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How strong are Ca^{2+}–heparin and Zn^{2+}–heparin interactions?

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ABSTRACT

The formation of the calcium and zinc salts from $CaCl_2$, $ZnCl_2$ and six monomeric structural units of heparin (1-OMe ΔUA-2S, 1-OMe GlcN-S6S, 1,4-DiOMe GlcA, 1,4-DiOMe GlcN-S3S6S, 1,4-DiOMe IdoA-2S, and 1,4-DiOMe GlcN-S6S) have been studied in gas phase and aqueous solution as model reactions for formation of heparin–Ca^{2+} and heparin–Zn^{2+} complexes. Gibbs reaction energies computed at the B3LYP/6-311++G(d,p) level of theory for the reactions studied in aqueous solution are positive and range from 20 to 250 kJ/mol.

1. Introduction

Heparin is a linear polysaccharide macromolecule polyanion present in several mammalian and other vertebrates tissues [1,2]. It is the member of a large group of polysaccharides named glycosaminoglycans, a major class of extracellular complex macromolecules [2,3]. Heparin is found only in mast cells, where it has a function in storage of granular components such as histamine and mast cell specific proteases. Heparin has been widely used in medicine for more than 75 years because of its anticoagulant, antithrombotic and antilipaemic activites [4–6]. Because of its highly acidic sulfate and carboxylate groups [7–9], heparin exists as an anion at physiologic pH values [7]. The activity of heparin can be modulated by metal cations such as Mg^{2+}, Ca^{2+}, Zn^{2+} and Cu^{2+} [8]. The heparin–metal cation interactions are a subject of several experimental investigations [8–16]. The interaction of sodium cations with monomeric and oligomeric units of heparin has been studied also theoretically using density functional theory (DFT) [17–21]. Hricovíni examined the effect of counterions Na^+ and Ca^{2+} and water on the conformational structure of heparin disaccharide containing iduronic acid residue using a hybrid quantum mechanical/molecular mechanical method [22]. More recently the interaction of Li^+, Na^+, K^+, Mg^{2+} and Ca^{2+} cations with dimeric and pentameric structural units of heparin was studied by using B3LYP hybrid DFT and it was shown that the ionic charge state, the number of metal ion adducts and the counterion radii are important factors that influence counterion-induced conformational changes in these structural units of heparin [23].

The present letter reports the results of a theoretical investigation of the elementary reactions of the Ca^{2+} and Zn^{2+}–heparin complexes formation in the gas phase and in water solution, studying the formation of the neutral complexes of these cations with six basic monomeric units of heparin (units A, D, E, F, G and H) and HCl from calcium chloride and zinc chloride and corresponding monomer units of heparin. Special attention was paid to geometries, reaction enthalpies, entropies and Gibbs energies and to how these quantities are changed by coordination effects induced by the Ca^{2+} and Zn^{2+} cations, and by water solvation.

2. Computational details

For modeling of the formation of calcium and zinc salts of heparin we selected six basic monomer structural units of heparin (1-OMe ΔUA-2S, 1-OMe GlcN-S6S, 1,4-DiOMe GlcA, 1,4-DiOMe GlcN-S3S6S, 1,4-DiOMe IdoA-2S, and 1,4-DiOMe GlcN-S6S), Figure 1. All studied compounds were geometrically optimized with the GAUSSIAN 09 program [24] employing DFT with the B3LYP hybrid functional [25–27] and the triple-ζ 6–311++G(d,p) basis set [28]. The relative B3LYP energies are in very good agreement with accurate ab initio results [29–31]. Enthalpies, entropies, and Gibbs energies were computed for all reactions studied (reactions 1–12).

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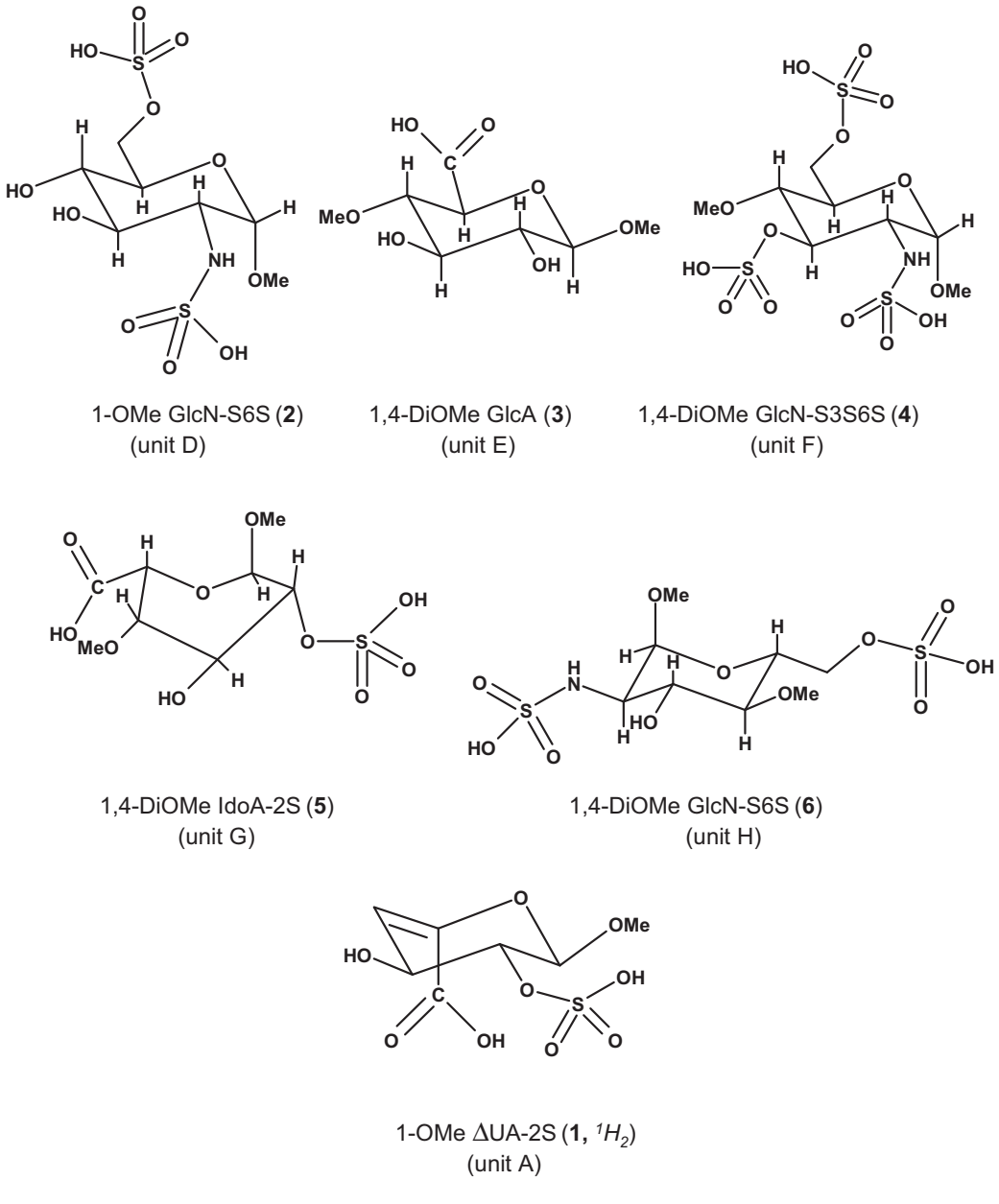

Figure 1. Structure of the heparin monomers studied.

$$\text{1-OMe}\Delta\text{UA-2S} + 2\text{CaCl}_2 \rightarrow \text{1-OMe}\Delta\text{UA-2SCa}_2\text{Cl}_2 + 2\text{HCl} \quad (1)$$

$$\text{1-OMe}\Delta\text{UA-2S} + 2\text{ZnCl}_2 \rightarrow \text{1-OMe}\Delta\text{UA-2SZn}_2\text{Cl}_2 + 2\text{HCl} \quad (2)$$

$$\text{1-OMeGlcN-S6S} + 2\text{CaCl}_2 \rightarrow \text{1-OMeGlcN-S6SCa}_2\text{Cl}_2 + 2\text{HCl} \quad (3)$$

$$\text{1-OMeGlcN-S6S} + 2\text{ZnCl}_2 \rightarrow \text{1-OMeGlcN-S6SZn}_2\text{Cl}_2 + 2\text{HCl} \quad (4)$$

$$\text{1, 4-DiOMeGlcA} + \text{CaCl}_2 \rightarrow \text{1, 4-DiOMeGlcACaCl} + \text{HCl} \quad (5)$$

$$\text{1, 4-DiOMeGlcA} + \text{ZnCl}_2 \rightarrow \text{1, 4-DiOMeGlcAZnCl} + \text{HCl} \quad (6)$$

$$\text{1, 4-DiOMeGlcN-S3S6S} + 3\text{CaCl}_2 \rightarrow \text{1, 4-DiOMeGlcN-S3S6SCa}_3\text{Cl}_3 + 3\text{HCl} \quad (7)$$

$$\text{1, 4-DiOMeGlcN-S3S6S} + 3\text{ZnCl}_2 \rightarrow \text{1, 4-DiOMeGlcN-S3S6SZn}_3\text{Cl}_3 + 3\text{HCl} \quad (8)$$

$$\text{1, 4-DiOMeIdoA-2S} + 2\text{CaCl}_2 \rightarrow \text{1, 4-DiOMeIdoA-2SCa}_2\text{Cl}_2 + 2\text{HCl} \quad (9)$$

$$\text{1, 4-DiOMeIdoA-2S} + 2\text{ZnCl}_2 \rightarrow \text{1, 4-DiOMeIdoA-2SZn}_2\text{Cl}_2 + 2\text{HCl} \quad (10)$$

$$\text{1, 4-DiOMeGlcN-S6S} + 2\text{CaCl}_2 \rightarrow \text{1, 4-DiOMeGlcN-S6SCa}_2\text{Cl}_2 + 2\text{HCl} \quad (11)$$

$$\text{1, 4-DiOMeGlcN-S6S} + 2\text{ZnCl}_2 \rightarrow \text{1, 4-DiOMeGlcN-S6SZn}_2\text{Cl}_2 + 2\text{HCl} \quad (12)$$

The optimized structures of monomeric structural units of heparin, $CaCl_2$, $ZnCl_2$, and their reaction products studied are shown in Figures A, B1–B6 and C1–C6 of the Supplementary material.

3. Results and discussion

3.1. General considerations

Initial conformations to use in theoretical calculations of the monomeric heparin species studied (heparin units 1-OMe ΔUA-2S (unit A), 1-OMe GlcN-S6S (unit D), 1,4-DiOMe GlcA (unit E), 1,4-DiOMe GlcN-S3S6S (unit F), 1,4-DiOMe IdoA-2S (unit G), and 1,4-DiOMe GlcN-S6S (unit H), respectively) were constructed using the Gauss View graphical interface of Gaussian and published molecular structures of sodium salts of basic monomeric building units of heparin [20]. The pyranose ring of the D-glucosamine in these monomers was considered in the more stable $^4C_1$ conformation. The starting conformation of the L-iduronic acid building unit G was set to the more stable skew-boat ^2S_o form [20,32]. Uronate residue A with an unsaturated 4,5 carbon bond according to the crystallographic data exists in two different forms $^2H_1$ and $^1H_2$ within the same unit cell [33]. The actual calculations of the thermodynamic parameters for reactions (1) and (2), respectively were carried out with the $^1H_2$ conformer of the 1-OMe ΔUA-2S preferably found in solution of heparin derived saccharide [34]. The geometry and energy of free acids was adapted from our recent publication [35]. The overall shape and the selected structural parameters of the fully optimized monomeric heparin species are given in the Supplementary material. Harmonic vibrational frequency calculations were conducted on all optimized geometries and their analysis revealed that all reactants and reaction products are minima (zero number of imaginary frequencies). As our previous investigations have shown, the polar OH, COOH, $NHSO_3H$, and OSO_3H groups in monomeric structural units of heparin of the most stable structures form, where possible, intramolecular hydrogen bonds [17,20,35].

The intramolecular $C(3)O-H\cdots OC(4)$ hydrogen bond with lengths of 2.1 – 2.3 Å stabilizes the optimized structure of the $^1H_2$ conformer in both Ca^{2+} and Zn^{2+} complexes of the 1-OMe ΔUA-2S monomer. Because of different stereochemistry for the $^2H_1$ conformation the existence of such hydrogen bond is impossible (Figures B1 and C1 of the Supplementary material). Initial conformations used in the DFT calculations of the metalated species investigated were based on the previous calculations of corresponding sodium salts [20]. Pairing the negatively charged carboxyl group with Ca^{2+} and Zn^{2+} cations results in bidentate bonding with an $O\cdots Ca$ separation range slightly longer (by about 0.1–0.2 Å) in comparison with the $O\cdots Zn$ ones, which correlates well with the general trends observed for other biologically important metal complexes [36]. The coordination of the anionic sulfate groups with bivalent calcium and zinc cations leads to structural rearrangement of the $NHSO_3^-$, and OSO_3^- moieties and to conformational stabilization of these moieties via bidendated and/or tridentaded coordination (Figures B1–B6 and C1–C6 of the Supplementary material). Besides the charged metallic complexes of 1-OMe ΔUA-2S, 1-OMe GlcN-S6S, 1,4-DiOMe GlcA, 1,4-DiOMe GlcN-S3S6S, 1,4-DiOMe IdoA-2S, and 1,4-DiOMe GlcN-S6S we also investigated neutral systems formed by adding corresponding chlorine anions in a stoichiometric ratio. The largest changes upon coordination of chlorine anions were observed for the $^2H_1$ conformer of the 1-OMe $\Delta UA\text{-}2SCa_2Cl_2$ and 1,4-DiOMe $GlcN\text{-}S3S6SCa_3Cl_3$ species (Figures B1 and B4 of the Supplementary material).

The effect of solvent (water) changes on the molecular structure of the species studied is treated with the CPCM salvation method [37–39]. It has been shown previously that the conductor-like polarizable continuum model CPCM is one of the most useful solvation methods [40], and reproduces hydration energies with accuracies in the order of a few kcal/mol but mostly (70% of the cases) even better than one kcal/mol [41]. For hydrated geometry optimizations, the solvation energy is computed as the difference between the energies of the optimized gas phase structure and the solvated structure that was optimized in aqueous solution (Table 1). Neutral monomers **1–7** are in aqueous solution stabilized at least about −45 kJ/mol. The charge complexes containing Ca^{2+} and Zn^{2+} cations (systems **8–19**) with a positive charge are most stable in water as expected because their dipole moments are significant. The computed solvation energies for neutral reaction products containing metal ions are substantially lower (systems **20–31**, Table 1). The solvation energy stabilization of the cations is approximately 400–1700 kJ/mol higher than the neutral complexes. This trend correlates well with the degree of ionization of the individual monomers and indicates considerable stabilization of the cationic species in aqueous solution (Table 1). The overall structural motifs of the in solution optimized complexes studied are also shown in the Supporting material (Figures B1–B6 and C1–C6, respectively). The larger structural changes upon hydration were observed especially at the sites of coordination of the metal cations. Hydration causes appreciable prolongation of the $O\cdots Ca$ and $Ca\cdots Cl$ bonds (by about 0.3 and 0.2 Å, respectively) compared to the bonds in isolated systems. A slightly shorter prolongation of about 0.1–0.2 Å is observed with stronger $O\cdots Zn$ and $Zn\cdots Cl$ bonds in hydrated zinc species.

3.2. Gas phase and solvated state interaction energies of the monomer$\cdots M^{n+}$ ($M^{n+} = Ca^{2+}$ and Zn^{2+}) complexes

Table 2 contains computed interaction enthalpies, entropies and Gibbs energies of the heparin monomer$\cdots M^{n+}$ ($M^{n+} = Ca^{2+}$ and Zn^{2+}) metallic complexes. Corrections for basis set superposition errors (BSSE) were not implemented in the results because the B3LYP/6–311++G(d,p) results for interaction energies of similar metal complexes of organic bases are close to the CBS-QB3 results with an average absolute deviation of approximately 20 kJ/mol [42]. Thus, the basis set used here is appropriate to reduce the basis set superposition error effects. The computed interaction energies represent values for dissociation energies of metal ions from different binding sites (carboxylate, N- and O-sulfate groups, respectively) in the monomers investigated. Because Ca^{2+} and Zn^{2+} ions and the anionic centers in these monomers are multiply coordinated, the interaction Gibbs energies are highly negative and span a broad energy interval from −1200 to −4700 kJ/mol. The Zn^{2+} complexes are always more stable than the Ca^{+2} complexes, which is consistent with their ionic sizes (Zn^{2+} (0.60 Å), Ca^{2+} (1.0 Å)) [43]. The gas-phase calcium and zinc affinities result from the dissociation of several metal cations from corresponding anionic sites of the monomers studied. Table 2 also contains corresponding values of Gibbs interaction energies calculated per cation. For both Ca^{2+} and Zn^{2+} ions the values of Gibbs interaction energies per cation differ from case to case and reflect the unique ability of various structural units of heparin to distinguish and discriminate individual metal cations. Metal cations with a formal charge of +2 (such as Mg^{2+}, Ca^{2+}, Zn^{2+}) in some cases participate in networks with the anionic residues of proteins and/or participate in territorial interactions [11]. Ca^{2+} cations moderate the anticoagulant activity of heparin [44] and potentiate the acceleration by heparin of thrombin inhibition by antithrombin III and the anti-Factor Xa activity of heparin [45]. The transition metal Zn^{2+} mediates high affinity binding of heparin with heparin cofactor II [12], fibrinogen [16] and ternary heparin–thrombin–fibrin complex [15]. Considering the same interaction sites for Ca^{2+} and Zn^{2+} ions our calculations show that the zinc cation is able to interfere with heparin and protein binding sites more effectively.

Table 1
Computed solvent stabilization energies ΔG^{CPCM} (kJ/mol) of the species studied in water solution (T = 298.15 K).

No.	System	ΔG^{CPCM}	Gas-phase dipole moment, Debye (D)
1	1-OMe ΔUA-2S, $^1H_2$ conformer	−59.7	3.38
2	1-OMe ΔUA-2S, $^2H_1$ conformer	−45.3	6.13
3	1-OMe GlcN-S6S	−75.1	6.10
4	1,4-DiOMe GlcA	−43.0	2.12
5	1,4-DiOMe GlcN-S3S6S	−86.1	4.72
6	1,4-DiOMe IdoA-2S	−64.6	2.97
7	1,4-DiOMe GlcN-S6S	−75.9	4.59
8	1-OMe ΔUA-2S$^{(2-)}\cdots$2Ca^{2+}, $^1H_2$ conformer	−1209.3	14.83
9	1-OMe ΔUA-2S$^{(2-)}\cdots$2Zn^{2+}, $^1H_2$ conformer	−1264.1	8.68
10	1-OMe GlcN-S6S$^{(2-)}\cdots$2Ca^{2+}	−1176.9	22.43
11	1-OMe GlcN-S6S$^{(2-)}\cdots$2Zn^{2+}	−1191.6	18.74
12	1,4-DiOMe GlcA$^{(-)}\cdots$Ca^{2+}	−529.5	19.24
13	1,4-DiOMe GlcA$^{(-)}\cdots$Zn^{2+}	−520.9	9.59
14	1,4-DiOMe GlcN-S3S6S$^{(3-)}\cdots$3Ca^{2+}	−2025.5	3.42
15	1,4-DiOMe GlcN-S3S6S$^{(3-)}\cdots$3Zn^{2+}	−2136.2	2.01
16	1,4-DiOMe IdoA-2S$^{(2-)}\cdots$2Ca^{2+}	−1145.5	19.41
17	1,4-DiOMe IdoA-2S$^{(2-)}\cdots$2Zn^{2+}	−1168.6	14.56
18	1,4-DiOMe GlcN-S6S$^{(2-)}\cdots$2Ca^{2+}	−1168.3	22.01
19	1,4-DiOMe GlcN-S6S$^{(2-)}\cdots$2Zn^{2+}	−1178.3	18.25
20	1-OMe ΔUA-2SCa_2Cl_2, $^1H_2$ conformer	−373.4	3.14
21	1-OMe ΔUA-2SZn_2Cl_2, $^1H_2$ conformer	−244.5	3.49
22	1-OMe GlcN-S6SCa_2Cl_2	−412.1	8.95
23	1-OMe GlcN-S6SZn_2Cl_2	−243.0	9.08
24	1,4-DiOMe GlcACaCl	−216.3	4.33
25	1,4-DiOMe GlcAZnCl	−130.4	3.95
26	1,4-DiOMe GlcN-S3S6SCa_3Cl_3	−554.9	4.51
27	1,4-DiOMe GlcN-S3S6SZn_3Cl_3	−419.3	5.29
28	1,4-DiOMe IdoA-2SCa_2Cl_2	−369.5	9.04
29	1,4-DiOMe IdoA-2SZn_2Cl_2	−208.1	4.97
30	1,4-DiOMe GlcN-S6SCa_2Cl_2	−406.6	7.35
31	1,4-DiOMe GlcN-S6SZn_2Cl_2	−236.9	8.74
32	$CaCl_2$	−340.7	0
33	$ZnCl_2$	−177.6	0
34	Ca^{2+}	−1576.6 (−1592.4)[a]	
35	Zn^{2+}	−1961.4 (−2041.3)[a]	

[a] Experimental single-ion standard hydration enthalpy [46].

Table 2 also contains the interaction Gibbs energies, ΔG^{298} (CPCM), for the systems studied in aqueous solution. Solvation has a dramatic effect on these interactions. In the gas phase, the binding interactions occur with high and negative (i.e. stabilizing) Gibbs energies resulting from the strong attractive Coulombic interactions between the positively charged Ca^{2+} and Zn^{2+} cations and the corresponding anionic groups of monomers studied. The computed Gibbs binding energies for the $Ca^{2+}\cdots$monomer $Zn^{2+}\cdots$monomer complexes in aqueous solution are positive, meaning that the readiness of calcium and zinc cations to coordinate site-specifically with the anionic groups of heparin is very low. The heparin species, owing their high negative charge, behave as polyelectrolytes. In water solution these polyelectrolytes can bind counterions either territorially or site-specifically [11]. Our calculations indicate a different balance between the heparin monomers–Ca^{2+} and Zn^{2+} interactions with respect to the cation–anion and cation-water interactions. In isolated systems both Ca^{2+} and Zn^{2+} interact with anionic heparin sites site-specifically. Despite of the much stronger interaction of the $Zn^{2+}\cdots$monomer complexes in gas-phase in comparison with the $Ca^{2+}\cdots$monomer species in aqueous solution both cations are delocalized in a volume around the polyanion as is also manifested by the positive Gibbs energies of solvation (Table 2). The higher Gibbs energy of solvation for Zn^{2+} (−1961.4 kJ/mol) in comparison with the Ca^{2+} (−1576.6 kJ/mol) counterbalances much stronger interaction in the gas-phase $Zn^{2+}\cdots$monomer complexes and results in an almost the same weakening of the cation-anion attraction upon hydration. The computed metal binding properties of the monomer heparin$\cdots M^{2+}$ (M^{2+} = Ca, Zn) systems, in which metal ions are arranged to the negatively charged carboxyl and sulfate groups, correlate well with the binding properties of these cations in biological systems. According to Mattai and Kwak the binding constants for these two cations are

Table 2
Calculated enthalpies, entropies and Gibbs energies at T = 298.15 K of the heparin monomer$\cdots M^{n+}$ (M^{n+} = Ca^{2+} and Zn^{2+}) metallic complexes.

No.	System	ΔH (kJ/mol)	ΔS (J/K mol)	ΔG (kJ/mol)	ΔG, per M^{2+} (kJ/mol)	ΔG^{CPCM} (kJ/mol)	ΔG^{CPCM}, per M^{2+} (kJ/mol)
1A	1-OMe ΔUA-2S$^{(2-)}\cdots$2Ca^{2+}	−2556.0	−233.6	−2486.4	−1243.2	145.8	72.9
2A	1-OMe ΔUA-2S$^{(2-)}\cdots$2Zn^{2+}	−3301.8	−237.9	−3230.1	−1615.0	128.6	64.3
3D	1-OMe GlcN-S6S$^{(2-)}\cdots$2Ca^{2+}	−2511.3	−232.5	−2441.7	−1220.8	174.7	87.3
4D	1-OMe GlcN-S6S$^{(2-)}\cdots$2Zn^{2+}	−3273.2	−236.9	−3202.6	−1601.3	174.1	87.0
5E	1,4-DiOMe GlcA$^{(-)}\cdots$Ca^{2+}	−1301.2	−111.6	−1267.7	−1267.7	49.0	49.0
6E	1,4-DiOMe GlcA$^{(-)}\cdots$Zn^{2+}	−1730.9	−113.6	−1697.0	−1697.0	16.7	16.7
7F	1,4-DiOMe GlcN-S3S6S$^{(3-)}\cdots$3Ca^{2+}	−3798.0	−339.8	−3696.7	−1232.2	269.1	89.7
8F	1,4-DiOMe GlcN-S3S6S$^{(3-)}\cdots$3Zn^{2+}	−4844.3	−357.5	−4737.8	−1579.2	276.3	92.1
9G	1,4-DiOMe IdoA-2S$^{(2-)}\cdots$2Ca^{2+}	−2613.7	−250.5	−2538.3	−1269.1	138.2	69.1
10G	1,4-DiOMe IdoA-2S$^{(2-)}\cdots$2Zn^{2+}	−3400.3	−253.3	−3324.8	−1662.4	104.4	52.2
11H	1,4-DiOMe GlcN-S6S$^{(2-)}\cdots$2Ca^{2+}	−2519.9	−257.5	−2443.3	−1221.6	175.4	87.7
12H	1,4-DiOMe GlcN-S6S$^{(2-)}\cdots$2Zn^{2+}	−3285.5	−264.7	−3206.1	−1603.0	175.8	87.9

Table 3
Computed reaction energies (kJ/mol), enthalpies (kJ/mol), entropies (J/K mol), Gibbs energies (kJ/mol) and solvent stabilization reaction energies ΔG^{CPCM} (kJ/mol) of the reactions studied (T = 298.15 K).

No.	Reaction	ΔH	ΔS	ΔG	ΔG^{CPCM}
1A	1-OMe ΔUA-2S + $2CaCl_2$ → 1-OMe ΔUA-2SCa_2Cl_2 + 2HCl	−105.3	110.3	−137.9	171.2
2A	1-OMe ΔUA-2S + $2ZnCl_2$ → 1-OMe ΔUA-2SZn_2Cl_2 + 2HCl	58.3	74.5	36.1	197.1
3D	1-OMe GlcN-S6S + $2CaCl_2$ → 1-OMe GlcN-S6SCa_2Cl_2 + 2HCl	−187.8	68.2	−208.1	116.4
4D	1-OMe GlcN-S6S + $2ZnCl_2$ → 1-OMe GlcN-S6SZn_2Cl_2 + 2HCl	22.1	58.5	4.6	18.9
5E	1,4-DiOMe GlcA + $CaCl_2$ → 1,4-DiOMe GlcACaCl + HCl	−32.7	61.9	−51.2	117.2
6E	1,4-DiOMe GlcA + $ZnCl_2$ → 1,4-DiOMe GlcAZnCl + HCl	35.3	42.4	22.7	84.1
7F	1,4-DiOMe GlcN-S3S6S + $3CaCl_2$ → 1,4-DiOMe GlcN-S3S6SCa_3Cl_3 + 3HCl	−318.5	119.6	−354.2	178.1
8F	1,4-DiOMe GlcN-S3S6S + $3ZnCl_2$ → 1,4-DiOMe GlcN-S3S6SZn_3Cl_3 + 3HCl	98.2	135.1	57.9	249.1
9G	1,4-DiOMe IdoA-2S + $2CaCl_2$ → 1,4-DiOMe IdoA-2SCa_2Cl_2 + 2HCl	−171.9	81.7	−196.2	166.2
10G	1,4-DiOMe IdoA-2S + $2ZnCl_2$ → 1,4-DiOMe IdoA-2SZn_2Cl_2 + 2HCl	11.2	50.6	−3.8	190.1
11H	1,4-DiOMe GlcN-S6S + $2CaCl_2$ → 1,4-DiOMe GlcN-S6SCa_2Cl_2 + 2HCl	−181.4	65.3	−200.9	129.2
12H	1,4-DiOMe GlcN-S6S + $2ZnCl_2$ → 1,4-DiOMe GlcN-S6SZn_2Cl_2 + 2HCl	20.5	32.0	10.9	180.7

identical, suggesting similar mechanisms for Zn^{2+} and Ca^{2+} binding to heparin [9]. In hemostasis, Ca^{2+} ions are one of the cofactors, which are essential in regulating the intricate balance between procoagulant and anticoagulant factors [47]. Zinc plays a role in blood clotting, promoting the interaction between heparin and heparin cofactor II [12] which increases the affinity of heparin to fibrin [15] and to the αC domain of fibrinogen [16].

3.3. Gas-phase and solution state reactivity

Binding of metals to heparin may play important role in the modulation of heparin's anticoagulant activities [8–16]. In binding experiments with metal salts of heparin species the metal compounds in the form of chlorides are usually used [8–16,48]. Reactions **1**–**12** model such interactions using basic structural units of heparin responsible for its anticoagulant activity. The calculated reaction enthalpies, entropies, and Gibbs energies at room temperature for the reactions **1**–**12** are reported in Table 3. The reaction energies were computed as the differences between the total energies of reaction products and reactants. In these energy calculations the fully-optimized geometry of the species was considered. These reactions represent reactions of heparin monomers with calcium chloride and zinc chloride in which an equimolar amount of corresponding monomer heparin salt and HCl is formed. The ΔS term is positive and therefore favorable. Gibbs energies for the gas-phase reaction of $CaCl_2$ with heparin units are large and negative, i.e. stabilizing. The highest reaction Gibbs energy (−354 kJ/mol) is found for the salt 1,4-DiOMe GlcN-S3S6SCa_3Cl_3 coordinating unit F of heparin. The structural unit F bearing one carboxyl group is the weakest center of salt formation with the reaction Gibbs energy of about −50 kJ/mol. The transition metal cation Zn^{2+} behaves differently. With regards to Zn^{2+} salts, the gas-phase enthalpies for the reactions of six monomers with the $ZnCl_2$ are positive. Positive changes of entropy are not sufficient to reverse the thermal effect for all but one reaction in gas-phase. The gas-phase Gibbs reaction energy of 1,4-DiOMe IdoA-2SZn_2Cl_2 is slightly negative (by about −4 kJ/mol), Table 3. Despite the greater metal affinity of Zn^{2+} ions compared to the Ca^{2+} ones (Table 2) the readiness of $ZnCl_2$ for gas-phase reactions with neutral acids of heparin monomer units is in comparison with the $CaCl_2$ much lower. In real experimental conditions reactions such as those studied by us involve charged species. Ionic reactions normally take place in liquid solutions, where solvent molecules assist the formation of charged intermediates. In polar solvents like water, the reactions **1**–**12** are completely endothermic. Thus the existence of neutral Ca^{2+} and Zn^{2+} complexes of heparin monomers is highly improbable. It is well known that the adhesion of metal cations to anionic centers of heparin results in the formation of essentially electrostatic bonds [3,10,11]. In addition to electrostatic interactions the dielectric properties of the environment play an important role in the process of the metal cation binding.

In the gas phase, the binding reactions of charged complexes could happen with high Gibbs binding energies (Table 2). This is due to the strong attractive Coulombic interactions between the oppositely charged Ca^{2+} and Zn^{2+} cations and the carboxylate, sulfate groups, respectively. The binding enthalpy in the gas phase is substantially larger in magnitude than in a polar solvent (water). The neutralization of a positive charge in those complexes by coordination of corresponding chlorine anions results in a considerable weakening of O···M^{2+} (M^{2+} = Ca, Zn) bonds and therewith in overall destabilization of neutral complexes. The computed Gibbs reaction energies for the reactions **1–12** in aqueous solution range from 20 to 200 kJ/mol. Thus there is little tendency for the calcium and zinc chloride to form the corresponding neutral salts with monomeric structural units of heparin. In a water-exposed environment the high hydration effect between Ca^{2+} and Zn^{2+} cations and anions results in a diminution of the direct electrostatic and ion-dipole interactions. It is probable that the addition of the Ca^{2+} and Zn^{2+} cations (in the form of their chlorides) to heparin in solution is accompanied by the intermediate dissolution of the putative complex and the Ca^{2+} and Zn^{2+} cations in these complexes appear to be territorially bound.

4. Summary and conclusions

The thermodynamics of 12 reactions modeling the reaction of heparin monomers (1-OMe ΔUA-2S, 1-OMe GlcN-S6S, 1,4-DiOMe GlcA, 1,4-DiOMe GlcN-S3S6S, 1,4-DiOMe IdoA-2S, and 1,4-DiOMe GlcN-S6S) with $CaCl_2$ and $ZnCl_2$ was studied with DFT using the B3LYP functional. Gibbs energies for the gas-phase reaction of $CaCl_2$ with heparin units are large and negative, i.e. stabilizing. The transition metal cation Zn^{2+} behaves differently. With regards to Zn^{2+} salts, the gas-phase enthalpies for the reactions of six monomers with the $ZnCl_2$ are positive. Despite of greater metal affinity of Zn^{2+} ions compared to the Ca^{2+} ones the readiness of $ZnCl_2$ for gas-phase reactions with neutral acids of heparin monomer units is, in comparison with the $CaCl_2$, much lower. In polar solvents like water, these reactions are endothermic. Thus there is little tendency for the calcium and zinc chloride to form the corresponding neutral salts with monomeric structural units of heparin. In a water-exposed environment the high hydration effect between Ca^{2+} and Zn^{2+} cations and anions results in a diminution of the direct electrostatic and ion-dipole interactions. It is probable that the addition of the Ca^{2+} and Zn^{2+} cations (in the form of their chlorides) to heparin in solution is accompanied by the intermediate dissolution of the putative complex and the Ca^{2+} and Zn^{2+} cations in these systems appear to be territorially bound.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cplett.2014.12.018.

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