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Note

Molecular modeling of cobalt(II) hyaluronate

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Abstract—Structural data for complexes of hyaluronic acid and 3d metals(II) of the fourth group of the periodic table are lacking. A combined QM/MM method was used to solve the structure of the first coordination sphere around the cobalt(II) ion. Some available experimental data were compared with the results obtained via computation and were found to be in good agreement. Our results open the way for using molecular modeling to solve the structure of other metal(II) hyaluronates.

Keywords: Cobalt(II) hyaluronate; Molecular modeling; EXAFS; QM/MM

Hyaluronan (hyaluronic acid, HA) is a naturally occurring biopolymer involved in many important biological processes. HA naturally occurs in the extracellular matrix of tissues in higher animals.[1] High concentrations of HA can be found in skin, vitreous humor, cartilage, and umbilical cord where it controls the tissue hydration level. HA also serves structural and mechanical functions.[2] In synovial fluid, where HA is essential for normal joint function, the concentration can reach 1.4–3.6 mg/mL. HA does not act only as a molecular filter, but it also confers the necessary rheological properties to synovial fluid (space filling, viscoelasticity, and lubrication).[3,4] The high water retention of HA also influences various cellular processes including cell migration and differentiation, cell recognition, and adhesion.[5,6]

The utility of this biopolymer is derived from a remarkably simple construct. HA is composed of linear, unbranched, polyanionic disaccharide units consisting of D-glucuronic acid (GlcA) and 2-acetamido-2-deoxy-D-glucose (*N*-acetylglucosamine, GlcNAc) joined alternately by β-(1→3) and β-(1→4) glycosidic bonds (Fig. 1).

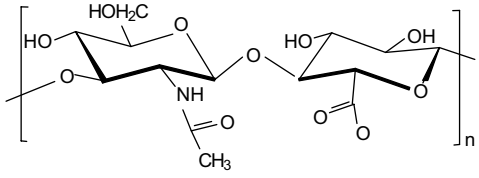

Figure 1. Disaccharide repeating unit of HA comprising GlcA and GlcNAc. The molecular weight of HA from different sources is highly variable ranging from 10^4 to 10^7 Da.

Under physiological conditions, hyaluronic acid is a negatively charged polyelectrolyte due to repeating anionic carboxylic sites.[7] The interaction of the polyanion with cations is an important factor for the overall supermolecular structure.[8] Metal complexation may change the biological activity of HA, the new properties being used in many pharmaceutical practices.[8–10]

In the literature few references are related to complexes of HA with 3d metal ions of the fourth period of the periodic table. HA has been shown to coordinate to Cu^{2+}, Ag^{1+}, Au^{3+}, Cd^{2+}, Pb^{2+}, Fe^{3+}, and Zn^{2+} in solution.[8,11–15] Hyaluronate complexes with heavy metals show microbicidal activity. Gold complexes are used in arthritis therapy, whereas platinum(II) complexes have antitumor activity.[16]

Biological activity of the hyaluronate molecule is strongly conformation dependent. Factors contributing

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to the conformation include the following: pH, temperature, extent of hydration, and the counter ion, the latter being the most important. Thus knowing the conformation of the hyaluronate chain(s) is very important.

In the past, X-ray diffraction on fibers and films was used to solve the solid-state structure of HA in univalent cationic environments, for example, sodium, potassium, and ammonium hyaluronates at different conditions (temperature, humidity) and calcium hyaluronate films as well.[17–22] Recent developments in the field of molecular modeling of polysaccharides offer new opportunities to examine the formation and stabilization of ordered structures and their interactions with cations.

The conformational analysis and electrostatic properties of mono-, di-, and trisaccharide units of HA using semiempirical AM1 and ab initio quantum molecular computations has shown good agreement between optimized geometries of the monosaccharides and the avalaible crystallographic data.[23,24] The molecular electrostatics potential around the polysaccharide chain caused by the polyanionic character of the hyaluronate is composed of isopotential lines with large and intense negative basins around carboxylate groups, which are able to interact with cationic species such as metal ions.[6]

The results of molecular dynamics (MD) simulations on hyaluronic acid dimer and trimer subunits in aqueous solution are in good agreement with NMR and X-ray data.[25]

Haxaire et al. re-examined the structural basis underlying the formation and stabilization of ordered hyaluronate structures and their interactions with counterions. The motility of the polysaccharide chain (illustrated by three different helical conformations 3_2, 4_3, 2_1) is explained by small energetic cost (0.7 kcal/mol per dimer) associated with small variations in glycosidic torsion angles leading to different types of helix.[26]

Recently, a group lead by Bayraktar used the ZINDO1 method and the program HYPERCHEM to model a complex between a hyaluronan disaccharide unit and hydrated chromium ion.[27]

In the past, methods used in molecular modeling were very limited. The system in question had to be relatively small, composed of approximately 50 atoms, but even in cases of small systems consisting of only a few atoms, the calculations took days if not months to complete. One problem was the inadequacy of the methods employed because a lot of processing power was lost on atoms that were not important, that is, atoms that did not participate in chemical reactions/interactions being studied.[28]

The QM/MM method is different. First the system is divided into two parts, namely QM and MM. MM stands for molecular mechanics and denotes the part that is computed using the laws of classical mechanics. The MM part is usually the majority of the system, the other part is QM. QM stands for quantum mechanics and describes the atoms that are computed using quantum mechanics (ab initio or semiempirically). Typically, the QM portion of the system consists of atoms on an enzyme active site or atoms that are important for interactions with other molecules. By dividing the system we provide a means to accelerate the computation as processing power is not used on irrelevant atoms. QM/MM methods make possible computations on very large systems, composed of many thousands of atoms.

Heavy metals in the hyaluronate chains network have not yet been studied using molecular modeling. In this article, we have used molecular modeling (the QM/MM method) to gain insights into the conformation of hyaluronate chain, which is bound to the cobalt(II) ion. The applied technique provides structural information, and the results obtained for the first coordination sphere can be compared with some experimental data (EXAFS) analysis reported previously.[29]

Figure 2 shows the segment under discussion of the hyaluronate molecule coordinated to calcium ions, each chain consisting of six disaccharide units. Calcium ions bind to two antiparallel HA chains. Water molecules around the calcium ion form a network of hydrogen bonds between each other and between unbound carboxylate oxygen atom and other nearest O-donor groups. Water molecules, together with calcium ions,

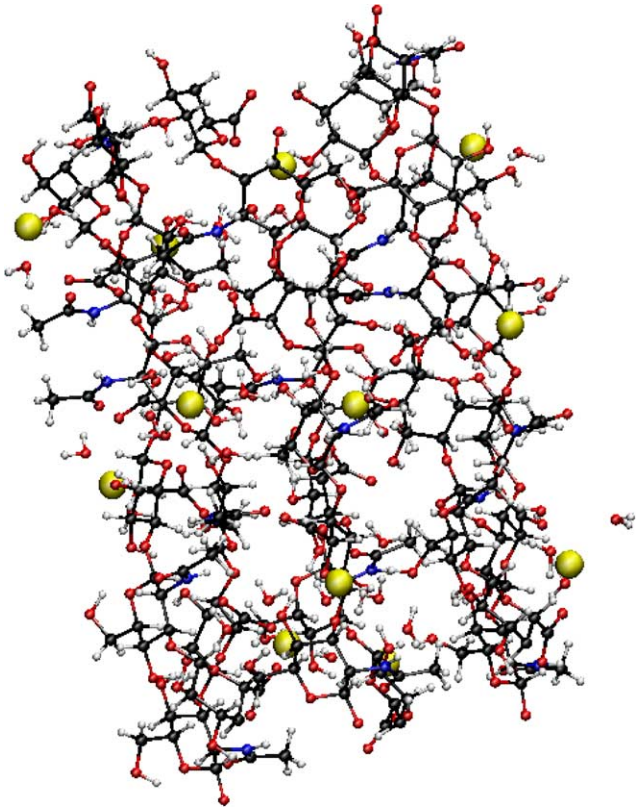

Figure 2. Network of six hyaluronate chains (viewed as CPK). Calcium ion (displayed as yellow sphere) binds the two antiparallel chains through carboxylate groups.

Table 1. Calculated and experimental distances in the first coordination sphere in calcium hyaluronate[a]

Residue	Atom	Distance to Ca^{2+} (Å)	
		QM/MM	Experimental
GCU 39	O6A	2.420	2.510
HOH 44	O	2.430	2.474
HOH 47	O	2.437	2.556
HOH 50	O	2.363	2.569
GCU 54	O6A	2.487	2.511
HOH 59	O	2.475	2.475
HOH 62	O	2.455	2.555
HOH 65	O	3.744	2.570

[a] GCU refers to D-glucuronic acid (GlcA) and HOH refers to water.

Table 2. Distances in the first coordination sphere in the cobalt(II) hyaluronate after the QM/MM and QM calculation[a]

Residue	Atom	Distance to Co^{2+} (Å)	
		QM/MM	QM only
GCU 39	O6A	1.989	1.956
HOH 44	O	2.351	2.150
HOH 47	O	2.021	2.108
HOH 50	O	2.006	2.119
GCU 54	O6A	1.988	1.982
HOH 59	O	3.820	4.253
HOH 62	O	3.586	3.795
HOH 65	O	2.238	2.142

[a] GCU refers to D-glucuronic acid (GlcA) and HOH refers to water.

link the hyaluronate chains together into an entangled three-dimensional framework. The calculated environment around the calcium ion after the last step of energy minimization is summarized in Table 1, where the interatomic distances are compared to experimental data.[21] During the minimization process the water molecule HOH 65 moved away from the first coordination sphere of Ca^{2+}, diminishing its coordination number from 8 to 7. The reason for this difference could be either poor X-ray data as shown by R and R' values (almost 0.30) or the method of sample preparation as mentioned further for Co^{2+}.[21,29]

After replacing the calcium ion with cobalt(II) the energy minimization was run again. The position of individual atoms in the first coordination sphere after the minimization process is shown in Figure 3. After approximately 300 steps, the minimization process was basically finished as the atoms only oscillate around their equilibrium positions. The calculated distances after the combined QM/MM computation and QM computation are displayed in Table 2. Two water molecules (labeled HOH 59 and HOH 62) are in the far distance from the central ion at ~3.7 Å, while the other two molecules (HOH 44 and HOH 65) are closer to the the central atom at ~2.3 Å. The first coordination sphere around cobalt(II) ion is shown in Figure 4. Carboxylate groups (from residue GCU 39 and GCU 54, respectively), are monodentately bound to cobalt(II) ion at an average distance of 2.00 Å. Water molecules HOH 44 and HOH 65 can be regarded as axial ligands, and from Figure 5 it is obvious that the ligands GCU 39, HOH 47, HOH 50, and GCU 54 are planar. The average calculated distances are in good agreement with EXAFS measurements on film samples of cobalt(II) hyaluronate, whereas measurements of cobalt(II) hyaluronate powder show only four neighboring atoms (Table 3). From the EXAFS results it is clear that sample preparation has a major impact on the local geometry around the cobalt(II) ion.[29]

The conformation of hyaluronate main chain has a major impact on the distances between the ligands and

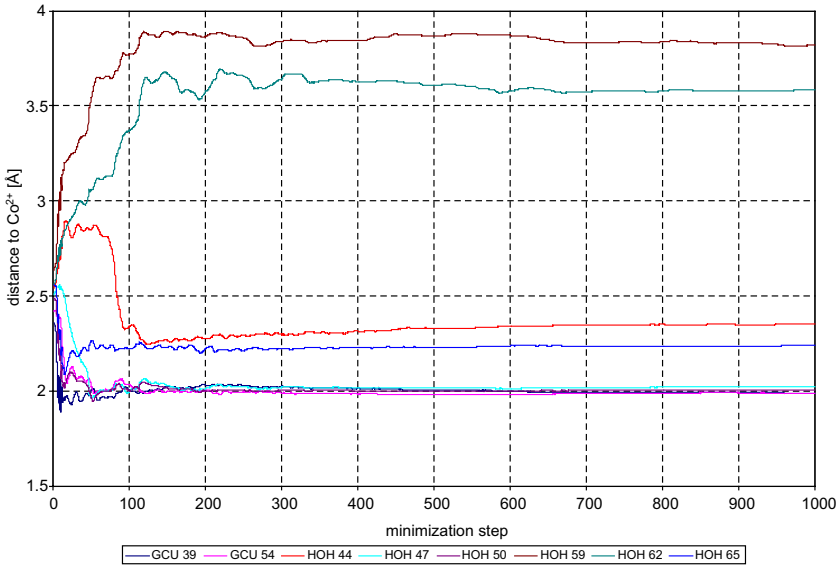

Figure 3. Distances in the 1000-step minimization process for the first coordination shell of cobalt(II). Note: GlcA = GCU in figure.

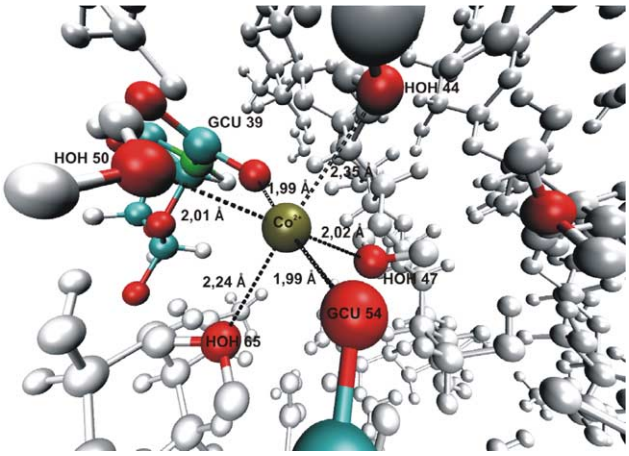

Figure 4. The first coordination sphere around cobalt(II) ion (viewed as CPK). Cobalt(II) ion is displayed as an orange sphere. QM atoms are displayed in colors, the rest are grayed. Note: GlcA = GCU in figure.

the central atom as evidenced by the smaller distances (see Table 2) obtained by computing the QM system alone as opposed to the QM/MM system.

The results of the hydrogen-bond analysis are summed up in Table 4. Many hydrogen bonds are formed because of the polyanionic nature of the main hyaluronate chain.

In summary, the aim of the work was to compare two different methods for the determination of the type, number, and positions of the atoms in the first coordination shell in cobalt(II) hyaluronate. Experimental data were gathered with EXAFS analysis, and the distances obtained were compared with the calculated ones using combined QM/MM modeling. The results of these two methods were found to be in very good agreement, thus opening the possibility for the evaluation of other metal(II) hyaluronates in the future. Our findings confirm the assumptions made by Sheehan et al. in which they assumed that the conformation of the hyaluronate chain is ionic species independent.[20–22]

Table 3. Parameters of the nearest coordination shell of oxygen atoms around cobalt(II) ion in cobalt(II) hyaluronate obtained from EXAFS analysis

N	*R* (Å)	Σ^2 (Å^2)
CoHA		
Film		
4.2(7)	2.07(1)	0.007(1)
2.0(7)	2.39(1)	0.033(1)
Powder		
4.0(1)	2.058(4)	0.00075(4)

N = average number; *R* = metal–oxygen distance, σ^2 = Debye–Waller factor; with uncertainty of the last digit is given in parentheses.

Table 4. Hydrogen bonds around the first coordination sphere of cobalt(II) ion[a]

Donor		Acceptor		Length (Å)	Angle (°)
Residue	Atom	Residue	Atom		
GCU 39	O6B	HOH 50	O	2.594	150.2
HOH 44	O	HOH 62	O	2.761	154.4
HOH 47	O	GCU 78	O3	2.705	155.2
HOH 50	O	GCU 39	O6B	2.594	150.2
HOH 50	O	NAG 55	O4	2.663	173.0
GCU 54	O6B	HOH 65	O	2.763	158.0
HOH 59	O	NAG 19	O7	2.732	175.6
HOH 62	O	GCU 18	O3	2.666	175.2
HOH 65	O	NAG 40	O4	2.786	160.9
HOH 65	O	GCU 54	O6B	2.763	158.0
GCU 39	O6B	HOH 50	O	2.594	150.2
HOH 44	O	HOH 62	O	2.761	154.4
HOH 47	O	GCU 78	O3	2.705	155.2
HOH 50	O	GCU 39	O6B	2.594	150.2
HOH 50	O	NAG 55	O4	2.663	173.0
GCU 54	O6B	HOH 65	O	2.763	158.0
HOH 59	O	NAG 19	O7	2.732	175.6

[a] GCU refers to D-glucuronic acid (GlcA), NAG refers to 2-acetamido-2-deoxy-D-glucose (GlcNAc, *N*-acetylglucosamine), and HOH refers to water.

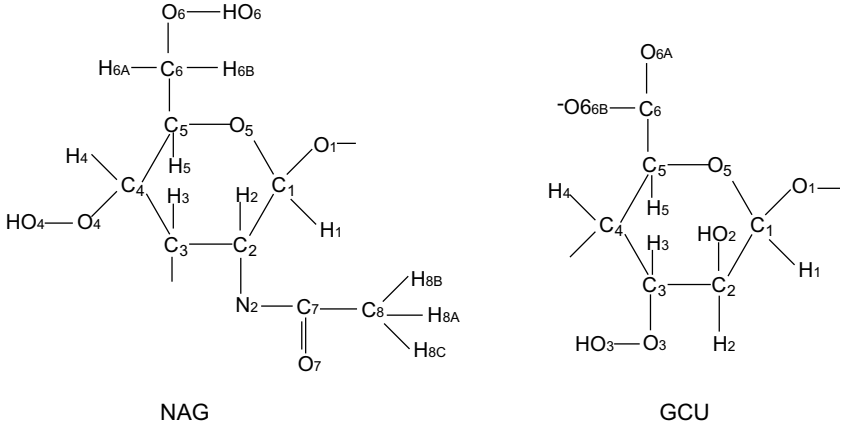

Figure 5. Atom numbering and labeling in GlcNAc (NAG) and GlcA (GCU).

1. Experimental

1.1. Computational methods

The only structure of a metal(II) hyaluronate was solved by Winter and Arnott for calcium hyaluronate films at high relative humidity. They reported a 3-fold helical form. Six chains are packed in a trigonal unit cell with symmetry $P3_212$. The polyanion conformation is stabilized by hydrogen bonds across the (1→4) and (1→3) glycosidic linkages, respectively. Adjacent antiparallel chains are held together through –$COO^-\cdots Ca^{2+}\cdots{}^-OOC$– bridges and three pairs of water molecules, extensively hydrogen bonded to the polyanion as well. It has been suggested that the secondary structure of the polymer will be similar to calcium hyaluronate also in cases of other divalent cations.[20,21]

First a suitable model was built using coordinates for one disaccharide repeating unit of HA from the Protein Data Bank (entry: 4HYA). Using the available crystallographic data and taking the symmetry of the system into account, a model system containing 938 atoms was built at a temperature of 298 K in vacuum.[20–22] Existing CHARMM empirical parameters for sugars were modified to include GlcNAc and GlcA residues. A short (100 ps) molecular dynamics (MD) calculation was run to assess whether the model built on the chosen parameters is correct. The three-dimensional structure calculated this way was again checked toward available experimental data for calcium(II) hyaluronate and found to be in good agreement.[20,21]

Sheehan et al. and Winter et al. presumed the 3-fold conformation of the hyaluronate chains to be insensitive to changes of relative humidity or ionic species. According to this, and taking our work on calcium hyaluronate (unpublished) into account, we replaced calcium ions with cobalt(II) ions.[19] For the computation of the structure of cobalt(II) hyaluronate a method combining both the classical and quantum mechanical approaches was used. The central atom and the positions of the potential ligands are computed quantum mechanically, and the rest of the system is computed using molecular mechanics.

The software that allows for this kind of computation is CHARMM (version 28a1). The expression for the classical potential field used in CHARMM is based on experimental data for proteins, nucleic acids, and some monosaccharides. Parameters for monosaccharides were taken from elsewhere and modified to support GlcNAc and GlcA.[30,31]

The QM part of the system was modeled using the program GAMESS, which solves the Schrödinger equation according to Hartree–Fock's approximation, with the basis function set 6-31G*.

The region computed quantum mechanically included the following: one cobalt(II) ion (label CO 95), four water molecules (labeled HOH 44, HOH 47, HOH 50, HOH 59, HOH 62, and HOH 65, respectively), and two carboxylate groups (labeled GCU 39 and GCU 54, respectively); for a total of 27 atoms. The atom numbers and labels for GlcNAc and GlcA (labeled NAG and GCU, respectively, in the figure) are shown in Figure 5. To separate the two regions, we added two linker atoms after each carboxylate group. QM/MM structural minimizations were run for 1000 steps. After minimization the hydrogen bonds were analyzed. For a valid hydrogen bond we assumed a distance of 2.2–3.3 Å and an angle of 150–180°. For comparison the MM part of the system was omitted, and the energy minimization was run again. The central atom–ligand distances were compared to available experimental data gathered with EXAFS.[29]

1.2. Computer equipment

Computing was performed using the GNU/Linux-based cluster of personal computers VRANA-5 at the National Institute of Chemistry in Ljubljana. Figures were made using VMD.[32]

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