Adhesion and Cohesion of Mussel Adhesive Protein on Glass and Gold Through Protein Removal Studies

Brian R. Baker1, Azim N. Laiwalla1, Jeong-Yeol Yoon2, Javier Cañavate3, and Robin L. Garrell1

1University of California, Los Angeles, Department of Chemistry and Biochemistry, Los Angeles, CA 90095
2University of California, Los Angeles, Biomedical Engineering Interdepartmental Program, Los Angeles, CA 90095
3Polytechnic University of Catalonia, Department of Chemical Engineering, Terrassa, Spain

INTRODUCTION

Mytilus edulis foot protein-1 (Mefp-1), a highly repetitive proline-rich protein, adheres to a variety of substrates, including wet and biofilm-coated surfaces.1,2 Its unusually strong adhesive and cohesive properties are at least partly attributable to dihydroxyphenylalanine (DOPA) residues, which can form crosslinks and bond to surfaces.3-4 Because of the prohibitive cost and environmental impact5 of using Mefp-1 itself as a biomolecular adhesive, many efforts have been made to design synthetic analogs of Mefp-1 with comparable or improved materials properties.6-8 A greater understanding of the chemical nature of the adhesive and cohesive properties of Mefp-1 may guide the design of improved biomaterials.

Mussel adhesion is also a severe environmental and economic nuisance.9,10 The prevention and remediation of biofouling by mussels and other marine organisms is an area of great concern. In the laboratory, adhesive proteins themselves are often an annoyance. Apparatus such as surface plasmon resonance (SPR) chips and quartz cuvettes can be irreversibly fouled by adhesive proteins; the lack of an effective cleaning technique can prove costly. In this paper, SPR, quartz crystal microbalance (QCM), and colorimetric assays are used to find the best method for removing adhesive proteins, including Mefp-1, to determine the kinetics of protein removal from various surfaces, and to correlate resistance to removal with adhesive and cohesive properties. The results also provide a fast, easy, and thorough method for removing adhesive proteins from common analytical laboratory equipment.

EXPERIMENTAL

Materials. Bovine serum albumin (BSA) was obtained from Sigma Chemical Company (St. Louis). Mefp-1 was isolated by a procedure similar to Waite’s.12 Commercially available dishwashing detergents, bleaches, and enzymatic contact lens cleaners were used as purchased or diluted with Super-Q filtered water (Super-Q Water System, Millipore Corp., Bedford, MA). A 75-W Xe lamp was used for UV irradiation. Protein concentration was determined by the Bradford assay.13

Crosslinking. Crosslinked Mefp-1 films were created by depositing a small droplet of Mefp-1 solution immediately followed by a droplet of 0.003% (w/v) aqueous hydrogen peroxide. This procedure yielded more extensive crosslinking than other crosslinking protocols such as UV irradiation, as determined by thickness shear mode (TSM) conductance measurements, described below.

Characterization. BSA and Mefp-1 adsorption and removal kinetics on gold were characterized by surface plasmon resonance (SPR) sensorgrams collected on a Biacore X system (Biacore AB, Uppsala, Sweden) using bare gold sensor chips (Pioneer Chip J1, Biacore AB, Uppsala, Sweden).

Protein Removal Assays. Protein removal from gold surfaces was measured with a TSM acoustic sensor, also known as a quartz crystal microbalance (QCM). The apparatus consisted of a HP 4192A LF Impedance Analyzer (Hewlett-Packard, Palo Alto, CA) and 5 MHz quartz crystals (International Crystal Manufacturing Co., Oklahoma City). A 5 µL droplet of Mefp-1 solution was deposited on the center of the QCM gold electrode and allowed to dry under nitrogen. The difference between the resonance frequency with and without the dry protein film, \( \Delta f \), was measured. The crystal was then subjected to a cleaning protocol, rinsed, and analyzed again. The percent residual protein was calculated as the ratio \( \frac{\Delta f}{\Delta f_{\text{before}} \text{cleaning}} \) before cleaning, times 100. The conductance of the protein-coated device was used as an assay of film rigidity, and interpreted as reflecting the extent of crosslinking.

The residual protein on glass was determined by a colorimetric assay. As shown in Figure 1, drops of protein solution were placed on a pre-cleaned glass slide (Fisher Scientific, Pittsburgh) and allowed to dry in air. The slides were then subjected to a cleaning agent, rinsed, and placed in a staining solution (1 g/L Coomassie Blue in 7.5% acetic acid / 40% methanol / 52.5% water) for 30 min. The slides were rinsed again, then digitally imaged to determine relative spot densities. The average spot density of control protein droplets (not subjected to a cleaning agent) was used to calculate the residual protein amount (%) for all other slides. Each data point represents the average of at least three measurements.

![Figure 1](image.png)

Figure 1. Colorimetric assay for residual protein on glass.

RESULTS AND DISCUSSION

Characterization of Mefp-1. The SPR sensorgrams of BSA and Mefp-1 (1.0 mg/mL) adsorption onto gold are shown in Figure 2. During exposure to the protein solutions (t=0 to 420), the amount and rate of adsorption for Mefp-1 are higher than for BSA, indicating that Mefp-1 is a strongly adhesive protein. After adsorption (t=420 to 800), little protein desorbed from the gold surface. However, after prolonged rinsing, the BSA-coated chip eventually returned to the original response, but the Mefp-1 never completely desorbed from the gold surface. Without removal of the tenaciously adhesive Mefp-1, the sensor chips could not be reused.

![Figure 2](image.png)

Figure 2. SPR sensorgrams of BSA and Mefp-1 adsorption onto gold.
Efficacy of Cleaning Agents. An initial study of protein removal from glass was used to determine which cleaning agents, if any, were capable of removing Mefp-1 and crosslinked Mefp-1 from various surfaces. Many cleaning agents were used on BSA, Mefp-1, and crosslinked Mefp-1 spots; the results are summarized in Figure 3. All results are after 30 min exposure to the cleaning agent, except the Xe lamp UV irradiation (5 min). While the BSA was completely removed in less than 30 min by almost all cleaning agents, Mefp-1 and crosslinked Mefp-1 remained on the surface for the majority of cleaning agents. Many cleaning agents were not completely effective after even 24 hr exposure. Only cleaning agents containing both detergent and bleach were completely effective in removing Mefp-1 and crosslinked Mefp-1 in less than 30 min. Undiluted automatic dishwashing detergent often completely removed Mefp-1 and crosslinked Mefp-1 in less than five minutes. Several other automatic dishwashing detergents were also tested and gave similar results to Cascade dishwashing detergent (results not shown). For the less effective cleaning agents, the non-crosslinked Mefp-1 is usually removed faster than the crosslinked Mefp-1. However, for bleach, the crosslinked Mefp-1 was removed faster than the non-crosslinked Mefp-1.

Protein Removal Kinetics. The kinetics of Mefp-1 removal from gold were measured by SPR. After treatment of BSA, Mefp-1, and crosslinked Mefp-1 with various cleaning agents, results similar to those in Figure 3 were obtained (data not shown). Multiple data points after different cleaning times were fitted to a first order decay to determine the pseudo-first order rate constants of protein removal. The pseudo-first order rate constants for Mefp-1 removal from glass were measured using the colorimetric assay described above. Some of the kinetic results are shown in Table 1. The kinetics of protein removal from glass were always slightly faster than from gold, suggesting that Mefp-1 adheres more strongly to the gold substrate. The bleach and detergent containing dishwashing detergents Cascade, Electrasol and Ralph’s brand were all much more effective at removing Mefp-1 than the enzyme and detergent-containing Cascade Complete. For the bleach and detergent-containing agents, the crosslinked Mefp-1 was almost always removed slightly faster than the non-crosslinked Mefp-1, suggesting that stronger cohesion is correlated with weaker adhesion. Furthermore, the pseudo-first order rate constant displayed a roughly first order dependence on the cleaning agent concentration.

![Figure 3](image-url) Residual BSA, Mefp-1, and crosslinked (XL) Mefp-1 on glass after 30 min exposure to A) Xe lamp UV irradiation (5 min/spot); B) 0.01 M HNO₃; C) Dawn dishwashing detergent; D) Renu 1 Step Enzymatic Lens Cleaner (subtilisin); E) Cascade Complete automatic dishwashing detergent; F) Dry Clorox Regular (perborate) bleach; G) Ralph’s Ultra bleach; H) 0.6% OCl⁻ / 0.1% SDS; I) Clorox Advantage bleach; J) Cascade automatic dishwashing detergent.

### Table 1. Kinetics of Mefp-1 Removal from Glass

<table>
<thead>
<tr>
<th>Cleaning Agent</th>
<th>Mefp-1</th>
<th>k (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Cascade Gel</td>
<td>non-XL</td>
<td>1.1</td>
</tr>
<tr>
<td>10% Cascade Gel</td>
<td>non-XL</td>
<td>0.066</td>
</tr>
<tr>
<td>1% Cascade Gel</td>
<td>non-XL</td>
<td>0.0057</td>
</tr>
<tr>
<td>100% Cascade Gel</td>
<td>XL</td>
<td>1.3</td>
</tr>
<tr>
<td>1% Cascade Gel</td>
<td>XL</td>
<td>0.069</td>
</tr>
<tr>
<td>10% Cascade Powder</td>
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<td>0.0053</td>
</tr>
<tr>
<td>10% Electrasol Gel</td>
<td>non-XL</td>
<td>0.091</td>
</tr>
<tr>
<td>10% Ralph’s Gel</td>
<td>non-XL</td>
<td>0.092</td>
</tr>
<tr>
<td>100% Cascade Complete Gel</td>
<td>non-XL</td>
<td>0.00025</td>
</tr>
</tbody>
</table>

Rate-Limiting Active Agent. As seen above in Table 1, the pseudo-first order rate constants for Mefp-1 removal follow only first order dependence on bleach/detergent cleaning agent concentration, even though efficient Mefp-1 removal requires both bleach and detergent. In order to determine whether bleach or detergent is a rate-limiting agent, several bleach/detergent solutions were prepared at different concentrations. A 0.06% OCl⁻ / 0.1% SDS solution left 67% of the Mefp-1 after 3 min. Increasing the detergent concentration by a factor of 10 had little effect on the Mefp-1 removal kinetics (69% after 3 min), while increasing the bleach concentration by a factor of 10 allowed complete removal within 3 min. Although detergent is necessary for efficient removal of Mefp-1 and crosslinked Mefp-1, bleach is the rate-limiting active agent.

### Summary and Conclusion

The tenaciously adhesive protein Mefp-1 proved resistant to many conventional protein removal protocols, including UV irradiation, acid, proteolytic enzymes, and several detergents. A thorough survey of cleaning agents showed that only those containing both bleach and detergent proved completely effective in removing Mefp-1 and crosslinked Mefp-1 within 30 minutes from glass and from gold. Whereas the non-crosslinked Mefp-1 is removed faster during the ineffective cleaning treatments, the crosslinked Mefp-1 is removed faster by bleach- and detergent-containing cleaning solutions. The relationship between cohesion and adhesion revealed by resistance to protein removal is complex and depends upon the method of removal.

Kinetic analysis of Mefp-1 removal shows that bleach is the rate-limiting active agent. Bleach and detergent-containing cleaning solutions can be used to quickly, easily, and completely remove adhesive proteins, including Mefp-1, from glass and gold surfaces, as used in SPR, QCM, reflectance FT-IR, CD, and UV-vis spectroscopies.

### References