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Solvent accessibility studies on glycosaminoglycans

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Abstract

Theoretical calculations on the solvent accessible surface area of the commonly occurring glycosaminoglycans (GAGs) have been carried out by employing the solvent accessibility technique of Lee and Richards. The results indicate that the average variation of solvent accessible surface area (ASA) for the different atoms is in the range of 2–28 Å^2. The average ASA for carbon (9.02 Å^2) and oxygen (19.3 Å^2) are relatively very high compared to that for nitrogen and sulfur. The total contribution of ASA by the buried atoms in all the molecules has been found to be small. Heparin complexed with the protein is less accessible to the solvent than the uncomplexed. The iduronic acid is also found to have a varied ASA due to its conformational flexibility. The sulfate groups in heparin have 50% of the total ASA of the molecule. Residue analysis indicates that in general D-configuration residues have higher ASA than L-configuration residues.

Keywords: Glycosaminoglycans; Solvent accessibility; Buriedness; Conformational change

1. Introduction

Carbohydrates are an important class of biopolymers which serve many biological functions. The last two decades have brought an enormous increase in our knowledge of their biological importance and their interactions with other macromolecules, such as proteins and nucleic acids. They occur as a component in proteins, in genetic materials, the RNA, DNA and in lipids. They include polysaccharides, proteoglycans, glycoproteins and glycolipids. The proteoglycans are specialized construction materials found in the cartilage and joints. Proteoglycans find their highest expression and widest variety in the animal kingdom. The carbohydrate components of proteoglycans are glycosaminoglycans (GAG). They are

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linear polymers of di/tri/tetra saccharide repeat containing sulfate and amino groups. The GAGs represent a class of animal polysaccharides, such as chondroitin sulfate and dermatan sulfate varieties which are components of the connective tissue and basement membrane. The following eight types of GAGs are found in nature: (1) heparin; (2) heparan sulfate; (3) hyaluronic acid; (4) chondroitin; (5) chondroitin-4-sulfate; (6) chondroitin-6-sulfate; (7) dermatan sulfate; and (8) keratan sulfate [1,2]. The knowledge of the conformational properties of carbohydrates in solution and their structure–activity relationships are important for understanding the mechanisms associated with their biological functions. Water is associated with biomolecules and plays a central role in both structure and function. An understanding of the extent of interactions between these molecules and water is therefore important. Hence, the conformational preferences of GAGs have long been the subject of theoretical study [3].

In this paper, we report on the results of our investigations on the commonly occurring GAGs regarding their accessible surface area to water by applying the solvent accessibility technique of Lee and Richards [4]. This approach has earlier been proved to give results in good accord with conformational properties of proteins [5,6] and nucleic acids [7,8].

2. Methods

2.1. Materials

The crystallographic data available for the GAGs are as follows: (i) chondroitin-4-sulfate (an alternating copolymer of β-D-glucuronic acid and 2-deoxy-2-acetamido-β-D-galactose-4-sulfate, 1C4S); (ii) hyaluronic acid (poly-D-glucuronic acid-*N*-acetyl-D-glucosamine, 1HYA); (iii) keratan sulfate [sulfated poly (galactosyl-*N*-acetyl glucosamine), 1KES]; and (iv) heparin [an alternating copolymer of 1–4 linked α-L-iduronic acid-2-

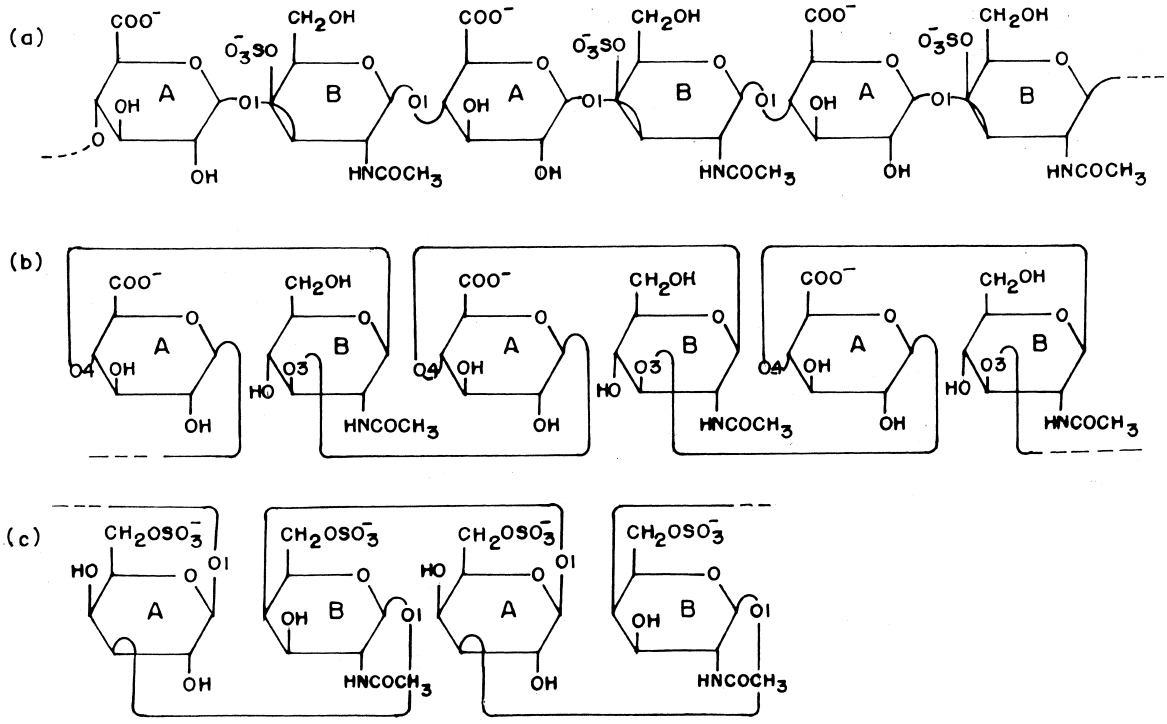

Fig. 1.

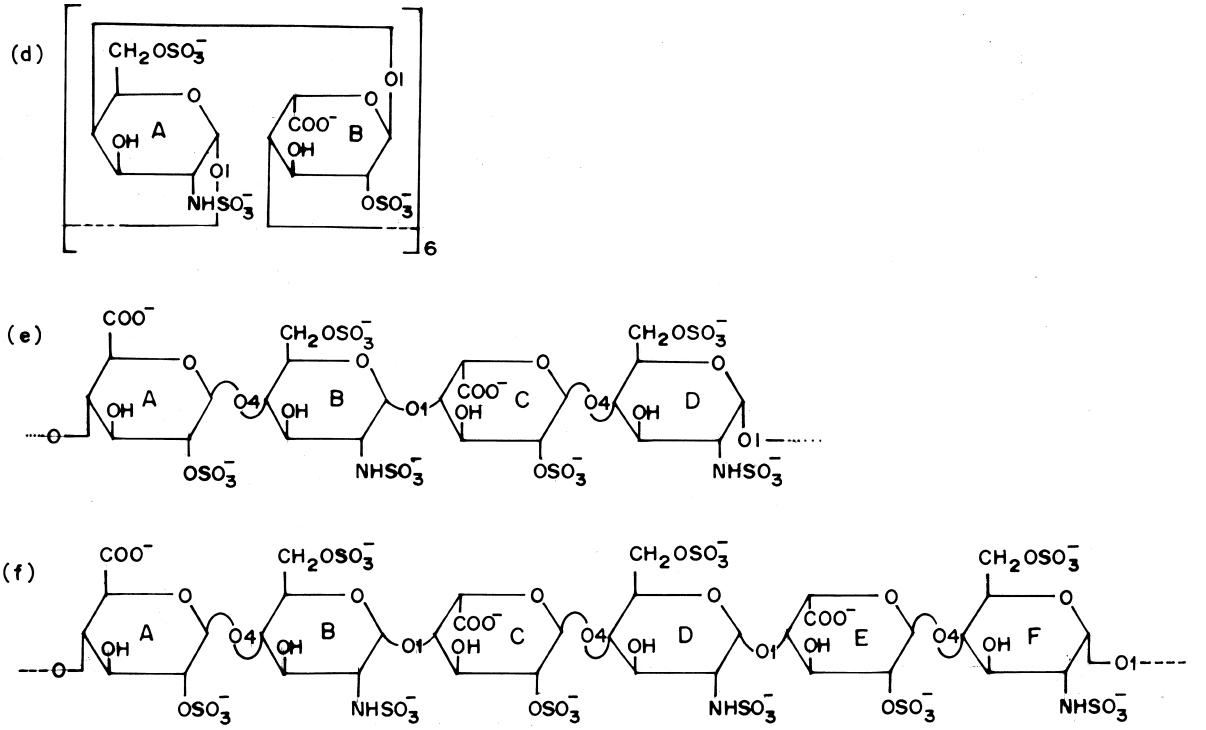

Fig. 1. (*Continued*) Representative glycosaminoglycan sequences. (a) Chondroitin 4-sulfate (1C4S); A, β-D-glucuronic acid (GCU); B, 2-deoxy-2-acetamido-β-D-Gal-4-sulfate (ASG). (b) Hyaluronic acid (1HYA); A, β-D-glucuronic acid (GCU); B, *N*-acetyl-β-D-glucosamine (NAG). (c) Keratan sulfate (1KES); A, β-D-Gal-6-sulfate (GLS); B, β-*N*-acetyl-D-glucosamine-6-sulfate (NGS). (d) Heparin uncomplexed (1HPN); A, N,O_6-disulfo-glucosamine (SGN); B, O_2-sulfo-glucuronic acid (IDS). (e) Heparin — complexed with fibroblast growth factor (1BFB); A, 1,4-dideoxy-5-dehydro-O_2-sulfo-glucuronic acid (UAP); B, N,O_6-disulfo-glucosamine (SGN); C, 1,4-dideoxy-O_2-sulfo-glucuronic acid (IDU); D, N,O_6-disulfo-glucosamine (SGN). (f) Heparin — complexed with fibroblast growth factor (1BFC); A, 1,4-dideoxy-5-dehydro-O_2-sulfo-glucuronic acid (UAP); B, N,O_6-disulfo-glucosamine (SGN); C, 1,4-dideoxy-O_2-sulfo-glucuronic acid (IDU); D, N,O_6-disulfo-glucosamine (SGN); E, 1,4-dideoxy-O_2-sulfo-glucuronic acid (IDU); F, N,O_6-disulfo-glucosamine (SGN).

sulfate and 2-deoxy-2-sulfamino-α-D-glucosamine-6-sulfate, 1HPN, and two other heparin fragments bound to the fibroblast growth factor (FGF) — a tetrasaccharide in 1BFB and a hexasaccharide in 1BFC] from the Protein Data Bank of Brookhaven National Laboratory [9,10] form the basis of our study (Fig. 1).

2.2. Computation of accessible surface area

We followed the method of Lee and Richards [4] to compute the solvent accessible surface area for all the atoms in the mentioned GAGs. In this method, an atom or a group of atoms in a solute molecule is said to be accessible to a solvent molecule if the solvent molecule of specified size can be brought into van der Waals contact with it without penetrating any other atoms of the molecule [11–13]. In the present study, the solvent molecule (water) has been assumed to be a sphere with a radius of 1.4 Å (Fig. 2). The solute molecule is represented by a set of interlocking spheres of appropriate van der Waals radii assigned to each atom and the solvent molecule is rolled along the envelope of the van der Waals surface at planes conveniently sectioned. The accessible surface area of an atom of radius r is then the area on the surface of sphere of radius $R = r + r_{\mathrm{solv}}$ on each point of which the center of the solvent molecule can be placed in contact

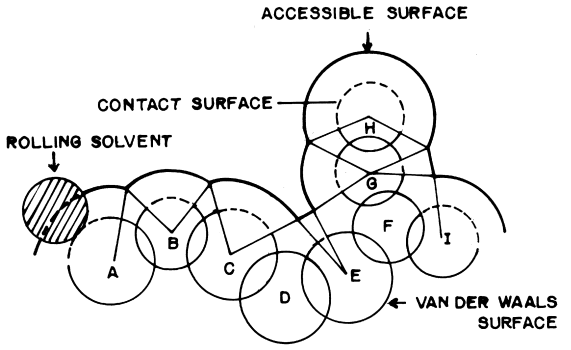

Fig. 2. A cross-section of a part of a macromolecule in space, A–I are atoms, van der Waals, contact and accessible surface areas are indicated. Atoms D and F are inaccessible to the solvent molecule.

with this atom without penetrating any other atoms of the solute molecule. The solvent accessible surface area (ASA) is calculated using the formula

$$\mathrm{ASA} = \sum \left[R/(R^2 - Z_i^2)^{1/2} \right] L_i \cdot D;$$

$$D = \nabla Z/2 + \nabla' Z \qquad (1)$$

where L_i is the length of the arc computed on a given section i, Z_i is the perpendicular distance from the center of the sphere to the section i, ∇Z is the spacing between the sections and $\nabla' Z$ is $\nabla Z/2$ or $R - Z_i$, whichever is smaller. Summation is over all of the arcs drawn for the given atom.

Based on these concepts, we have used the computer program ACCESS [12] to compute the solvent accessible areas of the atoms in the folded state of the GAGs. The atomic coordinates for the various GAG molecules were taken from the recent Protein Data Bank of Brookhaven National Laboratory and suitably adapted as required for running the ACCESS Program. The charged oxygen atoms in COO^- and SO_3^- groups in sugar residues were identified with the charged oxygens OE1 of glutamic acid in proteins.

3. Results and discussions

3.1. Accessible surface area of different atoms in GAGs

The ASA for all the atoms in the considered molecules were computed as described in the previous section. The variation of ASA for the different atoms, such as carbon (C), neutral oxygen (O), charged oxygen (O^-), nitrogen (N) and sulfur (S) present in the various GAGs and their average ASAs are shown in the histogram (Fig. 3). Their numerical values are given in Table 1. From the figure we observe that the charged oxygens acquire more ASA than all the other atoms. It is interesting to note that carbon and oxygen have almost nearly equal values whereas the O^- atoms have treble the ASA value than that for carbon or oxygen. For nitrogen and sulfur we note that their ASA values are relatively very small (lying between 1 and 7 Å^2 only).

In all the considered GAGs the weighted average variation of ASA for the different atoms is in the range of 2–28 Å^2 (Table 1). However, in the case of proteins a different trend is observed in which the variation of ASA values is less (17–35 Å^2) [14] which indicates that all the atoms compete more or less equally for favorable interactions with water. Also we note from Table 1 that the average contribution of oxygen (10.31 Å^2) is five times higher than that for sulfur (2.04 Å^2).

In 1C4S, the glycosidic oxygens O-1 of residues 3 and 5 involved in (1–3) linkage have zero ASAs which indicate that they are completely buried in a deep pocket whereas for O-1s of residues 2, 4 and 6 involved in the (1–4) linkage are partially buried; ASA = 3.7 Å^2 (the ASA values < 5 Å^2 are considered to indicate partial buriedness and zero indicates full buriedness [15]. The average ASA for carbon (15.02 Å^2) and O (18.65 Å^2 — the combined weighted average for oxygen and O^-) are comparatively higher than that for nitrogen and sulfur, which are very much less. In 1KES, the glycosidic oxygens involved in (1–3) linkages (of residues 2 and 4, NGS) are partially buried (3.7 Å^2) and that involved in (1–4) linkage (of residue 3, GLS) are on the surface (6.0 Å^2). These higher ASA values indicate that these atoms freely interact with the solvent molecules and hence the solute molecule may prefer to take up an extended conformation relatively. In 1HYA, it is interesting to note that all the ring oxygens (O-5) have very less ASA values (0.7–2.3 Å^2). Also, the other atoms in the partially buried region are the

Table 1
The average accessible surface area (ASA) of different atoms in GAGs

Molecule	Atoms				
	C	O	O^-	N	S
1KES	13.86 (28)	14.91 (20)	39.90 (12)	5.10 (2)	2.28 (4)
1C4S	15.02 (42)	13.10 (27)	28.65 (15)	3.87 (3)	1.40 (3)
1HYA	15.76 (42)	17.83 (27)	26.60 (6)	3.40 (3)	—
1HPN Model I	5.68 (72)	7.62 (49)	32.7 (66)	7.0 (6)	2.4 (18)
1HPN Model II	6.73 (72)	9.5 (49)	27.61 (66)	1.93 (6)	2.3 (18)
1BFB	4.85 (24)	8.56 (16)	24.45 (22)	4.9 (2)	1.45 (6)
1BFC	4.49 (36)	8.56 (19)	20.45 (38)	5 (3)	1.4 (9)
Weighted average	9.02 (316)	10.31 (207)	28.28 (225)	4.41 (25)	2.04 (58)

Note. The ASA values are in $Å^2$; the number of atoms is given in brackets.

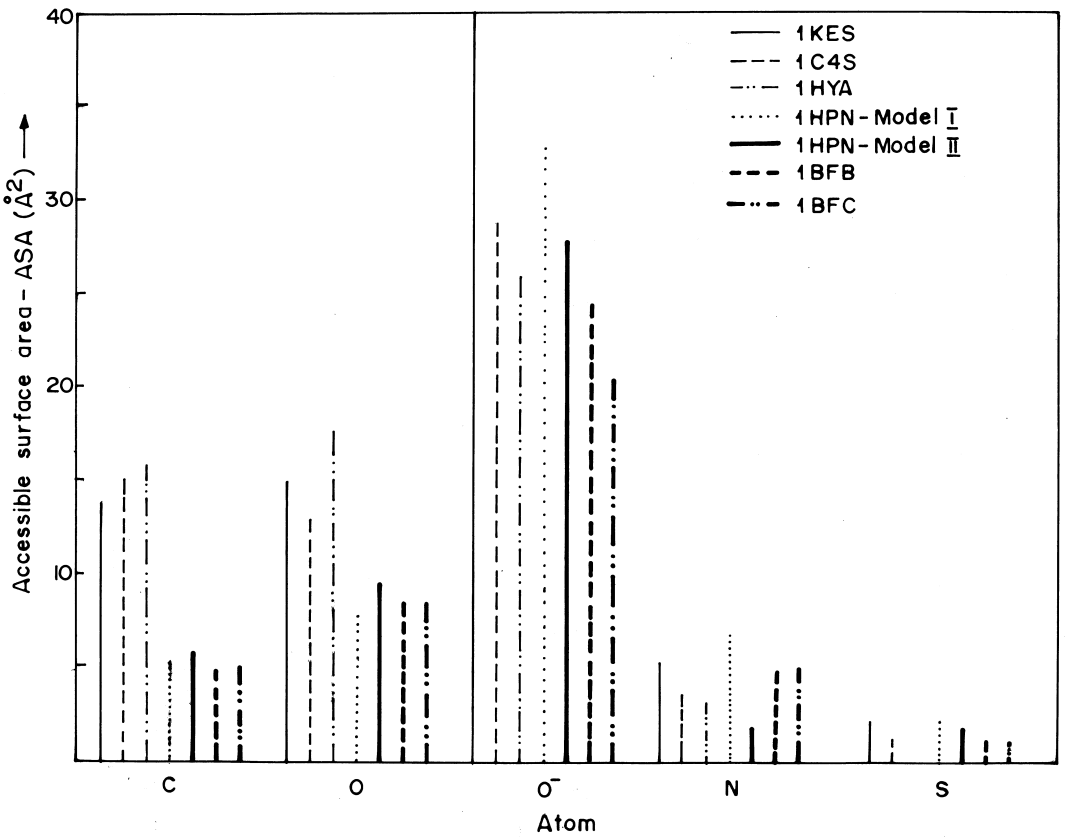

Fig. 3. Histogram showing the variation of average ASA for the atoms C, O, O^-, N and S in the various molecules.

glycosidic oxygens (O-3 of NAGs and O-4 of GCUs), the corresponding carbons (C-3 of NAGs and C-4 of GCUs) attached to these oxygens and nitrogens in NAGs. The ASA value for the methyl carbon (C-8) in NAG is observed to be 61 Å^2 which is nearly equal to that for carbon of methyl groups in the extended state of proteins [16]. In the uncomplexed heparin 1HPN, in both the models I and II, the glycosidic oxygens O-1s of SGN have ASAs 0.0–0.2 Å^2 and the O-1s of IDS have ASAs 0.3–0.9 Å^2 only, indicating that the O-1s in both the types of residues are buried inside. In the case of heparin complexed with 1BFB, the glycosidic oxygens O-4 and O-1 of SGN are also fully buried (ASA = 0.0 Å^2) and that in 1BFC are only partially buried (0.0–2.4 Å^2)

The total number of atoms present in the GAGs, the number of atoms in the buried regions and their corresponding ASAs are given in Table 2. From the table we note that 1KES has the smallest contribution (3.3%) and 1C4S and 1BFB have the highest contribution (6.6%) by buried atoms. In the case of uncomplexed heparin 1HPN, proposed by NMR and modeling the buried atoms contribute approx. 4–5% in both the models. However, in the case of heparin bound to FGF — a tetrasaccharide in 1BFB, the buried atoms contribute 6.57% and a hexasaccharide in 1BFC contribute 5.8%. In the uncomplexed heparin 1HPN, the buried atoms are only 36% where as in the bound heparin in 1BFB and in 1BFC the buried atoms are 50 and 48.6%, respectively. Also we note from the table that the ASA contribution per atom in the uncomplexed heparin is 14.33 Å^2 in model-I and 13.38 Å^2 in model-II. In the complexed heparin the corresponding values are 11.6 Å^2 in 1BFB and 10.8 Å^2 in 1BFC. Thus, the bound heparin molecule is less free to interact with the solvent due to restricted conformational freedom of the complex. Furthermore, it is seen from the table that in general the buried atoms in all the GAGs contribute $< 10\%$ of ASA acquired by all the atoms.

Table 2
Buriedness and contribution of ASA of atoms in GAGs

Molecule	N	ASAN	N_b	ASAN_b	B_a %	ASAB_a %
1KES	66	1184.3	13	38.5	20.0	3.3
1C4S	90	1430.0	32	93.8	36.0	6.6
1HYA	78	1313.0	20	70.5	25.6	5.4
1HPN-I	211	3025.0	76	119.0	36.0	3.9
1HPN-II	211	2825.0	75	136.5	35.5	4.8
1BFB	70	810.2	35	53.2	50.0	6.6
1BFC	105	1129.3	51	65.2	48.6	5.8

Abbreviations. N, total number of atoms; ASAN, ASA of N atoms (Å^2); N_b, number of buried atoms; ASAN_b, ASA of buried atoms (Å^2); B_a, percentage of buried atoms; ASAB_a, percentage contribution of ASA by buried atoms.

3.2. Accessible surface area of sugar residues in GAGs

The variation of ASA for the sugar residues present in the considered GAGs are shown in

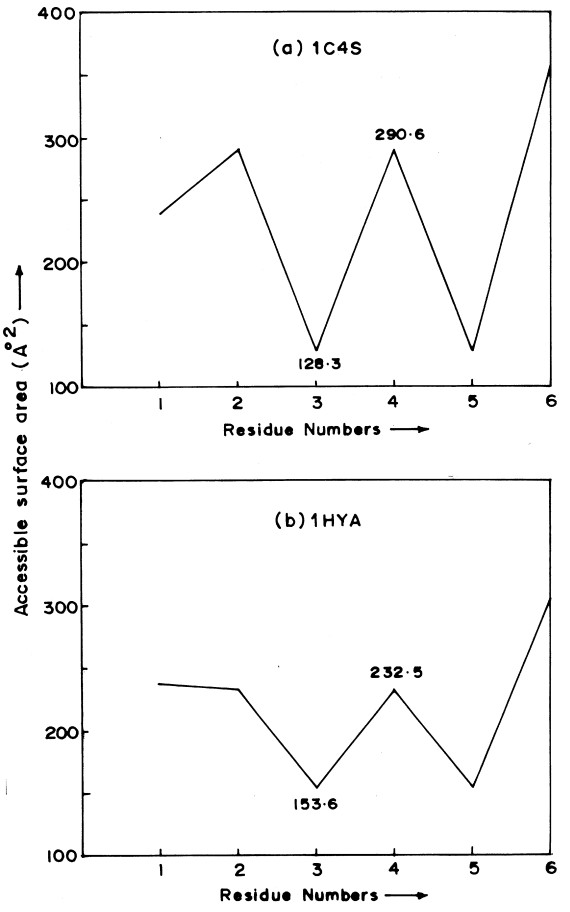

Fig. 4.

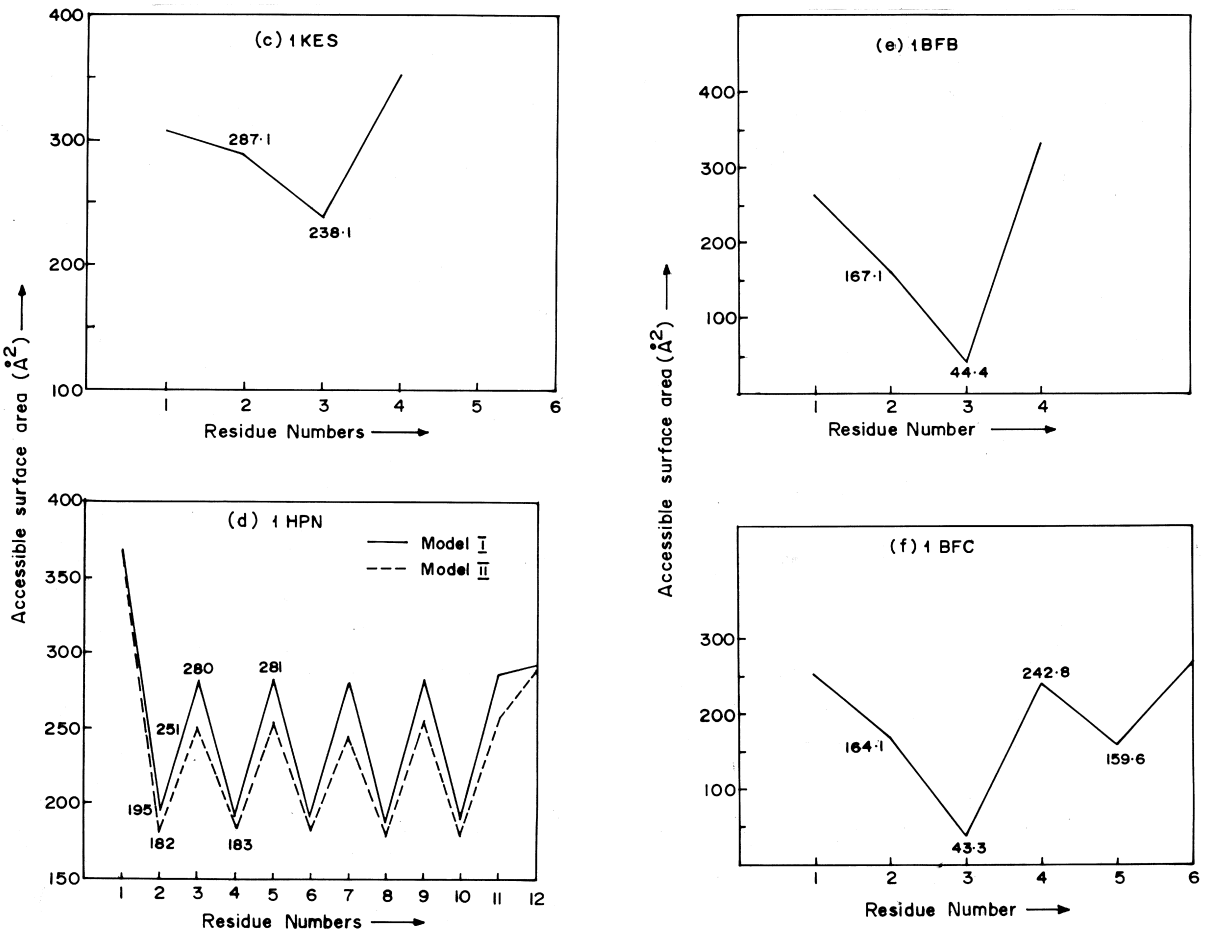

Fig. 4. (*Continued*) Plot of the accessible surface area of sugar residue in (a) 1C4S; (b) 1HYA; (c) 1KES; (d) 1HPN; (e) 1BFB; and (f) 1BFC.

Fig. 4(a–f). From Fig. 4a we note that in 1C4S, the residues 2 and 4 (ASG) have very high values, 290.5 and 290.6 Å^2 whereas the residues 3 and 5 (GCU) have low values, 128.4 and 128.3 Å^2 (the ASA of residues 1 and 6 are not taken for discussion since they are free at their ends and hence their computed ASA values may not represent the true picture of the residues in the actual chain).

Furthermore, we note that the ASA values vary alternately (high–low–high pattern) which is very much similar to the hydrophobic behavior of β strands in globular proteins [17,18]. A similar type of ASA variation is also observed in 1HYA (Fig. 4b). A comparison between the curves for 1C4S and 1HYA shows that the variation of ASA between minimum and maximum is approx. 162 Å^2 for 1C4S and for 1HYA it is 79 Å^2. This indicates that the solvent environment plays a key role in stabilizing their conformations, which is also the case for other biomacromolecules [19]. In 1HYA the residues 2 and 4 (NAG) have equal ASA values (232 Å^2) and the residues 3 and 5 (GCU) have also ASA values equal (153 Å^2). This emphasizes the fact about the occurrence of regular secondary structure of the molecule. The higher ASA value for the residues 2 and 4 is mainly due to the contribution by the methyl carbon (C-8) atoms in NAG (61.1 Å^2). In 1KES (Fig. 4c) the maximum difference in accessibility

Table 3
The conformations of iduronic acid residues heparin (unbound 1HPN and bound 1BFB and 1BFC) and their ASA values

	1HPN		1BFB	1BFC	
	IDS (2,4,6,8,10,12) residue Model-I	Model-II	IDU third residue	IDU third residue	Fifth residue
Conformation	$^{2}S_{O}$ (L)	$^{1}C_{4}$ (L)	$^{1}C_{4}$ (L)	$^{1}C_{4}$ (L)	$^{2}S_{O}$ (L)
ASA (Å^2)	(191.2)[a]	(182.0)[a]	(44.4)	(43.3)	(159.6)

[a]Average values of ASA for IDS.

is only 49 Å^2 (between residues 2 and 3; the end residues 1 and 4 are not considered due to the same reason explained above).

Similarly in the case of 1HPN in models I and II the ASAs for the sugar residues SGN are high (approx. 280 Å^2 in model-I and approx. 251 Å^2 in model-II). However, the iduronic acid residues (IDS) have ASAs ranging from 190 to 195 Å^2 in model-I and from 180 to 183 Å^2 in model-II. Thus, we observe that the IDS residues are less accessible to the solvent than the SGN residues. Also, the ASA values vary alternatively similar to that of 1C4S. In the case of bound heparin in 1BFB the ASA value for SGN (second residue only, fourth residue not considered) is 167 Å^2 and for the heparin bound in 1BFC the values for SGN — second and fourth residues are 164.1 and 242.8 Å^2. Similarly the iduronic acid residue (third one) in 1BFB has an ASA value of 44.4 Å^2 and in 1BFC for the third and fifth residues the ASA values are 43.3 and 159.6 Å^2, respectively. Thus, in both the molecules the iduronic acid residues

Table 4
Average ASA of sugar residues in GAGs

Residue	Molecule	ASA (Å^2)
1,4-Dideoxy-O_2-sulfo-glucuronic acid (IDU)(L)	1BFB	44.4
1,4-Dideoxy-O_2-sulfo-glucuronic acid (IDU)(L)	1BFC	101.5
β-D-Glucuronic acid (GCU)	1C4S	128.4
β-D-Glucuronic acid (GCU)	1HYA	153.5
N,O_6-disulfo-glucosamine (SGN)	1BFB	167.1
O_2-sulfo-glucuronic acid (IDS) (L)	1HPN-II	182.1
O_2-sulfo-glucuronic acid (IDS) (L)	1HPN-I	191.2
N,O_6-disulfo-glucosamine (SGN)	1BFC	203.5
N-acetyl-glucosamine (NAG)	1HYA	232.6
D-Gal-6-sulfate (GLS)	1KES	238.1
1,4-Dideoxy-5-dehydro-O_2-sulfo-glucuronic acid (UAP)	1BFC	250.3
N,O_6-disulfo-glucosamine (SGN)	1HPN-II	251.9
1,4-Dideoxy-5-dehydro-O_2-sulfo-glucuronic acid (UAP)	1BFB	267.0
N,O_6-disulfo glucosamine (SGN)	1HPN-I	281.9
Nac-D-Glu-6-sulfate (NGS)	1KES	287.1
Acetamido-β-D-gal-4-sulfate (ASG)	1C4S	290.6

have considerably less ASA values when compared to those in the unbound heparin 1HPN.

The conformations and the computed average ASAs of iduronic acid residues as found in the uncomplexed heparin 1HPN and the complexed heparin in 1BFB and in 1BFC are given in Table 3. It is interesting to note from the table that in the uncomplexed heparin, the iduronic acid residue has nearly equal ASA values in both the $^{2}S_{O}$ and $^{1}C_{4}$ (L) conformations. However, in the bound heparin in 1BFB, the iduronic acid residue in the $^{1}C_{4}$ (L) conformation has an ASA of 44.4 Å^2 only. However, in 1BFC, the third residue also in the $^{1}C_{4}$ (L) conformation has an ASA of 43.3 Å^2 , whereas the fifth residue in the $^{2}S_{O}$ conformation has an ASA value of 159.6 Å^2. This indicates that the IDU in the $^{2}S_{O}$ conformation is more accessible to the solvent than in the $^{1}C_{4}$(L) conformation.

A comparison of the ASA values for the iduronic acid and glucuronic acid (Table 4) reveals that in the bound state, the IDU residue in 1BFB and 1BFC have considerably less ASA values than that the glucuronic acid residues — UAP in 1BFB (267.0 Å^2) and 1BFC (250.0 Å^2) and in 1HYA and 1C4S. However, the iduronic acid (IDS) in the uncomplexed heparin (1HPN) is found to have higher values than that for glucuronic acid. From the above analysis one cannot conclude that IDU residues usually exhibit high or low ASA values than that of glucuronic acid residues which always exist in $^{4}C_{1}$ conformations.

From the average ASA for all the eight types of sugar residues present in the considered GAGs (Table 4) we note that the residues ASG (290.6 Å^2) and NGS (287.1 Å^2) have the highest ASA and IDU (44.4 Å^2) has the lowest ASA. Also, we note that sugar with an acidic group has ASA values in the range of 120–150 Å^2 whereas sugars with acidic and sulfated groups exhibit wide variations of ASA ranging from 44.4 to 282 Å^2 in different molecules.

3.3. Role of sulfate groups in GAGs

An analysis of the contribution of ASA by the sulfur atom and the oxygen atoms attached to sulfur, i.e. sulfate group and their role in the molecules 1KES, 1C4S, 1HPN,1BFB and 1BFC can also be made.

3.3.1. 1KES

In 1KES, the repeating disaccharide residues 1 (GLS) and 2 (NGS) are both 6-sulfated (Fig. 1c). The contribution of total ASA by the atoms in the SO_3^- groups (4) is 487.9 Å^2, i.e. 122 Å^2 per SO_3^- group, in which the four sulfur atoms contribute only 9.1 Å^2 and hence the major contribution is from the charged oxygens (478.8 Å^2, 40.4%). The contribution by the four SO_3^- groups (16 atoms) is 41.2% of the total ASA (1184.3 Å^2) acquired by all the atoms in 1KES. Hence, the 24.2% of the total number of atoms (66) contribute 41.2% of ASA in 1KES. The high value of ASA of the sulfate groups indicates that the groups exhibit strong interaction with the solvent and thus play a crucial role in preferring and stabilizing the suitable conformation of the molecule for its biological function [20,21].

Furthermore, it is seen that the average value of ASA for each charged oxygen, $O^{-1/3}$ (one negative charge is shared by the three oxygens) is found to be 39.9 Å^2, which is slightly on the higher side when compared to that for O^- (35 Å^2) in small proteins [6].

3.3.2. 1C4S

In 1C4S (Fig. 1a) the sulfate group is attached to the C-4 carbon like 1KES of ASG (residues 2, 4 and 6) through an oxygen atom. The total ASA for the nine charged oxygens attached to the sulfur is 286.3 Å^2, an average of 31.8 Å^2 per each charged oxygen which is relatively a high value when compared to that for other atoms (see Table 1) in the system, indicating a favorable interaction site, i.e similar to an active site leading to its role in the function of the molecule. However, their ASA contribution is only 20% of that of the whole molecule, which is relatively a very low contribution when compared to that in other molecules like 1KES (40.4%).

Also we note that the ASAs for the residues 2 and 4 are very high (290.5 Å^2) and that of residues 3 and 5 are less (128.4 Å^2; Fig. 4a). It is interesting to see that the residues 3 and 5 (GCU) inspite of having charged oxygens present in the form of

carboxylic acid group at C-6 position, their ASA values are less than half the ASA values for the residues with sulfate groups. This leads us to state that the SO_3^- groups pull out the residues to a greater extent to enhance their ASA values and thus the SO_3^- groups play a major role in stabilizing the conformation of the molecule. In these groups also the contribution of ASA by the sulfur atoms is very minimal and only the oxygens which are ionized, which is their state in biological pH, contribute more for the ASA.

3.3.3. 1HPN

In 1HPN there are totally 18 SO_3^- groups, two for each SGN and one for each IDS. The ASA contribution by all the SO_3^- groups is 1875.7 Å^2 which is 62% of the total ASA (3025.2 Å^2) of the molecule in model-I and the contribution by all the SO_3^- groups is 1148.9 Å^2, which is 54% in model-II (total ASA = 2825.4 Å^2). Here also the ASA contribution by the sulfur atoms varies from 2 to 5 Å^2 only and the major contribution is from the charged oxygen (O^-) atoms in both the models.

3.3.4. 1BFB and 1BFC

In the heparin fragments bound to FGF — a tetrasaccharide in 1BFB, there are six SO_3^- groups and contribute 56% of total ASA (810.2 Å^2) of the molecule. In the hexasaccharide fragment (1BFC) the nine SO_3^- groups contribute 61.3% of ASA of the molecule. Thus, in both unbound and bound heparin the SO_3^- groups' contribution to ASA is more or less equal. However, the SO_3^- groups present in IDU (third residues) in both 1BFB and 1BFC are found to contribute a very low ASA (6.4 Å^2), but the SO_3^- group in the fifth residue (IDU) in 1BFC contribute ASA of 103.5 Å^2.

3.4. Relation between accessible surface area and charge of oxygen

Consider the residues with charged oxygens attached to sulfur, in which one negative charge is shared by three oxygens (ASG in 1C4S and GLS and NGS in 1KES). Their average ASA values are given in Table 5. In residues with charged oxygens attached to carbon, one negative charge is shared by two oxygens (GCU in 1C4S and 1HYA). The ASA values for the oxygens of the carboxylate ion of GCU in 1C4S and 1HYA are respectively 23.9 and 26.6 Å^2 and for the charged oxygen in the SO_3^- groups the ASA varies from 32 to 41.5 Å^2 in 1C4S and 1KES.

Similarly, in 1HPN — models I and II the oxygens attached to the sulfur in the SGN residues have ASAs in the range 32–35 Å^2 whereas in the IDS residues the ASAs lie in the range 21–32 Å^2 and the carboxylate oxygens have ASA equal to approx. 27 Å^2. In the complexed heparin in 1BFB and in 1BFC the charged oxygens in the various residues other than IDU have ASAs in the range 26–37 Å^2, but in IDU the charged oxygens' contribution is as low as 2.1 in one case and 18 Å^2 in

Table 5
Charge and ASA of oxygen atoms in sulfate and carbonyl groups in GAGs

Molecule	Residue	Charge	ASA (Å^2)
1KES	GLS	−0.33	41.53
	NGS	−0.33	38.26
1C4S	ASG	−0.33	31.81
	GCU	−0.50	23.9
1HYA	GCU	−0.50	26.6
1HPN-I	SGN	−0.33	35.0
	IDS	−0.50	27.1
	IDS	−0.33	31.82
1HPN-II	SGN	−0.33	31.56
	IDS	−0.50	26.83
	IDS	−0.33	21.54
1BFB	UAP	−0.50	30.3
	UAP	−0.33	37.13
	SGN	−0.33	27.51
	IDU	−0.50	14.75
	IDU	−0.33	2.1
1BFC	UAP	−0.50	25.65
	UAP	−0.33	35.4
	SGN	−0.33	25.86
	IDU	−0.50	12.05
	IDU	−0.33	17.95

the other, showing a wide variation. An analysis of their charge and ASA values of these ionized oxygens reveals that there is a correlation between the charge and the ASA associated with these atoms, i.e. an increase in the negative charge associated with the atom decreases its ASA value except those in IDU/IDS which show both an increase and decrease of ASA values.

The decreased ASA values for the increased negative charge associated atoms, might be due to, that in the ionized state the specific atom might have favorable electrostatic interaction with the partial charge δ^+ associated with the carbon atoms of the rings and hence go inwards resulting in less average ASA. Similar behavior has also been reported with charged atoms in proteins, like O^- in Glu [6,22].

From the above analysis we note that the charged oxygens in the IDU residues are pushed away from the solvent environment due to large conformational flexibility of the IDU residues. Thus, the molecule heparin due to the presence of a large number of charged groups is able to perform subtle conformational maneuvers for efficient complexation with the protein.

3.5. D- and L-configuration and ASA

The iduronic acid residues in 1HPN, 1BFB and 1BFC molecules are found to exist in the L-configuration (Table 3). The IDS residue present in the unbound heparin, 1HPN are calculated to have an ASA value approx. 182–192 $Å^2$, whereas those in the bound heparin, 1BFB and 1BFC have average ASA values of 44.4 and 101.45 $Å^2$, respectively. Thus in the bound molecule the iduronic acid residues adjusts itself such that they are buried relatively inside enabling the molecules to have enough conformational flexibility. Hence, among the D- and L-residues when competing for interaction with the solvent the L-residues are pushed inside leading to more buriedness than the D-residues which in turn leads to large conformational change to the molecule.

Summarizing we conclude that the solvent accessible surface area was computed for the four molecules of GAGs and the ASAs of the residues in these molecules are found to vary alternately. The study reveals that the bound heparin is less accessible to the solvent compared to the uncomplexed which indicates that the complexed heparin acquires a compact conformation. The charged oxygens acquire treble the ASA that for carbon and neutral oxygen and the ASA value for sulfur atoms is very small. However, the ASA values for the sulfate groups as a whole is very large and they enhance the ASA of the residues to which they are attached indicating strong interaction with the solvent. Thus, they play a crucial role in stabilizing the conformations of the molecules for proper function. This is supported by the finding that in heparin the sulfate groups are essential for the binding to antithrombin III [23]. The L-configuration residues α(L) IDS are more buried than D-configuration residues. Thus, the detailed analysis on the ASA of GAGs provides a basis to understand glycosaminoglycan–solvent interactions.

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