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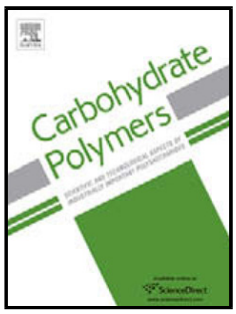


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# Hyaluronan random coils in electrolyte solutions – a molecular dynamics study

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## Highlights

- All-atom models of hyaluronan random coils were made based on molecular dynamics
- Computed radii of gyration agree with experiment for various conditions
- Simulated hyaluronan random coils shrink with growing ionic strength
- Interactions of polymer backbone with ions and water control the molecular shape


### Abstract

A computational method of modeling random coils of hyaluronan was developed based on the molecular-dynamics simulations. An oligosaccharide of 48 monosaccharide units was equilibrated within a 70-100 ns simulation and randomly chosen pieces of this molecule from different simulation frames were combined to constitute a long polysaccharide chain, both for hyaluronan and its non-ionic analog containing glucose instead of glucuronic acid. The dihedral angles of the glycoside connections of the pieces obeyed the statistics deduced from the simulation. The simulations were performed at various concentrations of NaCl and $MgCl_2$. The calculated radii of gyration show a striking agreement with experimental data from the literature and indicate a key importance of the polymer-ion interactions for the random-coil conformation, but a low influence of the excluded volume of the chain and the carboxylate-groups repulsion. The method has thus the potential to become a versatile tool of modeling macromolecules of various semirigid polymers.


**Keywords:** hyaluronan, molecular dynamics, dihedral angle, radius of gyration, electrolyte.

## 1. Introduction

Hyaluronic acid is a natural polysaccharide consisting of alternating units of β-D-glucuronic (GCU) acid and β-D-N-acetylglucosamine (NAG). The monomeric units are connected by β-1,3 glycosidic bond between C1 of GCU and C3 of NAG and by β-1,4 glycosidic bond between C1 of NAG and C4 of GCU [4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→]$_n$. At physiological conditions it mostly occurs in an ionized form (hyaluronan). Hyaluronic acid is a biologically active molecule occurring in connective tissues, especially the synovial fluid, vitreous fluid of eyes, umbilical cords and in chicken combs. Due to its biological functions it is an object of interest of both pharmaceutical and cosmetics industry. For more detailed information see current reviews (Allison & Grande-Allen, 2006; Jiang, Liang, & Noble, 2007; Necas, Bartosikova, Brauner, & Kolar, 2008). Hyaluronic acid is synthesized by membrane enzymes hyaluronan synthases and is catabolized by hydrolases called hyaluronidases (Stern, 2003). Industrially it is produced from animal tissues or genetically modified bacteria, but the cell-free technologies are being developed, too (Sze, Brownlie, & Love, 2016).

Hyaluronan is a hydrophilic polymer with a strong retention of water. In water environment it forms highly swollen random coils, the shape and dimensions of which are influenced by the solution composition. As hyaluronic acid is a polyelectrolyte practically fully dissociated in physiological conditions, it is conceivable that the concentration of ions is one of the key factors influencing the shape of its macromolecules. Indeed, Fouissac, Milas, Rinaudo, & Borsali (1992) studied the dependence of the radius of gyration ($R_g$) on the concentration of NaCl and confirmed a good agreement with the theory of Odijk, Skolnick and Fixman (Odijk, 1977, 1978; Odijk & Houwaart, 1978; Skolnick & Fixman, 1977), i.e. a continuous decrease of the random-coil dimensions when salt concentration increases. Later on numerous experimental studies were published presenting the measured values of different characteristics of hyaluronan random coils, especially the radius of gyration, hydrodynamic radius, diffusion coefficient, intrinsic viscosity of the solution or persistence length of the chain, often in dependence on ionic strength of the solution. Hayashi, Tsutsumi, Nakajima, Norisuye, & Teramoto (1995) studied the properties of the hyaluronan solutions in higher salt concentrations, 0.2 and 0.5M NaCl. Mendichi, Soltés, & Giacometti Schieroni (2003) carried out a complex study of hyaluronan properties at a single concentration of 0.15 M NaCl. In both these studies the dependences of $R_g$ and intrinsic viscosity on molecular weight as well as the scaling factors of both the quantities were determined. Sorci & Reed (2004) studied hyaluronan solutions at varying concentrations of NaCl and $CaCl_2$ up to the ionic strength of 0.1 M. They showed a continuous decrease of the radius of gyration with growing ionic strength as well as the formation of smaller coils in $CaCl_2$ with respect to NaCl. Buhler & Boué (2004) showed that the persistence length of a hyaluronan molecule decreases with growing ionic strength of the solution. In addition, interactions of hyaluronan with other compounds, especially quaternary ammonium salt surfactants, were studied (Bjoerling, Hersloef-Bjoerling, & Stilbs, 1995; Grundelova, Mracek, Kasparkova, Minarik, & Smolka, 2013).

The structure of free hyaluronan molecules has also been studied by means of theoretical chemistry. One of the first attempts was carried out by Holmbeck, Petillo, & Lerner (1994) who determined average values of the dihedral angles of the glycosidic bonds in hyaluronan by molecular-mechanics approach. Kaufmann, Möhle, Hofmann, & Arnold (1998) determined the pairs of dihedral angles of both the β-1,3 and β-1,4 glycosidic bonds and constructed the respective Ramachandran plots using the molecular-dynamics approach applied to short pieces of hyaluronan, namely dimers and trimers of the basic monosaccharide units. Pereira et al. (2006) generalized this method to several different disaccharides. Similar approach was used by Almond, Brass, & Sheehan (1998), who determined the average helix

of the polymer molecule, and Donati, Magnani, Bonechi, Barbucci, & Rossi (2001), who compared the MD simulations on short pieces of hyaluronan molecule with the experimental NMR data. Kirschner & Woods (2001) showed, using the quantum-mechanical approach, that explicit solvent is necessary for a good reproduction of the dihedral angles. Ivanov & Neamtu (2013) applied this technique to study the influence of dimethylsilanediol to the hyaluronan structure. Furlan, La Penna, Perico, & Cesàro (2004, 2005) used Monte Carlo simulation using all-atoms molecular potential to model the properties of hyaluronan random coils. In a different approach, Nyström et al. (2010) used Monte Carlo method to simulate larger hyaluronan random coils using the bead-and-spring model. This approach enabled simulations of molecules of real sizes, but without the use of the exact molecular potentials. Recently, Mutter et al. (2015) used molecular dynamics in combination with DFT in order to determine the vibrational spectra of hyaluronan.
In this work we apply the molecular-dynamics approach to an oligosaccharide of 48 monosaccharide units and compose large random coils connecting randomly chosen pieces of this chain from different frames of the simulation. This allows us to make all-atom models of random coils up to at least 10000 monosaccharide units and to determine some of their physico-chemical properties.

## 2. Methods

This study deals with the macromolecules of hyaluronan [4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→]$_n$ (hereafter abbreviated as HA) and its non-charged analog in which the carboxyl groups are substituted by hydroxymetyl groups, i.e. the glucuronic acid unit is substituted by glucose [4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*NAc-(1→]$_n$ (hereafter abbreviated as GlcHA). Equilibrium structures of 48 monosaccharide-units long chains of hyaluronan and its analog were generated by means of molecular-dynamics simulations in environments containing various concentrations of two salts, NaCl and $MgCl_2$. All MD simulations were performed in NAMD Version 2.10 program package (Phillips et al., 2005) using the CHARM36 carbohydrate topology and force field parameters (Guvench et al., 2008; Guvench, Hatcher, Venable, Pastor, & MacKerell, 2009). Interatomic distances and non-bonding interactions were evaluated using VMD 1.9.2 program (Humphrey, Dalke, & Schulten, 1996). High molecular weight random coils were generated by connecting randomly selected pieces of the simulated molecules by a method of selection of the glycoside-bond dihedral angles in accord with their distribution in the simulated molecules. For details see SI.

## 3. Results and discussion

### 3.1. Generation of equilibrium random coils.

MD simulations of polysaccharide molecules of 48 monosaccharide units were carried out for various systems. The chosen molecules were hyaluronan, simulated in 1M, 0.2M and 0M (polyelectrolyte only neutralized by counterions) NaCl and $MgCl_2$, and its non-charged analogue in which the glucuronic acid unit was substituted by glucose, simulated in 1M NaCl and $MgCl_2$ and in pure water.
The simulated molecules were used as the source material for building large macromolecular coils by a procedure similar to that used by Furlan et al. (2005) or Ivanov & Neamtu (2013), but applied to much larger molecules and using a specific statistical procedure to determine the dihedral angles of the glycosidic connections of the pieces (see SI). In order to monitor the equilibration of the system under the MD simulation, the construction of the random coils was performed in every interval of approx. 5 ns. For each such interval 5000 coils of a reference

length of 2000 monosaccharides (i.e. 379 kDa) were generated and the mean square value of the radius of gyration and its standard deviation of the mean were calculated. The chosen length is sufficient for the formation of a realistic random coil, but not too long for being biased by the neglect of the excluded volume of the chain (for the discussion of this point see section 3.2. Fig. 1 shows that the radius of gyration decreases during the course of the simulation reaching a stable region, biased only by random fluctuations, after about 60 ns. This supports the hypothesis that the equilibration of individual parts of the chain leads to the equilibrium structures of the whole generated random coils. Interestingly, together with the $R_g$ value its standard deviation of the mean decreases, too, in a highly regular manner (this phenomenon is discussed in detail in SI).

The initial configuration of the simulated molecules was an artificially constructed regular helix (Fig. S1). Although the molecule immediately adopted a more physically relevant shape of an irregular helix-like chain, the full equilibration lasted for tens nanoseconds, during which it still contained some remainders of the initial, non-physical structure. Hence, the set of frames from the equilibrated region that provided the $R_g$ value closest to the average of this region was further used to model the random coils of different sizes. As the random-coil models were constructed for different electrolyte concentrations, the reversibility of the conformation changes accompanying the transfer of the molecule from one concentration to another was tested on an example of the non-ionic analog of hyaluronan in $MgCl_2$. An oligosaccharide was initially equilibrated in 1M $MgCl_2$ and the random coils of 2000 monosaccharide units derived from it showed $R_g$ of (434.4 ± 1.6) Å (see also the next section). When an oligosaccharide of the same geometry was re-equilibrated in pure water, the $R_g$ of the constructed random coils started to increase immediately approaching the value of (802.7 ± 2.7) Å obtained for the same system after 70 ns of independent MD simulation. Hence, the model is well reversible when the electrolyte concentration is being varied.

### 3.2.The shape and dimensions of the macromolecules.

After finding the equilibrated set of frames, random coils were generated for a wide range of polymer lengths spanning from 20 to 10000 monosaccharide units. As can be seen in Figs. S3-S6, the resulting macromolecular coils are relatively rarely packed with a very low probability of chain crossing in the space. (Supplementary information (SI) contains also files in the XYZ format containing the coordinates of the random coils shown in Figs. S3-S6 that can be 3D-viewed in any relevant program.) Therefore, within this study we use only a simple model of a non-interacting coil in which the macromolecular chain can, in principle, intersect itself. To judge the applicability of the method for modelling macromolecules of various molecular weights, the comparison of the determined radii of gyration with miscellaneous experimental data was carried out. Mendichi et al. (2003) measured $R_g$ for a wide scale of molecular weights spanning from 40 kDa to 6 MDa in 0.15M NaCl. Figs. 2A and S2 indicate a good agreement of our model (0.2M NaCl) with this experiment in a substantial part of this interval. In addition, several discrete values of $R_g$ determined by Fouissac et al. (1992), measured at 0.3 M NaCl, agree with our model also well. In that study values for lower NaCl concentrations, 0.06 M and 0.01 M, are presented, too. They indicate a slight expansion of the coils when the salt concentration decreases, which is an identical trend that can be observed in our simulation when going from 0.2M NaCl to just neutralized hyaluronan molecules (Figs. 2B and S2). For higher salt concentrations less data are available. To our knowledge, no experiments carried out in 1M NaCl have been published yet, therefore a direct comparison of our simulation at these conditions cannot be done. However, Hayashi et al. (1995) published the values of $R_g$ in 0.5M NaCl for a wide range of molecular weights. It can be seen in Figs.

2C and S2 that these values fit between our values for 0.2M and 1.0M NaCl. This demonstrates a good applicability of the theoretical approach for molecules of the given size. At higher molecular weights this model may be in principle biased by the neglect of the interactions of the distant monosaccharide units and allowed intersection of the polymer chain, which might cause smaller $R_g$ values of the simulated molecules. However, the experimentally determined radius of gyration of a 1.8MDa molecule in 0.1M NaCl (Sorci & Reed, 2004) is about 1760 Å while the simulated values are 1530 Å for 0.2M NaCl and 1720 Å for just neutralized system. Considering the effect of electrolyte concentration on $R_g$ (see also section 3.3.), this deviation is obviously small even for molecules of this size. Hence, the resulting values show a striking agreement with the experimental data of various sources for molecules up to 10000 monosaccharide units, even under considerably varying conditions. This result, in addition, indicates that the excluded volume of the chain and the interactions of the distant parts of the molecule have only a minor influence on the overall shape of the macromolecule.

### 3.3.Random coils in varying electrolyte concentrations.

Reproducible differences of the molecular sizes can be found among the different simulated systems, especially among the molecules simulated in different electrolyte concentrations. For every system, i.e. a given polymer in a given salt, $R_g$ was determined for three salt concentrations, 1.0 M, 0.2 M and 0 M (in case of hyaluronan accurate number of counterions was present). Fig. 3 exhibits the $R_g$ dependence on the concentrations of NaCl and $MgCl_2$ and indicates an apparent decrease of this quantity with the increasing salt concentration. This result remarkably agrees with the experimental values (Figs. 2 and S2), although the experimental data are obtained from different sources.

The decrease of the radius of gyration with the growing salt concentration is reflected also by the changes of the persistence length of the polymer chain. Based on the Kratky-Porod model of a wormlike chain (Kratky & Porod, 1949) the persistence length can be calculated from the determined mean radius of gyration using the equation (Benoit & Doty, 1953)

$$\left\langle R_g^2 \right\rangle = l_p \left( \frac{L}{3} - l_p + \frac{2l_p^2}{L^2} \left[ L - l_p \left( 1 - \exp\left( -\frac{L}{l_p} \right) \right) \right] \right), \qquad (1)$$

where $l_p$ is the persistence length and $L$ is the contour length of the chain. Numerical solution of Eq. (1) carried out in the Wolfram Mathematica 9 package gives the resulting persistence lengths for the different systems summarized in Table S1, all of them calculated for the reference chain length of 2000 monosaccharide units and the contour length of 10 Å per a disaccharide unit.

As the persistence length reacts very sensitively on the changes in $R_g$, it decreases rapidly when the salt concentration grows. Hence, in low salt concentration the polymer chain is remarkably stiffer then in higher ionic strength which explains the tendency to form bigger random coils. The determined values of the total persistence length are roughly in agreement with the experimental findings of Buhler & Boué (2004) who also report values close to 200 Å in the salt-free solutions and the decrease of them when the salt concentration grows. They attribute this effect to the screening of the coulombic repulsion of the carboxylate groups in a high salt concentration and absence of this effect in low concentration. However, our simulations indicate that this effect is of a minor importance and the hyaluronan-salt interactions are facilitated especially by the other functional groups of the chain (see also section 3.5.).

The scaling factor of the radius of gyration, i.e. the power of $M_w$ the radius of gyration grows with, shows a remarkably different behavior in dependence on the salt concentration (Fig. 4). In every case it tends to 1 for very low $M_w$ which is a trivial consequence of the almost rod-like shape of very short molecules. On the other hand, as the model considers only a non-interacting random coil, the scaling factor must necessarily converge to the value of 0.5 when the chain length tends to infinity. In spite of this, considerable differences can be observed for different electrolyte concentrations. In the high electrolyte concentration the scaling factor reaches the value of 0.5 at about 500 monosaccharide units (95 kDa), while in the low concentration the decrease is much slower and the scaling factor does not approach the value of 0.5 below 4000 monosaccharide units (760 kDa) – see Fig. 4. Hence, the observed decrease of the scaling factor roughly correlates with the persistence length – the longer the persistence length, the slower the scaling-factor decrease. Accordingly, the characteristic ratio of the random coil, i.e. the ratio of the mean value of squares of the chain-ends distances $\langle r^2 \rangle$ to the mean value of squares of radii of gyration $\langle R_g^2 \rangle$, decreases in all cases to the value of 6.0 characteristic for an unperturbed random coil, but the decrease is significantly more rapid for macromolecules in high electrolyte concentration (see Fig. S7). However, comparison of the scaling factor with experimental data brings mixed results. Fouissac et al. (1992) present data roughly agreeing with our model, their scaling factor decreases from 0.585 at 0.01M NaCl towards 0.5 at 0.3M. On the contrary, the studies of Mendichi et al. (2003) and Hayashi et al. (1995) both provide the constant value of approx. 0.59 for 0.15M and 0.5M NaCl, respectively. Thus, according to those works the scaling factor is independent of the salt concentration and does not fall to 0.5 even if it is rather high. Unfortunately, it is not easy to explain this discrepancy as not enough independent data are available, and we can only speculate about its reasons. First of all, the dependencies of $R_g$ on $M_w$ for different scaling factors within the region of 0.5 and 0.6 are all quite similar and a minor inaccuracy can come from the experimental/theoretical setup and data evaluation. Furthermore, the experimental studies a priori expected a constant scaling factor independent on the chain length which might be inappropriate, especially for the short molecules. Finally, our theoretical model ignores the excluded-volume influence, which necessarily causes the convergence of the scaling factor to 0.5, the scaling factor can thus be underestimated for longer molecules. On the other hand, as can be deduced from the images of the modeled molecules (Figs. S3-S6), the chain crossing seems to be very rare, especially for shorter molecules, no matter what the salt concentration is. Therefore, it is unlikely that the excluded-volume effect has a significant influence except for the large molecules of at least MDa molecular weight. In addition, the results of the experimental study investigating the scaling factor consistently for three different salt concentrations (Fouissac et al., 1992) support our findings. Hence, it is not possible to make a clear an unambiguous conclusion in this point.

### 3.4.Hyaluronan molecules in different electrolytes.

Although the general polyelectrolyte theories are based on the concept of ionic strength, it is known from the pioneering work of Hofmeister (1891) that the influence of ions on the polyelectrolytic biomolecules is a more complex problem based on specific characteristics of individual ions. As was shown by Jungwirth and collaborators (Lund, Vácha, & Jungwirth, 2008; Vlachy et al., 2009), the interaction of an ion with a given group of the macromolecule depends on the electronic structure of both the ion and the group in a non-trivial way. Due to this fact even the originally proposed Hofmeister series is not valid generally for all systems. To show the influence of different salts on hyaluronan, we simulated its random coils in the

environment of two different salts, NaCl and $MgCl_2$. Both the salts were applied in the concentrations of 0 M (hyaluronan only neutralized by counterions), 0.2 M and 1 M for hyaluronan and 0 M and 1 M for its non-ionic analog. As can be seen in Fig. 3, both the molecules behave almost equally in solutions of both the salts, i.e. their radii of gyration are given only by the concentration but not by the identity of the electrolyte. This feature can be justified in the following way. First, Table S1 shows that the interaction of chloride ions is very small in comparison with any of the cations, their influence is, therefore, minor even though their concentration is twice higher at $MgCl_2$ in comparison with NaCl. Second, Fig. 5 indicates an apparent difference between the interactions of both the ions with the functional groups of the polymer. While $Na^+$ favors the direct interaction, more strongly solvated $Mg^{2+}$ prefers interactions across water molecules rather firmly attached to its surface and thus shielding the electrostatic interaction partially. This may also be a plausible explanation of the partial disruption of hyaluronan-water hydrogen bonds by high concentrations of NaCl, but not $MgCl_2$ (see Table S1). Therefore, the interactions of sodium and magnesium cations with the functional groups are approximately equal which results in a similar behavior of the hyaluronan random coil in equal concentrations of the two salts. However, simulations comprising more different electrolytes might be helpful in order to formulate general conclusions regarding the interaction of hyaluronan and similar molecules with ions, which is planned as a future perspective of this project.

### 3.5.Interactions driving the changes of the random coil shape.

The presented simulations, as well as the discussed experiments, show a strong response of the random coils of both the studied polysaccharides to the changes of the electrolyte concentration. Considering the polyelectrolytic essence of the molecule, the Coulombic interactions between the charged carboxylate groups and their shielding by the ions of the electrolyte may be considered as the most probable cause of this effect. However, as can be seen in Fig. 3, the influence of the salt concentration on the radius of gyration is approximately equal at both the charged molecule of hyaluronan and the neutral molecule of its non-charged analog. It indicates that this interaction is, in fact, minor, which is also documented by Fig. S8 showing that even the pure Coulombic interaction ($\varepsilon_r = 1$) between the carboxylate groups is smaller than other relevant interactions by several orders of magnitude. This is, actually, not surprising, considering that the average distance between these groups (the C6 atoms) is about 10 Å (Fig. S8B), at which the electrostatic force within the aqueous solution is already very week – the distance is, in fact, very close to the electrostatic cut-off of 10-12 Å applied generally in the MD simulations. However, the interactions of the simulated oligosaccharide chain with the surrounding water molecules and ions are strong and highly dependent on the electrolyte concentration. It indicates the key influence of these interactions on the polysaccharide conformation. Fig. S9 shows that the interaction with ions grows with the electrolyte concentration, while the interaction with water decreases. The non-ionic analog shows the same trend regarding the interaction with water, but in a lower extent (Fig. S10). When the interactions of the carboxylate groups (or hydroxymethyl groups of the analog) with the surrounding water molecules and ions are subtracted, the remaining interactions are roughly equal for both the systems, indicating that their influence on the conformation of the chain is the same at both the molecules (Figs. S11-S14). This finding justifies the hypothesis that the primary influence on the molecular conformation is given by the interactions of the ions and water molecules with the “backbone” of the molecule rather than the carboxylate groups, most likely via their influence on the flexible parts of the molecule, especially the glycoside bonds. Although, to our knowledge, no experimental comparison of this or another

structurally similar couple of charged and neutral polymer has been reported yet, numerous studies of different neutral water-soluble polymers in salt solutions indicate the plausibility of this hypothesis. A similar effect was observed at polyethylene glycol (PEG), a polymer resembling HA by the ether bond connecting the monomeric units, although its structure is simpler and more flexible. The increasing salt concentration causes the decrease of the intrinsic viscosity of PEG solutions with the intensities determined by the salt identity (Bailey & Callard, 1959; Brunchi & Ghimici, 2013) which indicates the shrinkage of the macromolecular coils. Another experiment showed the decreased PEG swelling in high salt concentrations by a direct measurement of the mass of the solvating water molecules using a quartz microbalance (Heeb, Lee, Venkataraman, & Spencer, 2009). An analogous behavior was found also for polyvinylpyrrolidone in various salt solutions (Guner, 1996). However, other polymers, e.g. polyvinylalcohol (Bianchi, Conio, & Ciferri, 1967) and polyacrylamide (Livney et al., 2003), showed mixed responses, i.e. swelling or shrinkage, to the electrolyte-concentration growth depending on the used salts. Hence, the electrolyte influence on neutral polymers is likely a consequence of a complex system of interactions of ions both with the solvation sphere of the macromolecule and with the individual polar groups of the polymer, which prevents a simple generalization of this phenomenon. In our case, separation of the total interaction energy to the contributions of water and ions interactions with the molecule shows that the increasing salt concentration leads not only to a stronger interaction of the chain with ions, but also to a weaker interaction with water. Thus, addition of salt causes restructuring of the solvation shell of hyaluronan which results in the tiny variations of the conformation of a short piece of the chain, but – consequently – in a substantial change of the radii of gyration of the large random coils. This phenomenon has, therefore, an analogous essence as the well-known salting-out effect. However, the salt concentration has only a minor effect on the number of hydrogen bonds. Table S1 shows that the number of hydrogen bonds between the groups of the chain is almost independent of the salt concentration for both hyaluronan and its analog. The number of hydrogen bonds between the chain and the surrounding water is also quite stable, although its moderate decrease of about 12.5% can be observed when NaCl concentration grows from 0 M to 1 M, which corresponds with the decrease of water molecules in the solvation shell of hyaluronan. On the other hand, in $MgCl_2$ solution this effect cannot be seen, probably due to the indirect interaction of the cation with the chain and thus smaller ability to repel water molecules. Hence, it seems that the formation or disruption of hydrogen bonds is not the key interaction forming the shape of hyaluronan and similar molecules.

## 4. Conclusion

A method of modeling the semirigid polysaccharide molecules was developed and applied on the macromolecules of hyaluronan and its non-charged analog in solutions of two electrolytes, NaCl and $MgCl_2$. The method is based on an equilibrium molecular-dynamics simulation of a 48 monosaccharide-units long molecule and combining randomly selected pieces of this molecule from different simulation frames in order to construct a bigger macromolecule. Using this method sets of random coils, each of a given molecular weight in a given environment, were generated and the averaged radius of gyration was determined for each set. The radius of gyration of the modeled random coils decreases and finally tends to a stable value as long as the MD-simulated oligosaccharide is drawing to equilibrium, indicating that the equilibration of the individual pieces of the macromolecule leads to the equilibration of the whole random coil. The stabilized radii of gyration show a strikingly good agreement with the experimentally observed values as well as the scaling with the molecular weight of the macromolecule. Moreover, the simulated random coils respond to the varying electrolyte

concentration in an obvious accordance with the experimental observations – both the radius of gyration and the persistence length of the chain grow when the electrolyte concentration is decreasing, similarly for both hyaluronan and its non-charged analog and for both the salts under consideration. Thanks to this excellent agreement of the model and experiment we can deduce that the main force driving the conformational changes of the macromolecule is the restructuring of the solvation shell of the polymer, i.e. the interactions of water molecules and the cations with the polymer backbone. On the contrary, the mutual repulsion of the carboxylate groups as well as the excluded volume of the chain has only a minor effect on the molecular conformation. Furthermore, the model-experiment agreement supports the hypothesis that the behavior of a selected piece of the chain is equal, no matter whether it exists as a free oligosaccharide or as a part of a long macromolecule. Therefore, the developed method is able to generate all-atom models of the random coils of semirigid polysaccharide molecules on the base of the complete set of their local pair interactions within the macromolecule itself and between the macromolecule and the environment. This feature gives the method the capability to become a versatile tool for the prediction of physical properties of random coils of semirigid polysaccharides.

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**Supplementary information**

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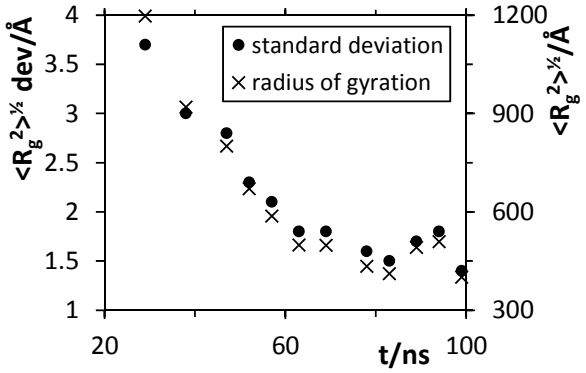
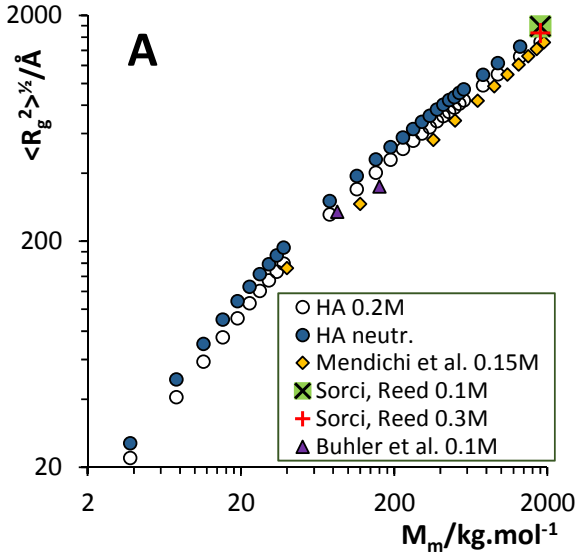
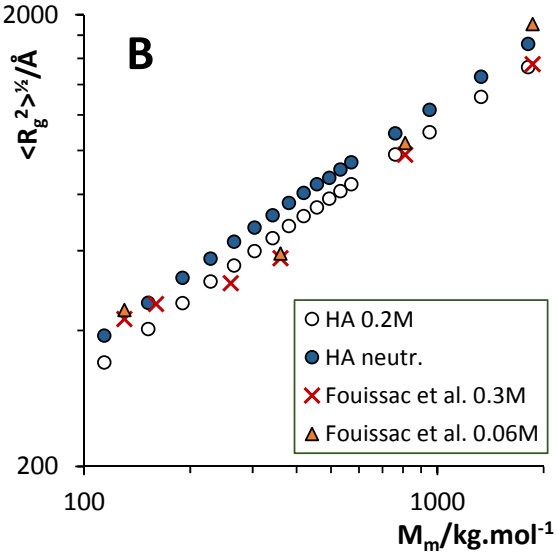



**Fig. 1:** Time evolution of the mean value (right axis) and the standard deviation (left axis) of the radius of gyration of a set of 5000 random coils of a 2000 monosaccharide-units long molecule of the non-charged hyaluronan analog in water with 1M $MgCl_2$. The simulation starts from the artificial conformation of a regular helix (Fig. S1, lower panel).



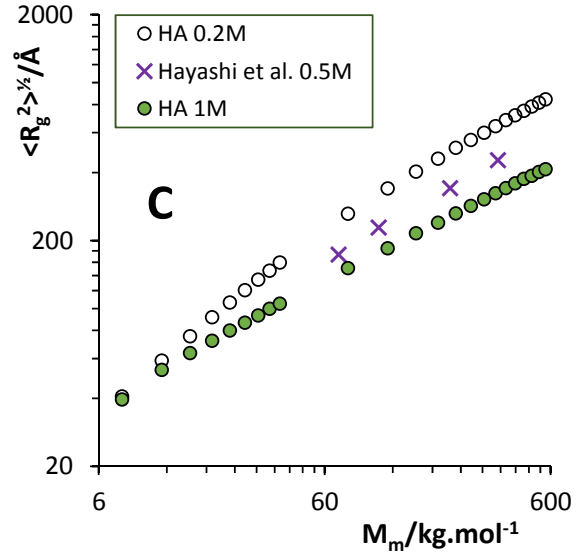



**Fig. 2:** Comparison of radii of gyration of the modeled hyaluronan random coils in water with various concentrations of NaCl (circles) with experimental data (other symbols) of different sources.

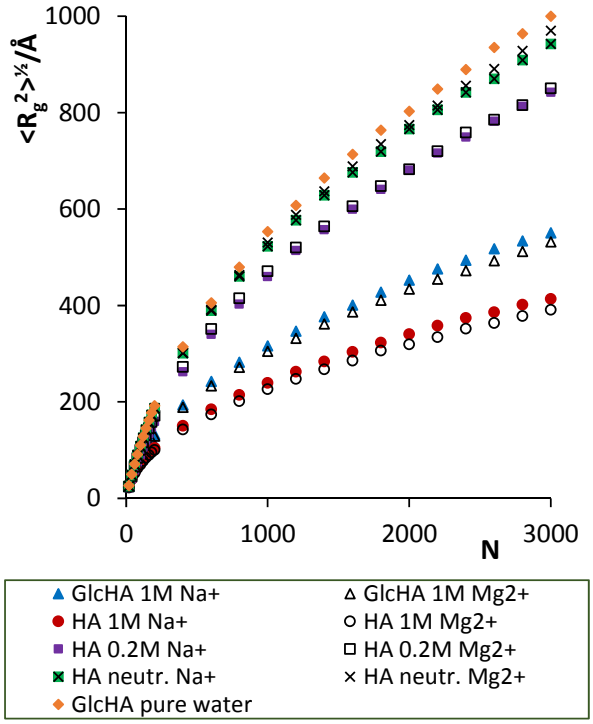



**Fig. 3:** Radius of gyration as a function of the number of monosaccharide units N for all the simulated systems.

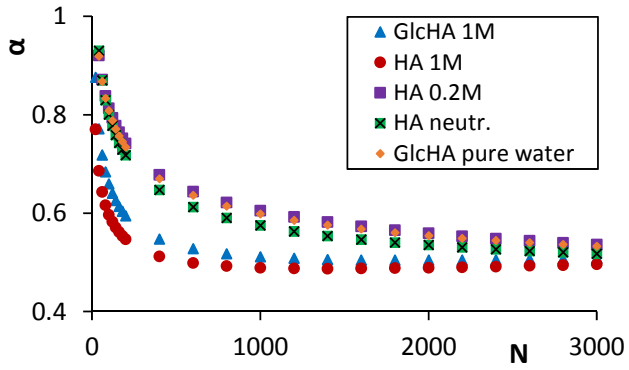



**Fig. 4:** Scaling factor as a function of the number of monosaccharide units N for the different simulated polysaccharides in NaCl solutions (concentrations are indicated in the legend).

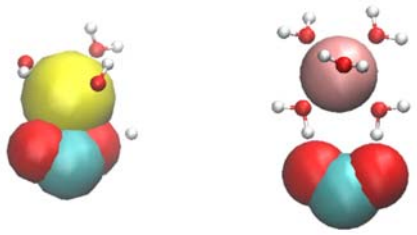


**Fig. 5:** A typical picture from MD simulations of the interaction of the sodium (left) and magnesium (right) cations with the carboxylate group.