Super-hydrophilic Mucin from Loach Skin Mucus and Its Adsorption on Gold Surface

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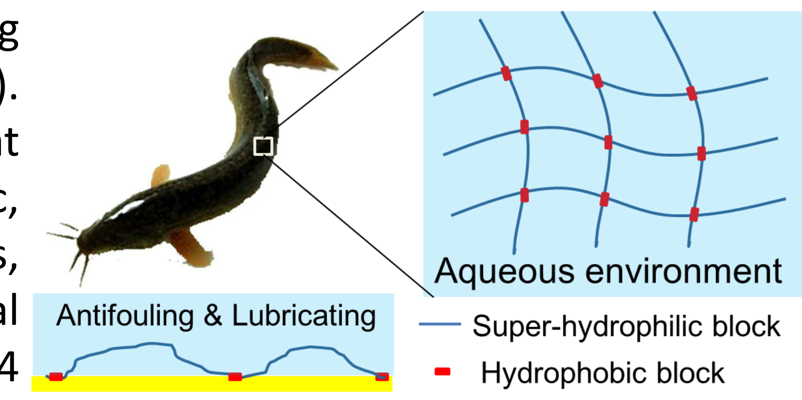
Abstract

Loach skin mucus is a weak hydrogel that exhibits excellent lubricating and antifouling functions, but its gel component and gel-forming matter properties remain obscure. In this study the gel-forming matter was isolated and found to be mucous glycoproteins (mucin). Colorimetric assay, sodium dodecyl sulfate polyacrylamide gel electrophoresis, rheological tests and fluorescence study suggested that the isolated loach skin mucin had low amino acid residue content (~10 wt%), high molecular weights, hydrogel-forming characteristic, and hydrophobic blocks. Loach skin mucin was also found to be much more hydrophilic than well-investigated mammalian mucins, possessing the feature of super-hydrophilicity. Its interfacial behavior on a gold surface was explored. Results of quartz crystal microbalance suggested that loach skin mucin adsorbed stably on the gold surface from 50 mM NaCl, with an adsorbed mass of ca. 4.4 mg/m^2. Results of scan probe microscopy showed that the adsorption of loach skin mucin lowered the roughness of the gold surface in aqueous environment. The adsorbed mucin aggregated and showed phase heterogeneity after dryness, demonstrating its intramolecular heterogeneity. The interfacial behavior of the adsorbed loach skin mucin should reflect its brush conformation on the gold–water interface. The interfacial behavior and super-hydrophilic nature give loach skin mucin potential as excellent material for use in solid–water interfaces for antifouling and lubrication, and should be crucial to the versatile functions of loach skin mucus.



Isolation of Mucin

Diluted loach skin mucin + Sevag reagent, 4CHCl$_3$:1^{n}BuOH, V/V → thoroughly mixing → centrifugation → Aqueous phase / Denatured proteins / Seveg reagent

Aqueous phase + 4-fold absolute alcohol → 4 °C for a week → precipitate → nuclease → freeze-drying → Mucous glycoproteins (loach skin mucin)

Mucin Characterization

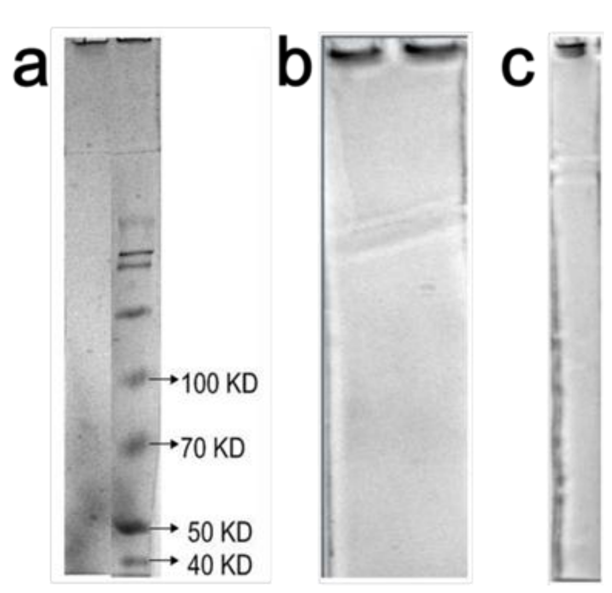


Figure 1. Results of SDS PAGE: (a) protein staining of native mucus and markers, (b) protein staining of Extract, (c) PAS staining of Extract

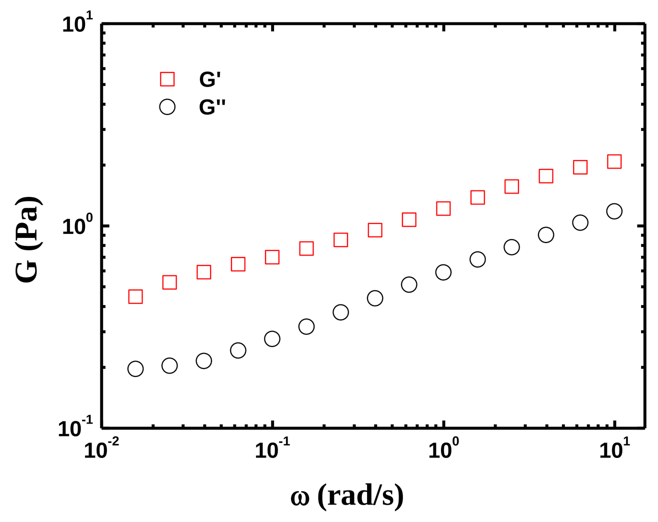


Figure 2. Dynamic frequency sweep behavior of 3 mg/ml Extract solution

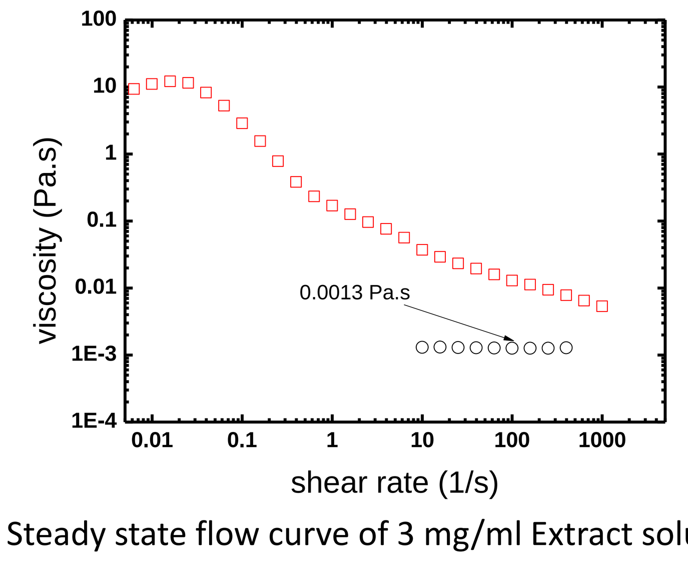


Figure 3. Steady state flow curve of 3 mg/ml Extract solution with (circle) and without (square) proteinase K treatment

Loach skin mucin possesses high molecular weights, and forms weak hydrogel in water.

Adsorption

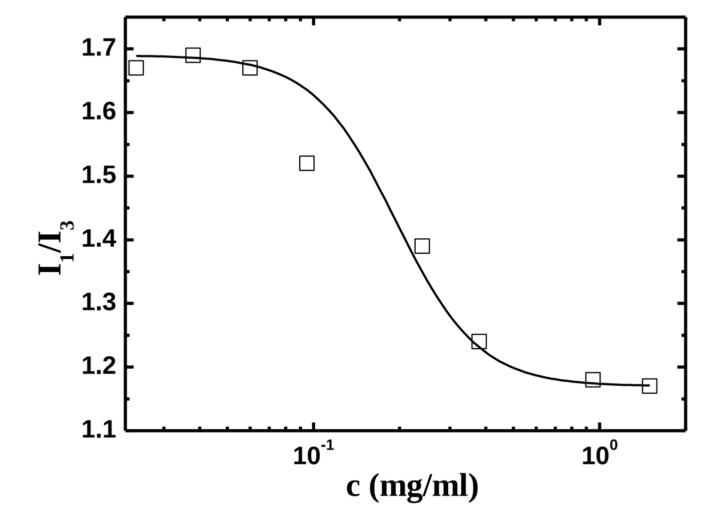


Figure 4. Intensity ratio (I_1/I_3) for pyrene in loach skin mucin solution in 0.1 M NaCl/0.02% NaN$_3$ as a function of loach skin mucin concentration

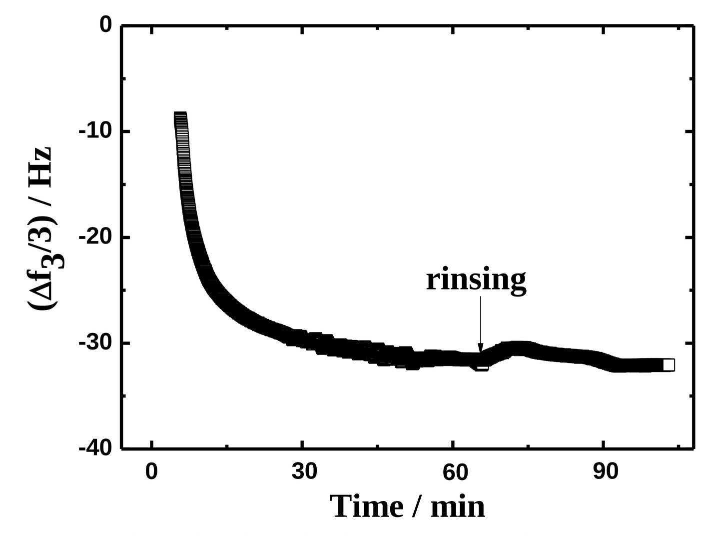


Figure 5. QCM data for loach skin mucin adsorption on a hydrophobic gold surface from 50 mM NaCl, followed by rinsing with 50 mM NaCl.

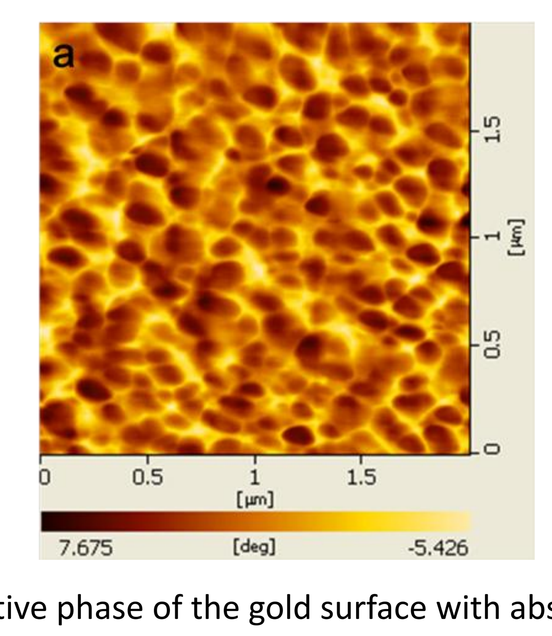


Figure 6. Relative phase of the gold surface with absorbed loach skin mucin in air.

Loach skin mucin possesses hydrophobic blocks, which enable it to form a stable adsorption layer on hydrophobic gold surface from aqueous solution.

Super-hydrophilicity

Loach skin mucin forms hydrogel in water under a much lower concentration than well investigated mammalian mucins, indicating their super hydrophilicity. Here we compare loach skin mucin with two typical mammalian mucins (i.e., human tracheobronchial mucin and porcine submaxillary mucin) on the basis of gel elasticity and swelling theories.

For an ionic gel absorbing water, Flory has proposed

$$q_m^{5/3} \cong \left[\left(i/2v_u S^{*1/2}\right)^2 + \left(1/2-\chi_1\right)/v_1\right] / \left(v_e/V\right)$$

$$Q \equiv \left(i/2v_u S^{*1/2}\right)^2 + \left(1/2-\chi_1\right)/v_1$$

A higher value of Q represents higher hydrophilicity of the gel-forming macromolecules.

The Q value of loach skin mucin is hundreds times higher than those of human tracheobronchial mucin and porcine submaxillary mucin, demonstrating the super-hydrophilic nature of loach skin mucin.

Conclusions

1. The gel forming matter is isolated from loach skin mucus with a convenient method, and proved to be mucous glycoproteins (mucin).
2. Loach skin mucin is similar to mammalian mucins in many aspects, except that they are much more hydrophilic.
3. Loach skin mucin can adsorb on a hydrophobic gold surface stably, which combining with their super-hydrophilc nature gives them potential as an excellent biolubricant etc.

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