mfp-1), contains a high DOPA content, yet in its native state, it is unable to bridge surfaces together. This suggests that the mere presence of DOPA is not sufficient to formulate an effective glue. The conformation of the protein must work in concert with its chemical components to enable its function as an adhesive. The dynamic response of mfp-1 to mechanical stresses can provide clues as to the adhesive properties of the protein in its turbulent environment. Recent research[2] using the surface forces apparatus (SFA) indicates that shearing the exposed side of mfp-1 films can promote adhesion in the films. Using an Atomic Force Microscope (AFM) in liquid, we have investigated in detail this phenomenon over varying parameters such as protein film thickness and shearing conditions. By doing so, we have identified the relevant factors which determine the magnitude and limitations of the adhesive bridging capability that can be induced in mfp-1.

Methods: Powders of mfp-1 were purified in the lab of Dr. J.H. Waite at UCSB according to published methods[3]. They were dissolved in a 0.1M sodium acetate, 0.25M potassium nitrate buffer (pH 5.5). Solutions of mfp-1 were deposited onto freshly-cleaved mica substrates and rinsed with buffer after various adsorption times. The topography of mfp-1 films was characterized using TappingMode® AFM in buffer (Agilent Technologies, Santa Clara CA). Shearing of the surface of mfp-1 was performed in the AFM by using a large, spherical silica bead (radius 300nm) – terminated tip (Novascan Technologies, Ames IA). During shearing, the friction between the silica tip and the mfp-1 film was measured through lateral force microscopy. Following each shearing cycle, adhesion spectroscopy was performed by recording normal force-distance profiles at the surface of the sheared film. All AFM experiments were performed in buffer.

Results: Globally, our results show an increase in adhesion resulting from shearing the mfp-1 films with a bare silica tip. Changes in adhesion were found to depend on both the shearing parameters and the sample preparation. More specifically, adhesion was affected by the number of shearing cycles and the thickness of the adsorbed mfp-1 films, which was modulated by the adsorption time of mfp-1 on the substrate. In *thick* films, the adhesion increased with initial shearing cycles, and was followed by a drop to a steady-state adhesive plateau, which was independent of shear (Fig. 1A). This phenomenon has been attributed to irreversible damage of the protein film, leading to removal of mfp-1 molecules from the film and their likely attachment to the AFM tip. Shearing the film with an mfp-1-covered AFM probe had almost no effect on adhesion (Fig 1B) and led to low and stable friction (data not shown). In *thin* films of mfp-1, the adhesion measured was significantly higher than in the thicker films, and monotonically increased with no drop in adhesion observed, even after prolonged shearing (data not shown).

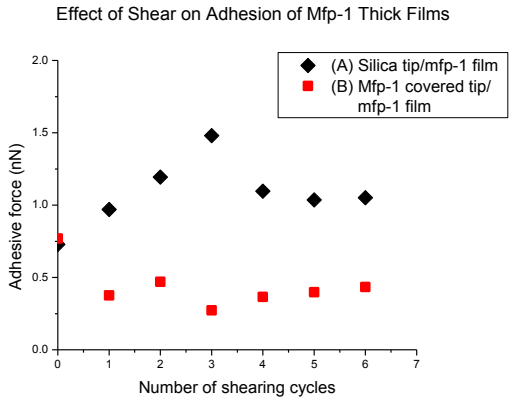

Figure 1. Change in adhesion to the mfp-1 film surface due to shearing using a bare vs. protein-covered AFM tip. (A) Shearing with a silica probe, adhesion increased before reaching a steady-state value. (B) With the mfp-1-coated probe, adhesion is low and barely affected by shear.

Conclusions: The shear-induced increase in adhesion has been attributed to the ability of the AFM tip to progressively rearrange the mfp-1 molecules that are initially bound to the mica substrate, thereby exposing the aforementioned adhesive DOPA groups to the film surface. This effect is more pronounced in thin rather than thick protein films. Prolonged shearing of thick films leads to irreversible detachment of proteins from the bulk film. Finally, when shearing the mfp-1 films with an mfp-1 covered tip, shearing ceases to impact the adhesion of the film. Instead, constant low adhesion and low friction- signs of efficient lubrication- are then exhibited at the new protein-protein interface.

References:

1. Lee, H *et al.* PNAS 2006; 103:12999-13003.
2. Lin, Q *et al*. PNAS 2007; 104:3782-3786.
3. Waite JH. Meth. Enzym. 1995; 258:1-20.