Supporting information

Mussel foot protein-1 (mcfp-1) interaction with titania surfaces

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Experimental materials and methods

Mcfp-1 purification

Mcfp-1 was purified from frozen *Mytilus californinus* feet according to published procedures.[1]Purified mcfp-1 was treated with 1 mM EDTA in 5% acetic acid and polished with C8 reverse phase colume to prevent residual metal ion contamination. Sample purity was assessed by acid urea polyacrylamide gel electrophoresis, amino acid analysis, and MALDI-TOF mass spectrometry. The DOPA in purified mfp-1 was ~13 mol% by amino acid analysis after a 1h hydrolysis in 6N HCl at 158 °C. Purified samples were freeze-dried and resuspended in a solution of 0.1 M acetic acid, pH 3.0 and thereafter divided into convenient aliquot volumes for storage in aluminum foil-covered vials at -80 °C prior to testing. Thin layer of TiO_2 (~15 nm) were deposited onto mica by e-beam evaporation (PVD-75, Kurt J. Lesker) at 0.05-0.1 nm/s with 1.5×10^{-5} Torr of O_2 and (2-8)×10-6 Torr of H2O. The root mean square (rms) roughness values of mica and TiO_2 determined by AFM (DI, Santa Barbara, CA, USA) with a AFM image (5μm *5μm) was about 0.16 and 0.8 nm, respectively.

SFA experiment

The normal force-distance profiles and adhesion forces of the mcfp-1 were determined using a surface forces apparatus (SFA) in a configuration reported previously.[2] The smooth and chemically inert surfaces of mica were used as the substrate surfaces for depositing thin films of the protein used in the experiment. Two thin mica sheets (thickness 1-5μm) were glued onto two cylindrical silica disks (radius R=2 cm).For mcfp-1 coating on the surfaces (mica and mica supported TiO_2), 100 μL of mcfp-1 solution (10 μg/mL) diluted in 0.1 M acetic acid (pH~3.0) wasdropped onto one the surfaces, and the surfaces are incubated until protein adsorption reaches equilibrium (20 min); then the surfaces are washed more than five times with a buffer that is appropriate for the specific experiment. The thickness of adsorbed mcfp-1 film on the surfaces measured by hardwall distance was consistant (8 ~10 nm). Since the mcfp-1 film thickness was less than the hydrodynamic diamter of mcfp-1 (~16 nm), we considered the mcfp-1 film as a monomolecular layers.Higher protein concentration (over ~10 μg/mL) or excessive incubation time with protein solution to surfaces (over ~20 min), generally lead to multi-layer deposition of protein and make the experimental results unreliable.one coated and one uncoated target surface (asymmetric configuration) were then mounted in the SFA chamber in a crossed-cylinder geometry, which roughly corresponds to a sphere of radius R approaching a flat surface based on the "Derjaguin approximation".The measured adhesion or "pull-off" force F_{ad} is related to the adhesion energy per unit area E_{ad} by $E_{ad}=F_{ad}/1.5\pi R$ (used in this study) for soft deformable surfaces with strong adhesive contact.[3]A constant rate of approach and separation (5~6 nm/sec) were used for each force run.The experiments were conducted at room temperature (20 °C).

Resonance Raman Spectroscopy

Raman spectra were collected from the surface of samples with a confocal Raman microscope (alpha300; WITec) equipped with a Nikon objective (100X) and using a laser excitation wavelength of 532 nm. Spectra were acquired with a CCD camera (DV401-BV; Andor) behind a spectrometer (UHTS 300; WITec) with a spectral resolution of 3 cm^1. Samples were prepared by incubating protein on TiO_2 surfaces as described in the SFA experimental section. Protein solutions of pH3 and pH5 were incubated for ~20 min after which the surfaces were washed with their respective solutions and dried prior to measurement. Spectra from bare mica, TiO_2 coated mica, and protein incubated on a bare mica surface were acquired as controls. Protein coated samples were sensitive to burning by the laser beam; therefore, laser power was restricted to 10–20 mW and only short integration times of 0.3 s were used for all measurements. The ScanCtrlSpectroscopyPlus software (version 1.38, Witec) was used for measurement and data analysis. Each collected spectrum consisted of 100 accumulations of a 0.3 s integration time. For each sample, three spectra were collected from different regions and averaged. Averaged spectra were smoothed with a Savitzky-Golay smoothing filter. To observe the resonance peaks more clearly in spectra from protein coated samples, the combined mica/TiO_2 background spectra was subtracted.

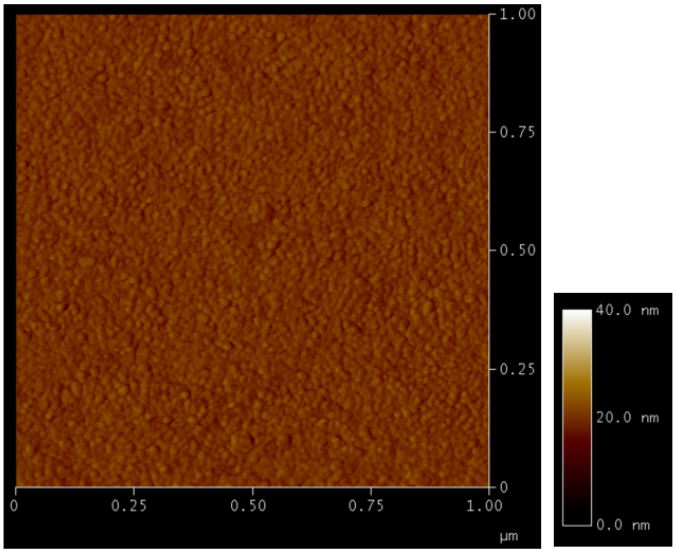

Figure S1. AFM tapping mode image of a mica supported TiO_2 surface in dry.

Mcfp-1 TiO2 (pH3)	Mcfp-1 TiO2 (pH5)	[b]Byssus coating	[c]Mefp-1 Fe^{3+}	[d](NH4)2[Ti(cat)3] •2H2O	[d]catechol-anatase	[e,f]assignments
518	536	550	531	505		δ,*v*-chelate CT
594	591	605	591	591		v Me-O_3
645	639	639	638	646		v Me-O_4
836	836	828	815			ring breathing
			1152	1153	1155	δCH
1274	1271	1271	1274	1270	1261	*v* C-O
1332	1332	1326	1326	1338	1329	*v* CC
1439	1456	1426	1426	1475		
1487	1487	1475	1491	1490	1483	*v* CC + δCH

Ref: [a]Present study [b]Harrington et al[4] [c]Taylor et al[5] [d]Lana-Villareal et al[6] [e]Michaud-Soret et al [7f]Ohhrstro€m et al [8]

Table S1. Spectral assignments for DOPA-TiO_2 resonance Raman peaks in comparison with similar complexes

Supplementrary references

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