Comparative Mucomics for the Discovery and Design of Materials

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Introduction

The metabolic costs of mucus production can exceed 1/4 of mollusks' energy budgets, indicating how important these materials are for survival. However, molluscan mucus composition, and how they contribute to function, are not well understood. It remains unclear how differences in protein structure, ion concentration, glycosylation, and other factors operate synergistically to account for the substantial diversity in mucus material properties. We applied a systematic comparative analysis of adhesive, lubricating, and protective mucus isolated from *C. aspersum*. Glycomic mass spectrometry was employed to identify the structures of the glycans decorating proteins. Comparison of these datasets reveal how *C. aspersum* exploit differential protein expression — including a series of previously uncharacterized proteins — glycosylation, and ion concentration are used to explain how these hydrogels behave as adhesives, lubricants, or protective barriers.

Research Question

What are the structures and properties of mucins identified in snail mucus, and can these be utilized as an analog for mammalian mucus?

Methods

- Mucus samples were purified with GdnHCl and CsCl and ultracentrifugation.
- Purified mucins were subjected to reduction with 25 mM DTT and alkylation with 90 mM IAA, then cleaned using 3 kDa MWCO filters.
- Glycoprotein was then incubated with PNGase F for 48 hrs at 37°C, with additional PNGase F being added after 24 hrs.
- Released N-glycans were purified using C18 SPE cartridge, and O-glycoprotein was dried.
- O-glycans were then released utilizing reductive β-elimination for 52 hrs at 50°C.
- N- and O-glycans were permethylated with DMSO-NaOH base and iodomethane-D3. Permethylated glycans were washed with dichloromethane, and dried.
- Samples were resuspended in MeOH and analyzed via MALDI-TOF-MS. GlycoWorkbench 2.0 and manual interpretation were used for data analysis.

Results

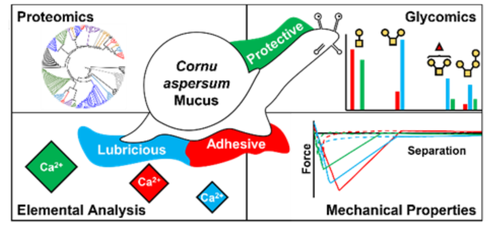

Figure 1. Mucomic analysis of *Cornu aspersum* snail mucus composition. Adhesive, lubricating, and protective mucus are subjected to an omics-style analysis to understand the composition and properties.

Table 1. O-glycans identified in each mucus type with relative ratios of each individual glycan

Sample	Glycan m/z	Monosaccharide Composition	Proposed Structure	Relative Percentage
Adhesive	561.5	Gal1GalNAc1		48.22%
	768.7	Gal2GalNAc1 + 2Me		14.56%
	771.7	Gal2GalNAc1 + 1Me		14.66%
	774.7	Gal2GalNAc1		9.56%
	981.9	Gal3GalNAc1 + 1Me		5.30%
	1192.1	NeuAc1Gal1HexNAc1GalNAc1		5.45%
	1552.4	NeuAc1Fuc2Gal1HexNAc1GalNAc1		2.25%
Lubricating	768.7	Gal2GalNAc1 + 2Me		14.56%
	954.97	Fuc1Gal2GalNAc1		5.30%
	982.8	Gal3GalNAc1 + 1Me		5.30%
Protective	534.5	Gal1GalNAc1		45.10%
	738.8	Gal2GalNAc1 + 2Me		48.90%
	942.8	Fuc1Gal2GalNAc1		2.80%
	983.9	Gal3GalNAc1 + 1Me		2.20%

- Naturally methylated N- and O-glycans were identified in all mucus types
- The overall extent of glycosylation is low in the mucus if snails (<2%)
- Sialic acid containing structures were identified in snail mucus, which is a novel finding. This was further confirmed by LC-MS/MS of tryptic peptides

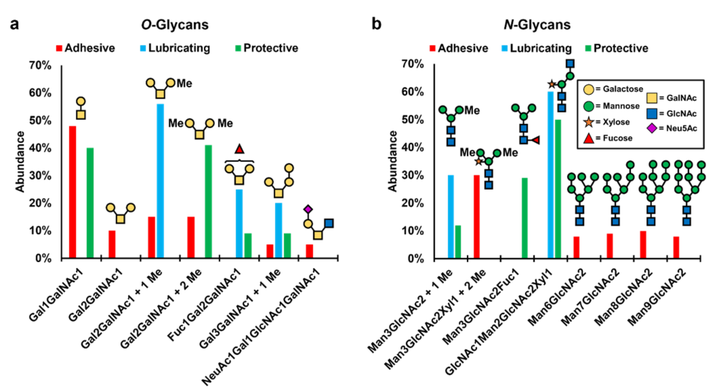

Figure 2. Structures and relative abundance of a) *O*-glycans and b) *N*-glycans found in each isolated mucus secretion. Glycans shown comprised > 5% of glycomic abundance. Trace glycans (< 5 % abundance) are listed in Supplementary Tables 4 and 5. Inset in 'b' lists monosaccharide structures as defined by the symbolic nomenclature for glycans (SNFG).

Conclusion

- Secreted snail mucus are majorly comprised of collagens and glycoproteins.
- A relatively simple set of *O*-glycan structures decorate these proteins (8 in snail mucus, compared to 24 in human GI tracts).
- The *O*-glycan length is modulated between mucus, possibly altering stiffness and lubricity.
- Each mucus has drastically different *N*-glycosylation. Sialic acids, which are uncommon in mollusks, were detected in *O*- and *N*-glycans of the adhesive mucus.
- The comparative mucomics strategy applied here for *C. aspersum* can be used to determine how compositions of other animal secretions account for their ecological function or to assist in the development of synthetic analogues with similarly advantageous biological and chemical properties.

Acknowledgement

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References

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