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How acidic are monomeric structural units of heparin?

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ABSTRACT

Density functional theory methods with the B3LYP functional have been used to letter the acidity of carboxyl, *O*-sulfo and *N*-sulfo groups in six basic 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). The predicted gas-phase acidity of the acidic functional groups in the monomeric structural units of heparin is: *O*-sulfo > *N*-sulfo > carboxyl. The computed pK_a values provide the same order of acidity as was observed in water solution. This implies that hydration does not change ordering of acidity of major acidic groups of monomeric structural units of heparin.

1. Introduction

Heparin is a linear polysaccharide macromolecule containing of variable proportion of disaccharide sequences consisting of either uronic acid or glucuronic acid and glucosamine residues. Few hydroxyl groups on each of these monosaccharide residues may be sulfated giving rise to a polymer that is highly negatively charged. The average negative charge of individual saccharide residues is about 2.3 [1,2]. This complex organic acid, that is found especially in lung and intestinal tissue, has a mucopolysaccharide as its active constituent, prevents blood clotting, and is used in the form of its sodium salt in the treatment of thrombosis and in heart surgery [3,4]. The heparin polysaccharide chain is not orally bioavailable and must therefore be administered intravenously or subcutaneously (it is not degraded in stomach acid). Its antithrombotic activity is explained by its ability to potentiate the activity of the serine protease inhibitor antithrombin (AT), which inactivates a number of serine proteases (such as thrombin and factor Xa) in the coagulation cascade [1,5]. It was found that a unique pentasaccharide fragment constitutes the minimal binding domain for AT [6,7]. It contains *O*-sulfo, *N*-sulfo groups and carboxyl moieties, which are in the antithrombin–pentasaccharide complex completely ionized and interact with the complementary (Arg, Lys, Glu and Asn) side chains on the protein [7–12]. It was shown experimentally, that Arg and Lys residues of proteins are clearly in position to have hydrogen bond interaction with the negatively charged sulfo and carboxyl groups of heparin pentasaccharide [8,13–17]. The bulk of these hydrogen bonds are sulfo group-mediated interactions. Therefore, determination of important physicochemical properties such as acidity of sulfo and carboxyl groups of heparin can provide more insights into the nature of the protein–heparin interactions. With regards to experimental studies, the pK_a values of carboxyl moieties of heparin-derived glycosaminoglycans have been determined using nuclear magnetic resonance spectroscopy [18–20]. Experimental gas-phase acidities have not been reported so far for basic monomeric structural units of heparin.

In this Letter we have used large-scale theoretical calculations for the determination of the gas-phase acidities and pK_a values for *O*-sulfo, *N*-sulfo and carboxyl functional groups of six monomeric structural units of heparin: 1-OMe ΔUA-2S (**1**), 1-OMe GlcN-S6S (**2**), 1,4-DiOMe GlcA (**3**), 1,4-DiOMe GlcN-S3S6S (**4**), 1,4-DiOMe IdoA-2S (**5**), and 1,4-DiOMe GlcN-S6S (**6**) (Figure 1: GlcN is glucosamine, IdoA is iduronic acid, GlcA is glucuronic acid, S is sulfo, and Me is methyl). The results of this letter are analyzed and compared with the available experimental data for structurally related systems. They are also discussed in the context of the present theories of action of these glycosaminoglycans.

2. Computational details

The geometry of the six monomeric structural units of heparin (1-OMe ΔUA-2S (**1**), 1-OMe GlcN-S6S (**2**), 1,4-DiOMe GlcA (**3**), 1,4-DiOMe GlcN-S3S6S (**4**), 1,4-DiOMe IdoA-2S (**5**), and 1,4-DiOMe GlcN-S6S (**6**)), respectively and their anionic forms (Figure 1) has been completely optimized with the GAUSSIAN 09 program system [21], using density functional theory [22–24], employing the B3LYP/6-311++G(d,p) method [25–27]. Calculations of gas-phase acidities of model acids CH_3–NH–SO_3H and CH_3–O–SO_3H were also carried out by means of the more accurate CBS-QB3 approach [28]. The CBS-QB3 method uses B3LYP coupled to the CBSB7 defined basis set for all geometry optimizations and frequency calculations.

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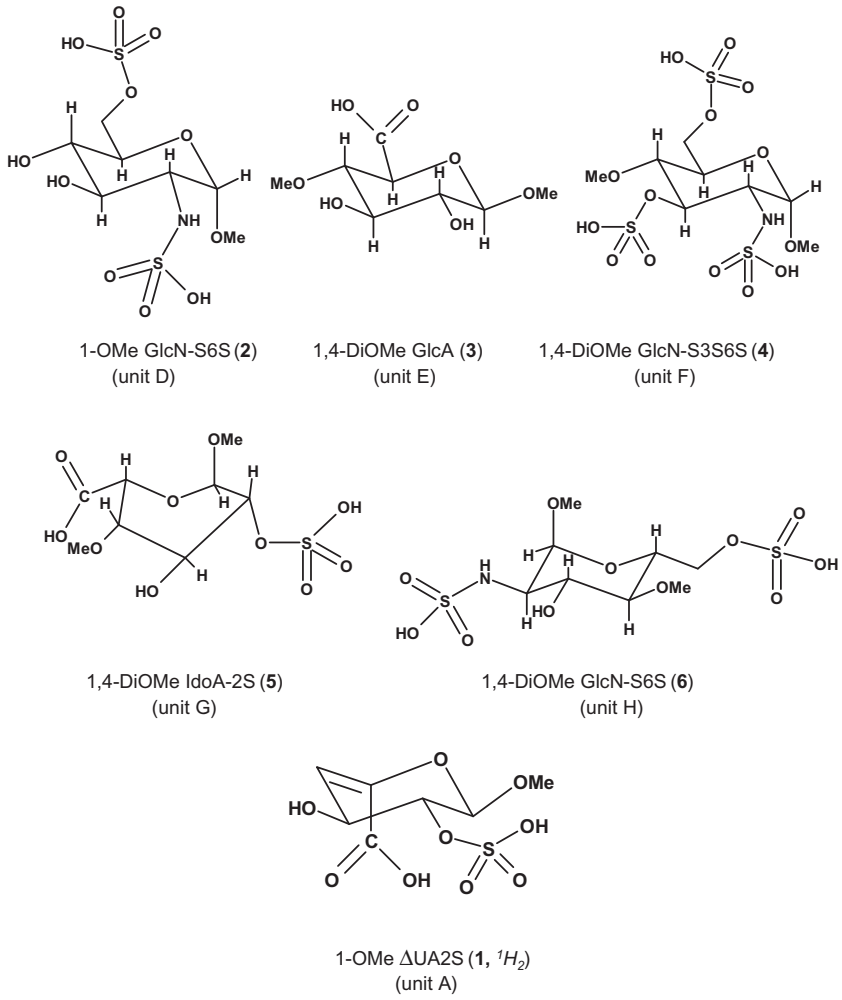

Figure 1. Structure of the acids studied.

The gas-phase acidity $\Delta E(\mathrm{A})$ was defined as the energy of deprotonation ΔE for reaction (A):

$$\mathrm{AH(g)} \rightarrow \mathrm{A^-(g)} + \mathrm{H^+(g)} \quad \text{(A)}$$

The enthalpy of deprotonation, ΔH^{298}, was computed using Eqs. (1) and (2):

$$\Delta H^{298}(\mathrm{A}) = \Delta E^{298}(\mathrm{A}) + \Delta(pV) \quad (1)$$

$$\Delta E^{298} = [E^{298}(\mathrm{A^-}) + 3/2\,RT] - E^{298}(\mathrm{AH}) \quad (2)$$

where E^{298} stands for the total energies of the stable conformations of the acids and their anions (including the thermal energy correction at $T = 298.15$ K). In Eq. (1) we substituted $\Delta(pV) = RT$ (1 mol of gas is obtained in the reaction (A)). The gas-phase Gibbs energy, ΔG^{298}, of the proton abstraction reaction may be calculated from:

$$\Delta G^{298} = \Delta H^{298} - T\Delta S^{298} \quad (3)$$

The enthalpy of deprotonation was calculated using expression (1). The entropy contribution is given by:

$$-T\Delta S^{298} = -T[S(\mathrm{A^-}) + S(\mathrm{H^+}) - S(\mathrm{AH})] \quad (4)$$

For $T = 298$ K at the standard pressure, the second term $TS(\mathrm{H^+}) = 32.5$ kJ mol^{-1} [29]. Thus:

$$\Delta G^{298} = \Delta H^{298} - T[S(\mathrm{A^-}) - S(\mathrm{AH})] - 32.5 \quad (5)$$

Notice that there is an inverse relationship between the magnitude of ΔG and the strength of the acid. The more positive the value of the ΔG, the weaker the acid. The calculations of the macroscopic pK_a of the studied species were performed using the program SPARC developed by Carreira et al. [30–32]. The computer program SPARC uses computational algorithms based on fundamental chemical structure theory to estimate a variety of chemical reactivity parameters (such as ionization pK_a, kinetics, heat of vaporization, boiling point, diffusion coefficient, etc.). SPARC costs the user only a few minutes of computer time and provides greater accuracy than is possible with other conventional methods [33].

3. Results and discussion

3.1. Geometry

Although heparin is structurally often described in terms of its disaccharide structural units [1,2], the main monosaccharide 'building blocks' of this polymer were identified using current experimental methods [1,34]. It was shown [34,35] that degradation of heparin by heparinase produces at the non-reducing end a terminal uronate with an unsaturated 4,5 carbon bond (structure 1-OMe ΔUA-2S (**1**), Figure 1), which may exist in two different forms $^2H_1$ and $^1H_2$, respectively. According to the NMR solution conformation investigations the uronate (A) residue is

Table 1
Gas-phase acidities (enthalpies ΔH, entropies ΔS and Gibbs energies ΔG) of the monomeric structural units of heparin (at 298.15 K).

No.	Reaction	ΔH kJ mol^{-1}	ΔS J K^{-1} mol^{-1}	ΔG kJ mol^{-1}
1 ($^1H_2$)	1-OMe ΔUA-2S			
	5-COOH–H$^+$ → COO$^{(-)}$	1376.7	125.0	1339.4
	2-OSO$_3$H–H$^+$ → OSO$_3^{(-)}$	1257.1	115.4	1222.7
	COOHSO$_3$H–2H$^+$ → COOSO$_3^{(2-)}$	2869.9	241.9	2797.8
2 ($^4C_1$)	1-OMe GlcN-S6S			
	5-OSO$_3$H–H$^+$ → OSO$_3^{(-)}$	1225.2	108.7	1192.8
	2-NHSO$_3$H–H$^+$ → NHSO$_3^{(-)}$	1310.9	113.3	1277.1
	O(NH)SO$_3$H–2H$^+$ → O(NH)SO$_3^{(2-)}$	2729.4	224.6	2662.5
3 ($^4C_1$)	1,4-DiOMe GlcA			
	5-COOH–H$^+$ → COO$^{(-)}$	1395.2	124.4	1358.1
4 ($^4C_1$)	1,4-DiOMe GlcN-S3S6S			
	3-OSO$_3$H–H$^+$ → OSO$_3^{(-)}$	1177.5	79.2	1153.9
	5-OSO$_3$H–H$^+$ → OSO$_3^-$	1259.1	112.4	1225.6
	2-NHSO$_3$H–H$^+$ → NHSO$_3^{(-)}$	1306.0	124.7	1268.8
	O(NH)SO$_3$H–3H$^+$ → O(NH)SO$_3^{(3-)}$	4442.7	380.3	4329.4
5 ($^2S_0$)	1,4-DiOMe IdoA-2S			
	5-COOH–H$^+$ → COO$^{(-)}$	1380.6	120.6	1344.7
	2-OSO$_3$H–H$^+$ → OSO$_3^{(-)}$	1240.7	111.3	1207.5
	COOHSO$_3$H–2H$^+$ → COOSO$_3^{(2-)}$	2849.4	237.3	2778.7
6 ($^4C_1$)	1,4-DiOMe GlcN-S6S			
	5-OSO$_3$H–H$^+$ → OSO$_3^{(-)}$	1229.1	77.0	1206.1
	2-NHSO$_3$H–H$^+$ → NHSO$_3^{(-)}$	1274.0	111.2	1240.8
	O(NH)SO$_3$H–2H$^+$ → O(NH)SO$_3^{(2-)}$	2734.2	223.8	2667.5
7	CH$_3$OSO$_3$H–H$^+$ → CH$_3$OSO$_3^{(-)}$	1296.6	118.2	1261.4
		1302.2[a]	118.7[a]	1266.8[a]
8	CH$_3$NHSO$_3$H–H$^+$ → CH$_3$NHSO$_3^{(-)}$	1330.7	115.4	1296.3
		1327.2[a]	115.0[a]	1292.9[a]
9	CH$_3$CO$_2$H–H$^+$ → CH$_3$CO$_2^{(-)}$	1446.1	134.8	1405.9
		1453.4[a]	130.7[a]	1414.4[a]
		1457 ± 6[b]	115 ± 9[b]	1422 ± 7[b]

[a] CBS-QB3 method.
[b] Gas-phase experimental value [41].

predominantly represented by $^1H_2$ conformation with minor contribution from the $^2H_1$ form [36,37]. The α-L-idopyranosyluronate structural unit of heparin (structure 1,4-DiOMe IdoA-2S (**5**), Figure 1) is likewise internally flexible, whereas glucuronic acid and glucosamine residues are conformationally rigid. The molecular structure of 1,4-DiOMe IdoA-2S species may oscillate among the three conformations ($^4C_1$, $^1C_4$ and $^2S_0$), and the $^2S_0$ conformation of L-iduronic acid is the preferred conformation in the biologically active pentasaccharides [7], as well as in simple monomeric species [38]. All glucosamine residues studied (1-OMe GlcN-S6S (**2**), 1,4-DiOMe GlcA (**3**), 1,4-DiOMe GlcN-S3S6S (**4**), and 1,4-DiOMe GlcN-S6S (**6**), respectively) are in the stable $^4C_1$ chair conformation [7,35–37]. These conformations were taken for the calculations as they are the prevalent forms in the species studied (Figure 1). An analysis of the harmonic vibrational frequencies at the B3LYP/6-311++G(d,p) level of theory of the optimized monomers and complexes revealed that these systems are minima (zero number of imaginary frequencies). The molecules studied model units A, D, E, F, G and H of typical fragment of heparin, in which two neighboring structural units of heparin bound by the (1–4) glycosidic bonds are substituted by the methyl groups (Figure 1). Examination of the space models of the optimized conformations of those compounds (Figure A of the Supplementary material) indicate that gas-phase conformers of these acids are stabilized via intramolecular hydrogen bonds O–H···O and O–H···N with bond lengths in the relatively short O···H and N···H separation range (1.7–2.5 Å), which is less than the sum of the van der Waals radii [39] (2.75 and 2.7 Å for H···N and H···O contacts, respectively). However, the acidic carboxyl and sulfo groups in monomeric units studied are free of such intramolecular contacts (Figure A of the Supplementary material).

Table 2
pK_a values of the monomeric structural units of heparin (at 298.15 K).

No.	Reaction	pK_a
1 ($^1H_2$)	1-OMe ΔUA-2SH$_2$	
	5-COOH–H$^+$ → COO$^{(-)}$	3.38
	2-OSO$_3$H–H$^+$ → OSO$_3^{(-)}$	0.51
2 ($^4C_1$)	1-OMe GlcN-S6SH$_2$	
	5-OSO$_3$H–H$^+$ → OSO$_3^{(-)}$	0.80
	2-NHSO$_3$H–H$^+$ → NHSO$_3^{(-)}$	1.41
3 ($^4C_1$)	1,4-DiOMe GlcAH	
	COOH–H$^+$ → COO$^{(-)}$	3.19
4 ($^4C_1$)	1,4-DiOMe GlcN-S3S6SH$_3$	
	3-OSO$_3$H–H$^+$ → OSO$_3^{(-)}$	0.70
	5-OSO$_3$H–H$^+$ → OSO$_3^{(-)}$	1.23
	2-NHSO$_3$H–H$^+$ → NHSO$_3^{(-)}$	1.85
5 ($^2S_0$)	1,4-DiOMe IdoA-2SH$_2$	
	5-COOH–H$^+$ → COO$^{(-)}$	3.09
	2-OSO$_3$H–H$^+$ → OSO$_3^{(-)}$	0.51
6 ($^4C_1$)	1,4-DiOMe GlcN-S6SH$_2$	
	5-OSO$_3$H–H$^+$ → OSO$_3^{(-)}$	0.80
	2-NHSO$_3$H–H$^+$ → NHSO$_3^{(-)}$	1.41

3.2. Gas-phase acidities

The glycosaminoglycans studied contain an acidic CO_2H, OSO_3H and $NHSO_3H$ groups and thus they may undergo deprotonation reactions. It is well known [7–12] that the polyanion of heparin is bound to the protein active site and therefore represents the active species. However, the deprotonation reactions of glycosaminoglycans in condensed phase have not been intensively investigated experimentally [18–20]. The gas phase proton affinities of simple carboxylic acids have been experimentally determined [40,41]. Table 1 contains gas-phase acidities of glycosaminoglycans studied. The density functional Becke3LYP enthalpies and free energies are close to the CBS-QB3 results of simpler model acids studied. The CBS-QB3 method approximates a high-level calculation with a very large basis set. The comparison of the B3LYP results with this very accurate method shows that density functional theory performs quite well and can thus be used as relatively inexpensive alternative for the investigation of acidity of larger systems. The enthalpy (proton affinity) and Gibbs energy (acidity) of deprotonation was computed as the difference between the fully optimized neutral acid and respective monoanions and polyanions. Thus the acidities shown in Table 1 represent acidity of respective functional groups and total acidities of the glycosaminoglycans by dissociation of all acidic (CO_2H, $NHSO_3H$ and OSO_3H) groups. Different acidity was found for individual monomeric structural units (Table 1). As regards of model acids **7**, **8** and **9**, Table 1 the gas-phase acidity increase in the order: acetic acid < methylsulfamic acid < methyl bisulfate and correlates well with the pK_a values of these acids [12]. When considering monoionization only, substitution of methyl groups by more complex glucosamine, iduronic acid and glucuronic acid substituents in the monomeric structural units of heparin results in an increase of the acidity of these groups (Table 1). The greater acidity of CO_2H, $NHSO_3H$ and OSO_3H groups, respectively in heparin structural units can be attributed, in part, to the extra electron-attracting effect of the pyranose ring. Table 1 also shows total acidities of monomeric structural units of heparin calculated as the difference between the fully optimized neutral acid and respective polyanion. The ionization of the second acidic group in 1-OMe ΔUA-2S (**1**), 1-OMe GlcN-S6S (**2**), 1,4-DiOMe IdoA-2S (**5**), and 1,4-DiOMe GlcN-S6S (**6**) occurs less readily than the first. This effect arises because more energy is required to deprotonate anions than neutral molecules. Thus the Gibbs energy of deprotonation of two acidic groups in those monomers is by about 190–235 kJ/mol greater than the sum of corresponding Gibbs energies resulting from deprotonation of individual acidic groups (Table 1). The destabilization effect of the trianion of monomer 1,4-DiOMe GlcN-S3S6S (**4**) containing three ionizable groups is even more noticeable. Deprotonation of three acidic groups results in considerable lowering of acidity by about 681 kJ/mol (Table 1).

3.3. Dissociation constants

The gas-phase acidities (Table 1) allow exploration of the reactivity of heparin structural units without the effect of solvent. However, in solution the dissociation constant or the pK_a is a measure of the strength of an acid or a base. Therefore this parameter is very useful in understanding the behavior of heparin at the site of action. We used the SPARC program [30] developed by Carreira et al. [31,32] to compute dissociation constants of monomeric structural units of heparin. This program computes pK_a values strictly from their molecular structure [31]. Of the two different sulfo groups present the more acidic is always *O*-sulfo group with calculated pK_a values 0.51–1.23 (Table 2). The pK_a values of the *N*-sulfo moieties present in 1-OMe GlcN-S6S (**2**), 1,4-DiOMe GlcN-S3S6S (**4**), and 1,4-DiOMe GlcN-S6S (**6**) are slightly greater (1.4–1.85) indicating that this group is less acidic. A carboxyl group is present in 1-OMe ΔUA-2S (**1**), 1,4-DiOMe GlcA (**3**), and 1,4-DiOMe IdoA-2S monomers, respectively. The pK_a values of this group in these monomers are close to each other (3.1–3.4) and much lower than the pK_a of model acetic acid (4.76) [12]. Thus the carboxyl moiety is the least acidic functional group of heparin structural units. However, at the physiological pH = 7.4 all acidic (CO_2H, $NHSO_3H$ and OSO_3H) moieties are completely ionized. The computed trends of aqueous ionization of individual functional groups of monomeric structural units of heparin correlate well with the gas-phase acidities of these species (Tables 1 and 2). The effect of water solute does not play any important part in the acidity ordering of CO_2H, $NHSO_3H$ and OSO_3H groups, respectively.

As regards of experimental data, the pK_a values for carboxy groups in several oligomeric structures of heparin have been studied [18–20]. The experimental pK_a values of their carboxyl groups were in the interval 3.13–2.79 [18], which is close to our calculated data (Table 2). However, exact comparison of pK_a values computed for monomeric structural units by us and available experimental results is not possible because the most important factor in determining the relative acid strengths of various heparin compounds is the nature of ions formed. The nature of the anions varies markedly when considering monomers, dimmers, and or higher oligomers of heparin [42,43].

4. Conclusions

A theoretical letter was set out to determine stable conformations, gas-phase acidity and pK_a 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 monomeric structural units of heparin for which a very limited amount of experimental physicochemical data exist. Using the theoretical methods the following conclusions can be drawn.

The investigated glycosaminoglycans are in gas-phase weak organic acids with calculated acidity of individual acidic groups about 1150–1350 kJ/mol. The ionization of the second acidic group in 1-OMe ΔUA-2S (**1**), 1-OMe GlcN-S6S (**2**), 1,4-DiOMe IdoA-2S (**5**), and 1,4-DiOMe GlcN-S6S (**6**) occurs less readily than the first. Thus the Gibbs energy of deprotonation of two acidic groups in those monomers is by about 190–235 kJ/mol greater than the sum of corresponding Gibbs energies resulting from deprotonation of individual acidic groups. Deprotonation of three acidic groups in 1,4-DiOMe GlcN-S3S6S (**4**) results in considerable lowering of acidity by about 681 kJ/mol. The predicted relative acidity of the three major acidic functional groups in the monomeric structural units of heparin (*O*-sulfo, *N*-sulfo and carboxyl) is: *O*-sulfo > *N*-sulfo > carboxyl. The same order of acidity was observed also in water solution. This implies that solvent effect does not change ordering of acidity of major acidic groups of monomeric structural units of heparin.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cplett.2013.10.062.

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