Investigating the Impact of Heparan Sulfate Domain Structure on Interleukin 8 Heparan Sulfate interactions

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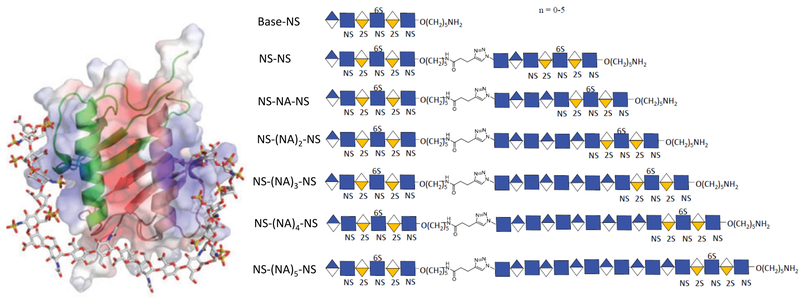
Abstract

- Heparan sulfate (HS) is a linear, complex polysaccharide that contains regions with high levels of sulfation, N-sulfated (NS) domains, and regions without sulfation, N-acetylated (NA) domains which is important for regulating HS-protein interactions.
- Interleukin-8, a member of the CXC chemokine family, has been shown to bind to glycosaminoglycans.
- We investigated the interactions of a series of chemoenzymatically synthesized HS-mimetics having defined domain structure with HS-binding protein interleukin 8
- A combination of native mass spectrometry, ion mobility distributions and collision unfolding profiles proves that at low concentrations HS-mimetics with two sulfated domains shift the oligomerization state of IL8 from monomer to dimer**.**

Background



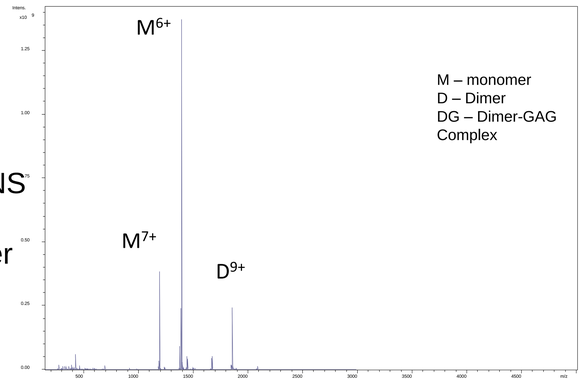
- Schematic showing binding of Interleukin 8 to Heparan Sulfate
- Chemoenzymatically synthesized HS-mimetics

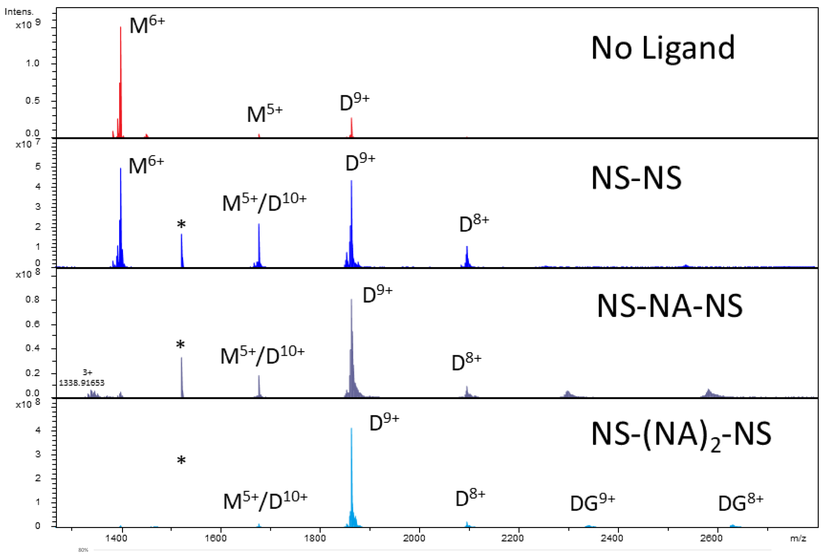
Methods

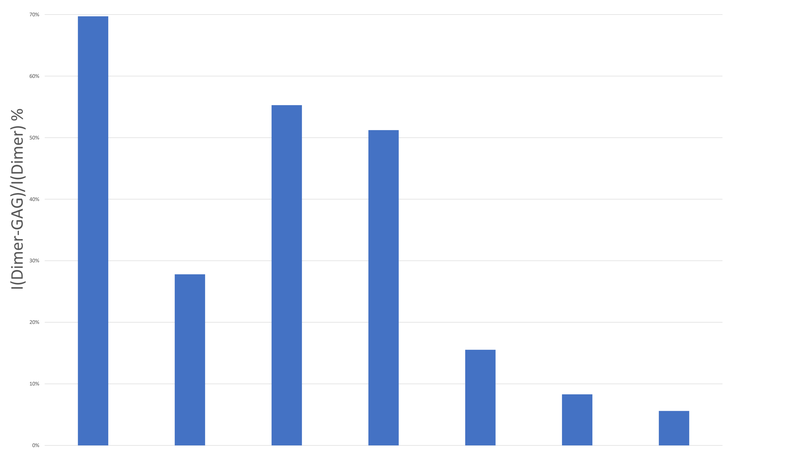
- Mass spectrometry was performed using 12T FT-ICR. A solution of 5 uM Interleukin 8, 5uM HS-mimetics was introduced by direct infusion nESI at a flow rate of 0.3-0.5 μL/min.
- Ion mobility mass spectrometry was performed using a Waters Synapt G2 (q-TWIMS-TOF) with the traveling wave velocity of 300 m/s and wave height of 25, 28, and 30 V.
- Collision induced unfolding experiments were performed by increasing the trap collision energy from 5 V to 80 V in 5 V steps.
- CCS values were determined by calibration with ubiquitin, cytochrome c, and myoglobin.

Native MS HS-mimetics induce dimerization of IL8

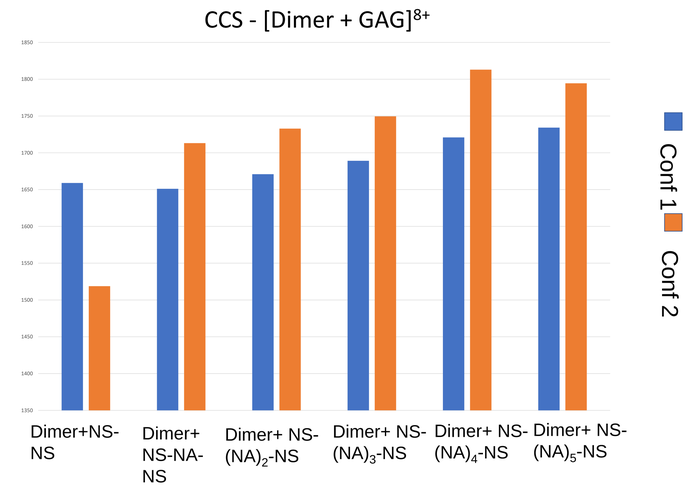
- In the absence of ligand IL8 is predominantly monomeric at this concentration.
- The addition of 2-NS domain ligands shifted the oligomer distribution to predominantly dimeric IL8.



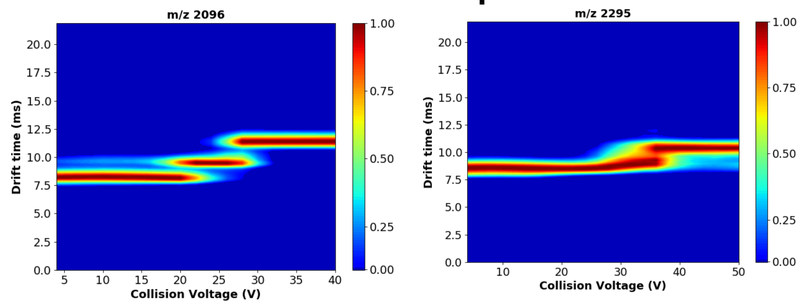




Ion Mobility shows two conformations of Interleukin 8 Dimer + HS-mimetics



Collision induced unfolding demonstrates increased stability of the Interleukin 8 + HS-mimetics complex



- CIU plot of Interleukin 8 shows three unfolded species.
- The Interleukin 8 + HS-mimetics complex shows a similar collisional unfolding pathway, however both transitions have shifted to higher collision voltages

Conclusions

Investigation of bivalent interactions of multiprotein complex with heparan sulfate using native ion mobility mass spectrometry.

References

[1] Goger, B., et al. (2002). " Different Affinities of Glycosaminoglycan Oligosaccharides for Monomeric and Dimeric Interleukin-8: A Model for Chemokine Regulation at Inflammatory Sites." American Chemical Society.
[2] Xue, D., et al. (2014). " Demystifying HeparanSulfate–Protein Interactions.".

Acknowledgments

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