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Rapp, MV
Maier, GP
Dobbs, HA
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Defining the Catechol–Cation Synergy for Enhanced Wet Adhesion to Mineral Surfaces

Michael V. Rapp,[†,⊥] Greg P. Maier,[‡,⊥] Howard A. Dobbs,[†] Nicholas J. Higdon,[‡] J. Herbert Waite,[*,§] Alison Butler,[*,‡] and Jacob N. Israelachvili[*,†,∥]

[†]Department of Chemical Engineering, [‡]Department of Chemistry and Biochemistry, [§]Molecular, Cellular, and Developmental Biology, and [∥]Materials Department, University of California, Santa Barbara, California 93106, United States

Supporting Information

ABSTRACT: Mussel foot proteins (Mfps) exhibit remarkably adaptive adhesion and bridging between polar surfaces in aqueous solution despite the strong hydration barriers at the solid–liquid interface. Recently, catechols and amines—two functionalities that account for >50 mol % of the amino acid side chains in surface-priming Mfps—were shown to cooperatively displace the interfacial hydration and mediate robust adhesion between mineral surfaces. Here we demonstrate that (1) synergy between catecholic and guanidinium side chains similarly promotes adhesion, (2) increasing the ratio of cationic amines to catechols in a molecule reduces adhesion, and (3) the catechol–cation synergy is greatest when both functionalities are present within the same molecule.

Water undermines polymer adhesion to surfaces. Water and hydrated salt ions strongly bind to hydrophilic surfaces (e.g., minerals, metals, oxides, fabrics, and biological interfaces) to form a thin hydration film that impedes the intimate contact between the polymer and the surface that is necessary for durable adhesion.[1−3] To surmount this obstacle, wet adhesives and coatings must displace the hydration layer, bond to the underlying surface, and resist deterioration. Marine mussels routinely accomplish this feat on intertidal rocks with a quick-curing blend of intrinsically disordered proteins known as mussel foot proteins (Mfps).[4,5] Of the >15 known Mfps, two vanguard proteins—Mfp-3 and Mfp-5—are deposited first to prime the wet surface and form the interfacial bridge that couples the rest of the holdfast to the surface.[4,6] Mfp-3 and -5 both consist of unique amino acid compositions, containing 20−30 mol % 3,4-dihydroxyphenylalanine (Dopa), a catecholic amino acid, with stoichiometric levels of cationic residues that are primarily lysine (Lys) and arginine (Arg). Dopa and cationic residues commonly occur in adjacent positions along the protein backbone. Over the past decade, the multifaceted catecholic functionality of Dopa[7]—which adheres to polar surfaces through hydrogen or coordination bonds,[8] and chelates metals or covalently cross-links to form cohesive glues[9,10]—has led to a surge of mussel-inspired adhesives and coatings for applications such as medical or dental adhesives,[11−13] self-healing hydrogels,[14,15] biopolymer scaffolds,[16,17] and biocompatible coatings.[18−20] However, until recently the role of cationic residues in mussel adhesives has been poorly understood and underutilized in bioinspired adhesives.

We recently demonstrated that synergistic interactions between catechol and Lys groups promote adhesion to a wet mineral surface, rationalizing the high Lys composition of interfacial Mfps.[21] To reduce the complexity of studying full proteins, we measured the adhesive interactions of a bacterial siderophore—cyclic trichrysobactin,[22] an iron-chelating small molecule (1053 g/mol) comprising catecholic 2,3-dihydroxybenzoic acid (DHBA) and Lys functionalities—and synthetic siderophore analogues assembled around the tris(2-aminoethyl)amine (Tren) scaffold, such as Tren-Lys-Cam (TLC) (Figure 1A). Through direct force measurements with a surface forces apparatus (SFA), we determined that the siderophore and its analogues mediate robust adhesion between two mica surfaces by displacing hydrated salt ions from the surface with their cationic Lys groups, allowing catechols to form bidentate bonds to the underlying aluminosilicate surface. Removing either catechol or amine functionalities from the analogues significantly reduced or eliminated adhesion, respectively.[21]

We report herein the nanoscale adhesive properties of new siderophore analogues that further explore the synergy between catechols and cationic moieties in wet mineral surface priming. The guanidinium cation in Arg displaces surface salt and promotes adhesion but is less effective than Lys. Doubling the number of Lys groups per molecule (from three to six) while retaining the same number of catechol groups (three) decreases the overall adhesion between surfaces, as the ratio of catechol binding groups to total molecular area decreases. Finally, we demonstrate that comixtures of two separate molecules that contain only catechols (appended to a Tren core) and only amines do not recreate the same adhesion synergy as the intramolecular configuration, suggesting that the adsorbate geometry and configurational entropy contribute significantly to the adhesion. Overall, these results suggest a rationale for the molecular compositions of Mfp adhesion priming proteins and offer design criteria for functional bioadhesives and coatings.

Certain interfacial priming proteins secreted from mussels, such as Mfp-3f in *Mytilus edulis*,[23] contain stoichiometric compositions of Arg and Dopa, which parallel the high compositions of Lys and Dopa found in other variants, such as Mfp-5 in *Mytilus californianus*.[4] To determine whether Arg is functionally similar to Lys in synergy with catechol in wet adhesion, we synthesized a siderophore analogue, Tren-Arg-

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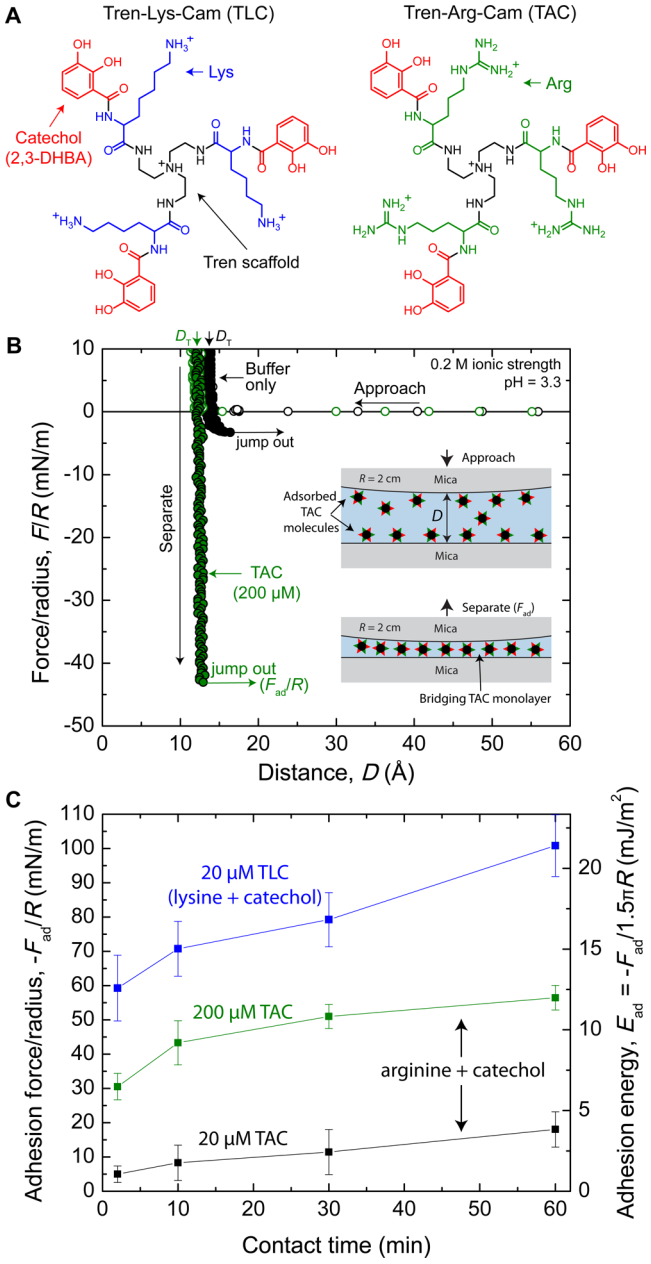

Figure 1. (A) Structures of the siderophore analogues TLC and TAC. (B) SFA force–distance interactions for two mica surfaces in aqueous buffer (150 mM KNO_3 + 50 mM acetate, pH 3.3) (black circles) and in 200 μM TAC (green circles). Open circles represent measurements during the approach of the two surfaces, while solid circles represent measurements during separation. The inset depicts the surfaces as they interact throughout the measurement. (C) TLC- and TAC-mediated adhesion forces, F_{ad}, and energies, E_{ad}, required to separate two mica surfaces in aqueous solution, as functions of the time the surfaces were left in adhesive contact. Error bars represent one standard deviation.

Cam (TAC), in which the Lys residues of TLC are replaced with Arg (Figure 1A; synthesis and characterization are shown in Figures S1–S4 in the Supporting Information). A SFA was used to measure the radius-normalized force (F/R) versus distance (D) profile for two cleaved mica surfaces during approach, compression, and separation in a buffered solution of TAC (Figure 1B). Mica is a molecularly smooth aluminosilicate mineral (Si:Al ratio of 3:1) that serves as an ideal model for the shale and clay minerals to which mussels commonly attach. The smooth and well-studied interface of mica allows for molecular-level insights into the adhesive mechanisms of adsorbates. Mica possesses a negative structural charge at surface Al sites and strongly adsorbs hydrated cations (e.g., K^+) and water to form a tightly bound hydration layer that is characteristic of most polar surfaces in solution.[1,24,25] In aqueous environments, robust attachment to surfaces is contingent upon displacement of this hydration layer and binding of molecules to the underlying surface.

In a buffer that mimics the solution imposed by mussels during plaque deposition (150 mM KNO_3 + 50 mM acetate, pH 3.3),[26] a hydration layer with D_T = 13 ± 1 Å formed between mica surfaces at 10 mN/m compression (Figure 1B, black circles). Upon separation of the two surfaces, only a weak van der Waals adhesion force was measured.[2] However, after nanomolar amounts of TAC were injected into the gap solution between the surfaces (resulting concentration of 200 μM) and equilibrated, the thickness of the intervening layer *decreased* to 12 ± 1 Å, and a large adhesion force, F_{ad} = −43 ± 6 mN m^{-1}, was measured upon separation after 10 min in contact, which increased slightly with additional contact time (Figure 1C). The measured adhesion forces between two crossed-cylinder surfaces in SFA experiments were converted into adhesion energies using the Johnson–Kendall–Roberts theory ($E_{ad} = F_{ad}/1.5\pi R$).[27] Overall, the decrease in the thickness of the intervening layer between mica surfaces, the increase in adhesion, and the sharply vertical shape of the force curve are consistent with a monolayer of TAC that bridges the mica surfaces and mediates adhesion.[2]

The force–distance profiles confirm that the guanidinium cations in TAC displace hydrated salt layers and promote adhesive synergy with catechols at mineral surfaces, similar to the amine cations in TLC yet with subtle differences. First, TAC demonstrates a critical adsorption concentration (CAC) approximately 10 times higher than that of TLC before the molecules adsorb to the mica interface (Figure 1C). Moreover, TAC mediates adhesion that is only 50–60% of the maximum TLC adhesion. The intervening TAC film thickness (12 ± 1 Å) is comparable to that of TLC (9 ± 1 Å), indicating comparable adsorption densities. We conclude that the bulkier structure and delocalized charge of guanidinium[28] decrease the magnitude of the cation's electrostatic interaction with the negatively charged sites on mica, as compared with amine. This effect is analogous to the more favorable adsorption free energy of K^+ (smaller radius) compared with Cs^+ (larger radius) to mica.[3] This conclusion further indicates that the cationic residues Lys and Arg contribute significantly to the equilibrium adhesion energy, rather than solely catechol.

To observe the effect of increasing the number of cationic groups per molecule on the adhesion and surface affinity, we synthesized a siderophore analogue, Tren-Lys-Lys-Cam (TLLC), that doubles the ratio of lysine cations to catechols (Figures 2A and S5–S9). SFA force–distance measurements were performed with TLLC using the same procedure as for TAC. Similar interaction profiles and adhesion forces were measured with TLLC (Figure 2B); the thickness of the intervening layer between the mica surfaces decreased to D_T = 12 ± 1 Å, and a comparable adhesion force, F_{ad} = −47 ± 9 mN/m, was measured after 10 min in contact.

The lower adhesion energy measured with TLLC is further evidence of the importance of catechols in robust adhesion. Doubling the number of Lys groups increases the molecule's projected area at a surface yet keeps the number of catechol

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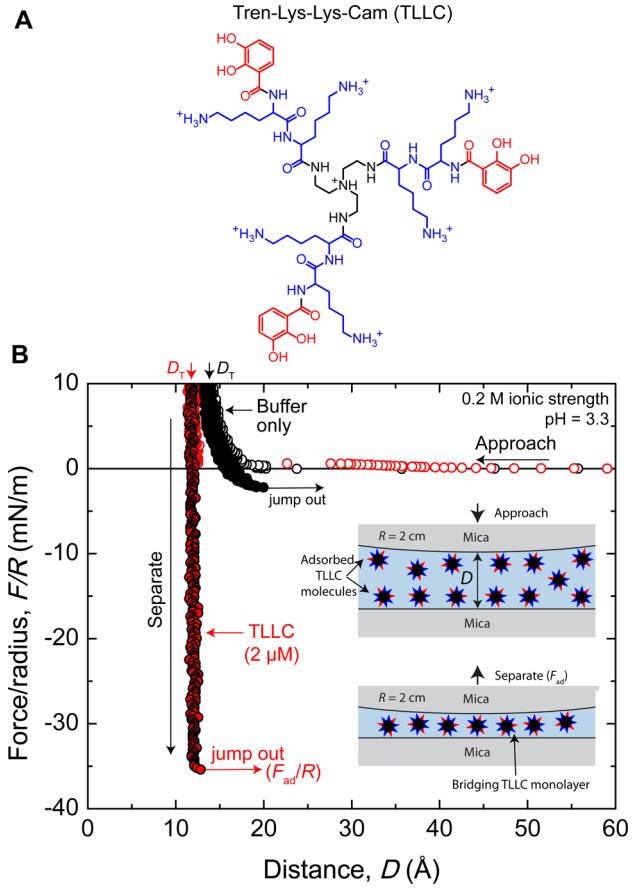

Figure 2. (A) Structure of the siderophore analogue TLLC. (B) SFA force–distance interactions for two mica surfaces in aqueous buffer (150 mM KNO_3 + 50 mM acetate, pH 3.3) (black circles) and in 2 μM TLLC (red circles). Open circles represent measurements during the approach of the two surfaces, whereas solid circles represent measurements during separation. The inset depicts the surfaces as they interact throughout the measurement.

groups the same, thereby decreasing the density of robust bidentate interactions per unit area across the surface. However, the three additional Lys groups increase the electrostatic charge density within the molecule and lower the CAC for TLLC by an order of magnitude (Figure S10). TLLC's adhesion performance is an interesting alternative result to the work of Wang et al.,[29] where increasing the concentration of Dopa groups in a cationic polymer had a negligible effect on the polymer's total adhesion. We ascribe these contrary results to differences in the geometries in our respective adhesive molecules; the excluded volume of random-coil polymers sterically hinders high catechol surface densities, while small molecules may assemble into more dense films.

Do cation and catechol functionalities require intramolecular proximity to enhance adhesion? To determine whether a mixture of two siderophore analogues—one without amine (Tren-Cam, TC) and the other without catechol (Tren-Lys-Bam, TLB)—promotes adhesion at aqueous mineral surfaces, we performed further SFA force–distance measurements between mica surfaces, as shown in Figure S11. When the ratio of TC (0.02–1 mM) and TLB (0.02–0.2 mM) was varied, no enhanced adhesion or synergy between the siderophore analogues was detected. At a concentration of 0.2 mM, TLB preferentially adsorbed onto the mica surface over TC, displaced the hydration layer, and mediated modest adhesive forces that were identical to those measured in solutions of only TLB (Figure S11C-i). Additional adhesion measurements were performed on mixtures of TC and amine compounds (tetramethylamine, lysine, isopropylamine, aniline, 1,3-diaminopropane, diethylenetriamine, TREN, and 2,4,6-triethyl-1,3,5-benzenetrimethanamine [TEBMA]); however, no evidence of synergy or adhesion was measured in any of these mixtures. In mixtures with TC and TREN or TEBMA, the highly charged amine compounds adsorbed on the mica surface, but no influence from TC was observed (Figure S11C-ii,iii). The inability to recreate the adhesive performance of TLC with mixtures of singly functionalized molecules suggests that the molecular geometry and configurational entropy[30] upon adsorption contribute significantly to the surface phenomena of the siderophore analogues.

The specific binding mode of 2,3-dihydroxycatechol to the surface and the resulting geometry of the siderophore analogues are not known. The first hydroxyl pK_a of the 2,3-dihydroxycatechol in TLC or TAC is expected to be similar to the pK_a of the analogous siderophores chrysobactin (2,3-dihydroxybenzoyl-D-Lys-L-Ser; pK_a = 6.73) and vanchrobactin ((2,3-dihydroxybenzoyl-D-Arg-L-Ser; pK_a = 6.79);[31] thus, at pH 3.3 the catechols in TLC, TAC, and TLLC are fully protonated. By virtue of single-molecule atomic force microscopy measurements, Li et al.[32] suggested that Dopa adsorbs on mica via bidentate hydrogen bonds. Alternatively, catechols may form mononuclear bidentate coordination complexes with mica's alumina sites,[33−35] but this interaction is unconfirmed at mica's crystalline surface. Similarly, a salicylate-type interaction involving the *o*-hydroxyl oxygen and the carbonyl group is possible[36] but not expected.[37] After displacement of the hydrated salt layer, the role of the cationic amine group is unconfirmed; however, we strongly suspect that the cationic groups participate in adhesion to the mica surface through Coulomb interactions at the negatively charged alumina sites.

Over the first few molecular layers extending from an aqueous mineral surface, paired catechol–cation functionalities cooperate to displace hydration layers and promote robust adhesion between the underlying surfaces. This synergy is not unique to amine cations: guanidinium groups likewise enhance aqueous adhesion, which provides a rationale for the high content of Arg residues in certain Mfps. In small-molecule adhesives, increasing the number of cationic groups per molecule increases the affinity for negatively charged surfaces but decreases the equilibrium adhesion energy by lowering the density of bidentate-binding catechol groups. Although the specific surface conformations of paired catechol–cation siderophores and analogues await characterization in future studies, it is apparent that the geometry and configurational entropy significantly affect their intermolecular interactions. Furthermore, while small changes to the separation distance between the catechol and cation do not significantly alter the adhesion synergy, a cutoff separation should exist beyond which synergy is not observed. In analogy to the remarkably high Fe^{3+} stability constant of triscatechol siderophores compared with monocatechol compounds,[38] intramolecular adjacency of binding functionalities contributes a significant energetic gain upon adsorption to a wet surface.

■ ASSOCIATED CONTENT

Supporting Information

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Synthesis and characterization of siderophore analogues and additional SFA adhesion measurements (PDF)

AUTHOR INFORMATION

Corresponding Authors

*herbert.waite@lifesci@ucsb.edu
*butler@chem.ucsb.edu
*jacob@engineering.ucsb.edu

Author Contributions

⊥M.V.R. and G.P.M. contributed equally.

Notes

The authors declare no competing financial interest.

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