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Hyaluronan random coils in electrolyte solutions—a molecular dynamics study

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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.

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, and 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, and Teramoto (1995) studied the properties of the hyaluronan solutions in higher salt concentrations, 0.2 and 0.5 M NaCl. Mendichi, Soltés, and 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 grow-

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ing 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, and Lerner (1994) who determined average values of the dihedral angles of the glycosidic bonds in hyaluronan by molecular-mechanics approach. Kaufmann, Möhle, Hofmann, and 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, and Sheehan (1998), who determined the average helix of the polymer molecule, and Donati, Magnani, Bonechi, Barbucci, and 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, and 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.

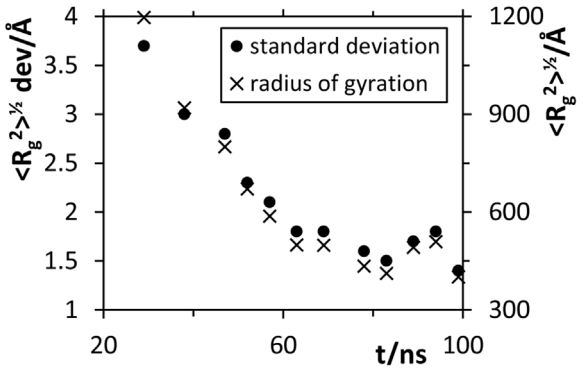

Fig. 1. Time evolution of the mean value (right axis) and the standard deviation (left axis) of the radius of gyration of a set of 5000 random coils of a 2000 monosaccharide-units long molecule of the non-charged hyaluronan analog in water with 1 M $MgCl_2$. The simulation starts from the artificial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

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 1 M, 0.2 M and 0 M (polyelectrolyte only neutralized by counterions) NaCl and $MgCl_2$, and its non-charged analog in which the glucuronic acid unit was substituted by glucose, simulated in 1 M 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