Double-helical structures of hyaluronan-like molecules revealed by molecular dynamics.

Marek Ingr[1], Eva Kutálková[1], Roman Witasek[1], Václav Buš[1], Josef Hrnčiřík[1]

[1]Tomas Bata University in Zlín, Faculty of Technology, Department of Physics and Materials Engineering, Nám. T.G. Masaryka 5555, 76001 Zlín, Czech Republic

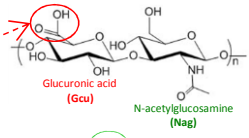
Introduction

Polysaccharides are natural biopolymers of an intrinsically disordered structure in solution. Studying their random coils theoretically requires generation of statistical ensembles of the molecules and determining mean values of their characteristic quantities, especially the radius of gyration R_g. Previously, we introduced a method generating an ensemble of kDa – MDa random coils based on a molecular-dynamics (MD) simulation of a 48 monosaccharide units long oligosaccharide [1]. The obtained results for hyaluronic acid (HA) [2] and its neutral analog are in a striking agreement with experiment. However, this approach neglects any interactions of distant regions of the polysaccharide chain. Experimental scaling factor of random coil, that is different from 0.5, indicates that such interactions may exist. Nevertheless, they were never identified in solution, neither experimentally, nor theoretically. On the other hand, double-helical structures of two hyaluronan chains have previously been reported in crystal structures as well as in computational simulation at zero temperature [3]. Hence, our intention is to investigate whether these structures may be stable also in aqueous environment under some conditions. At identified semi-stable structures we try to determine the contribution of hydrogen bonds vs. hydrophobic interactions to the stability of the structures.

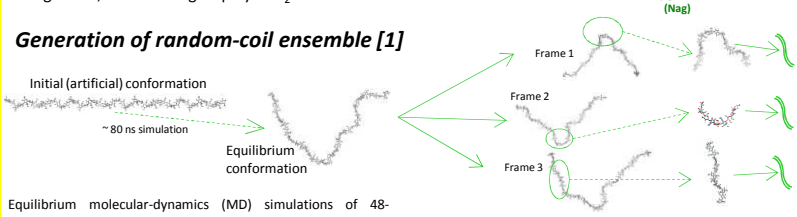
Hyaluronic acid

$[4)$-β-D-Glc*p*A-$(1\rightarrow3)$-β-D-Glc*p*NAc-$(1\rightarrow]_n$

In case of the neutral analog glucuronic acid is substituted by glucose, i.e. –COOH group by $–CH_2OH$

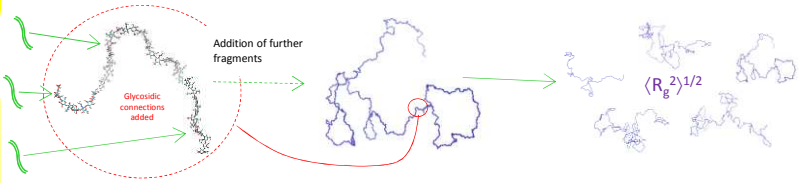


Generation of random-coil ensemble [1]



Equilibrium molecular-dynamics (MD) simulations of 48-monosaccharide-units long oligosaccharide in a corresponding environment (approx. 80 ns).

Selection of randomly chosen fragments from randomly chosen frames of the equilibrated part of the simulation.

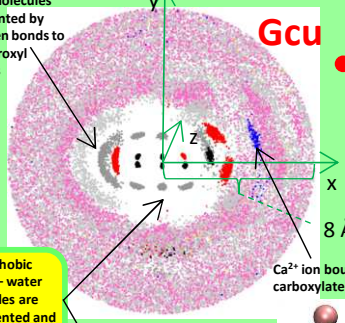


Connection of the selected fragments to a large random coil – the dihedral angles of the glycosidic connections obey the statistics obtained from the simulation. The self-avoidance of the chain in the space is not assumed.

An ensemble of random coils is generated and the mean value of the radius of gyration is calculated.

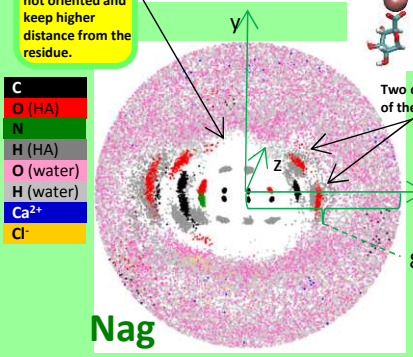
Cumulative solvation-shell diagram

Hydrophilic region – water molecules are oriented by hydrogen bonds to the hydroxyl oxygens

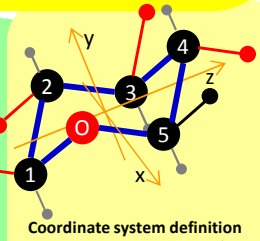


Hydrophobic region – water molecules are not oriented and keep higher distance from the residue.

Ca^{2+} ion bound on the carboxylate group



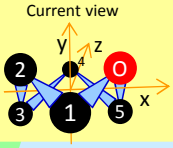
Two conformations of the $–CH_2OH$ group



Coordinate system definition

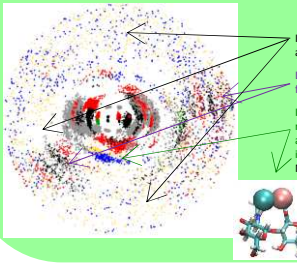
- origin in the geometric center of the six atoms of the monosaccharide "chair"
- z-axis given by the perpendicular projection of the 1→4 direction to the plane given by the average of planes 2-4-O and 1-3-5
- x-axis perpendicular to z, within the above mentioned plane
- y-axis – perpendicular to x and z

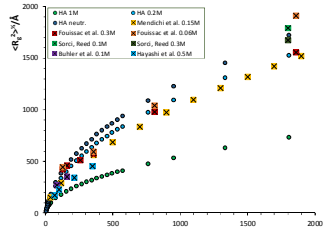
Current view



The diagram is composed of superimposed views of the near surrounding of all (or selected) monosaccharide units (residues) of a given kind (Glc or Nag) taken from several frames of an MD simulation. The diagram can be constructed in any orientation of the residue and for any selection of the ranges in axial (along the z-axis), radial (distance from the z-axis), and angular (angular sector in the x-y plane) dimensions. The plot can be composed of all atoms including the macromolecule and the solvent, or only of a limited selection of them (e.g. without water). Using the diagram distribution and orientation of the solvent molecules can be determined as well as the differences in the rigidity and conformation of the polymer chain.

Strucrural elements of the solvation shell observable in the diagram





Comparison of random-coil simulations with experiment

Random coils constructed on the base of the MD simulations were compared with experimental values from the literature for three NaCl concentrations: **0 M (system only neutralized by Na^+)**, **0.2 M** and **1 M**. The simulated results show a remarkable agreement with experiment.

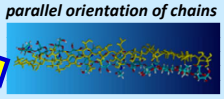
For NaCl, as well as $MgCl_2$, increasing salt concentration leads to a lower R_g values.

Similar simulations for the neutral analog show a similar dependence ⇒ the effect is not primarily mediated by the electrostatic repulsion od carboxylate groups.

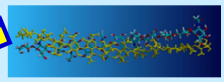
Hydrophobic regions above and below the residue indicate the possibility of hydrophobic interaction of two chains

Observed only in non-physical simulations with the switched-off Coulomb interaction between the molecule and water.

parallel orientation of chains



anti-parallel orientation of chains



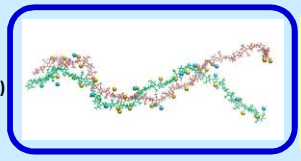
Double helices exist in both chains orientations for both HA and GlcHA, they are highly ordered.

With a complete force field the double helices are unstable for HA, they dissociate in les than 1ns. For GlcHA at low temperature (2 °C) some structures remain complexed for ~ 20 – 40 ns, then they dissociate, too.

Question: Is it possible to form a stable complex of two HA chains?

In higher salt solution interactions (ionic, hydrophobic, H-bonds) may be altered and the duplexes may get stabilized.

So far, the only example of a stable duplex is HA in 1M $ZnCl_2$!



Duplex of hyaluronic acid in 1M NaCl. The structure is stable in time (tens of ns), the double helix is considerably disordered.

Future perspectives

To investigate the hyaluronan duplexes in a set of different environments and temperatures and determine common features of their solvation shell.

To evaluate the influence of eventual duplex formation on the structure of hyaluronan macromolecules in solution.

Conclusions

1. Cumulative solvation-shell diagram allows us to determine significant structures and interactions within the solvation shell – hydrophilic and hydrophobic regions of macromolecules, hydrogen bonds, ion interactions, etc.
2. Hyaluronic-acid has hydrophobic patterns in its structure – this way it may potentially form double-helical duplexes.
3. Double helixes of hyaluronic acid are strongly bound only in non-physical conditions when the molecule-solvent reactions are switched off. After solvation they are mostly unstable and decay.
4. A partially stable double helical structure was found in the 1M solution of $ZnCl_2$. It is rather disordered, but does not decay during tens of ns.
5. Secondary structures of hyaluronic acid may exist in dependence on the environment and other factors, especially temperature. Their stability is, however, limited and their structure partially disordered.

Acknowledgment: Access to computing and storage facilities owned by parties and projects contributing to the National Grid Infrastructure MetaCentrum provided under the program "Projects of Large Research, Development, and Innovations Infrastructures" (CESNET LM2015042), is greatly appreciated. RW was supported by the Internal Funding Agency of Tomas Bata University in Zlín, project IGA/FT/2016/011.

References:

[1] M. Ingr, E. Kutálková, J. Hrnčiřík, *Carbohyd. Polym.* **170**, 289 (2017).
[2] J. Necas, L. Bartosíková, P. Brauner, J. Kolar, *Vet. Med.-Czech* **53**, 397 (2008).
[3] Haxaire, K. Braccini, I., Milas, M., Rinaudo, M., Peréz, M. *Glycobiology* **10**, 587 (2000).