Distribution and Clustering of Sialic Acids on Single Mucins and Mucous Gels

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Abstract:

Atomic force microscopy (AFM) is used to image biological samples with sub-nanometre resolution, and measure inter-molecular forces with picoNewton sensitivity. The tip, functionalized with a molecular probe, maps the epitope and allows bond energies to be measured. In mucins terminal sugars of oligosaccharide chains are often binding sites for bacteria, viruses and cells of the immune system. We used AFM to map the distribution of sialic acids (Neu5Ac) in isolated ocular mucins and mucus gels.
Tips were functionalised with the lectins Maackia amurensis (MAA) and Sambucus nigra (SNA). Spatially-correlated topographic and force-spectroscopy data were obtained in HEPES buffer. The localization, number, rupture forces and distances, and koff rates were calculated for all recognition events. Median rupture forces were conserved, irrespective of sample origin, and were 172 pN ± 4 pN for MAA- α-2, 3 Neu5Ac and 121 pN ± 8 pN for SNA- α-2, 6 Neu5Ac bonds, with koff rates of 156.6s-1 and 148.7 s-1, respectively. Blocking sugars injected in situ caused a 61% decrease in the frequency of interactions: washing reversed this effect. Force volume maps show 55% more α-2, 6 than α-2, 3-Neu5Ac in ocular impressions. MAA interactions occurred in larger clusters than SNA on single molecules, and the converse in gels. The observed clustering of sialic acids is expected when probing a highly glycosylated region of the mucin molecule. Not all α-2, 3 Neu5Ac

are available to the probing lectin when mucins are part of a gel, as reflected in the smaller cluster size.

To conduct CRBS experiments, a special experimental setup has been designed, fabricated, and installed at the end-station of the RBS-beamline at the University of Jordan Van de Graaff accelerator (JUVAC). Different inert gases can be used as targets, and energetic ions of different charge states and energies as projectiles.