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Molecular dynamics of a tetrasaccharide subunit of chondroitin 4-sulfate in water

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Abstract

Molecular dynamics (MD) simulations on a tetrasaccharide subunit of chondroitin 4-sulfate (CS4) in aqueous solution were carried out to study its interactions with water. Pair distribution functions and diffusion coefficients were calculated from a 4 ns trajectory and the hydration of different molecular groups was analysed. The average values of the interglycosidic torsion angles found in the simulations are $\phi_{13} = -10°$, $\psi_{13} = -85°$ and $\phi_{13} = 80°$, $\psi_{13} = 90°$ for the β-(1→3) linkage, and $\phi_{14} = -10°$, $\psi_{14} = -70°$ for the β-(1→4) linkage. Hydrophobic patches formed by sugar ring CH groups were found. The diffusion coefficients of the water molecules vary from 1.4×10^{-9} to 2.3×10^{-9} m^2 s^{-1} depending on the distances between the water molecules and the atoms of the CS4 molecule and the type of CS4 atoms, respectively. Reorientation correlation times of the water molecules in the vicinity of different CS4 atoms were estimated to be about 1 ps at a polymer concentration of 4 wt.% CS4. The number of hydrogen bonds between the water molecules and the acceptor atoms of CS4 was determined to be about 20 per disaccharide unit, indicating a higher hydration ability of chondroitin sulfate in comparison with non-sulfated oligosaccharides. Substructures, where water molecules are involved in hydrogen bonds to different sugar rings, were found, which may be important for the stabilisation of the secondary structure of the CS4 molecule.

Keywords: Chondroitin sulfate; Hydration; Molecular dynamics; Conformational analysis; Cartilage components

1. Introduction

Chondroitin 4-sulfate (CS4) is a linear glycosaminoglycan with a molecular mass between 10 and 50 kDa [1–3] consisting of alternating *N*-acetyl-D-galactosamine β-(1→4) and D-glucuronate β-(1→3) residues (Fig. 1). CS4, chondroitin-6-sulfate and keratan-sulfate are covalently attached to binding proteins and form the proteoglycans with molecular masses of about 10^6. These are noncovalently linked to the hyaluronic acid (HA) backbone in proteoglycan aggregates. Thus, the proteoglycan gains its space-filling capacity, e.g., as a pressure-resistant 'filling' responsible for the cartilage function [4], or keeps collagen fibrils apart, e.g., to preserve the transparency of cornea and, as in young tendons, to inhibit the growth and fusion of the fibrils [5].

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Together with collagen, the proteoglycans are the main components of cartilage. The viscoelastic properties of cartilage probably result from the large ability of association with water. The CS4 and HA polymer chains are essentially involved in the hydration of the proteoglycan. They are able to form secondary structures as a regular multihydrogen-bond array throughout the entire molecule involving the acetamido, carboxylic and hydroxy groups and the sugar ring oxygen atoms [6]. On the one hand, CS4 and HA exhibit an extended hydrogen-bonded system represented by a two-fold helix with elements of cooperativity including water bridges between neighbouring sugar residues as indicated by NMR studies [7]. On the other hand, large hydrophobic patches with up to nine CH units exist along the polymer and repeat on alternate sides of the molecule [8].

Experimental investigations [8,9] and molecular dynamics (MD) simulations [8] reveal a strong influence of water on the structure of CS4 and HA. The molecular structure of CS4 was determined by Winter et al. [10] using X-ray diffraction. Depending on its chemical environment, CS4 forms different helical conformations [6,7,10]. As a free acid, it builds a 2_1-helix with an identity period of 19.6 Å, where the length of one structure element projected on the helical axis is 9.8 Å. Contrary to this, a 3_2-helix with an identity period of 28.8 Å is formed in the sodium salt. The NMR, electron microscopy and molecular dynamics (MD) studies on CS4 oligosaccharides suggest a stabilising effect of the water structure on the two-fold helix. The negative charges of the polyanions seem to prevent aggregate formation [8]. Calculations by Scott et al. [8] showed that duplexes were formed with decreasing stability if the charge density increases and if the charge is concentrated towards the centre of the polymer.

The viscoelastic properties of cartilage mainly result from the swelling of the proteoglycans. Since numerous specific functions of CS4 depend on the water content, information on the hydration behaviour seems to be a prerequisite for an understanding of the role of CS4 in several biological phenomena. Therefore, we performed MD simulations on a CS4–water model system to gain insights into structure effects in aqueous solution and the interaction with the water environment.

2. Results and discussion

Conformational properties.—A schematic representation of chondroitin-4-sulfate (CS4), along with the labelling of the atoms and the torsional angles of interest, is shown in Fig. 1. The relaxed CHARMM energy map for the potential surface of the glycosidic linkage β-(1→3) is presented in Fig. 2(a). Fig. 2(b) shows the corresponding MD trajectory. There are two energy minima at $\phi_{13} = -10°$, $\psi_{13} = -85°$ (minimum 1) and $\phi_{13} = 80°$, $\psi_{13} = 90°$ (minimum 2), respectively. The averaged torsion angles from the MD trajectory in Fig. 2(b) are in good agreement with these energy minima. At about 2.5 ns simulation time a conformation change from minimum 1 to 2 occurs, which changes again into minimum 1 after 2.7 ns. Fig. 3(a) shows the CHARMM energy map for the potential surface of the glycosidic linkage β-(1→4). The comparison with the corresponding MD trajectory [see Fig. 3(b)] indicates a broad low-energy region centred at about $\phi_{14} = -10°$, $\psi_{14} = -70°$. The high fluctuations of the torsion angles correspond to this situation. X-ray

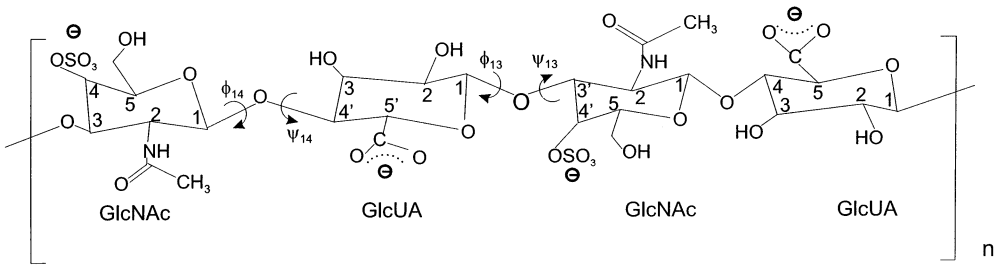

Fig. 1. Basic unit of chondroitin 4-sulfate (CS4).

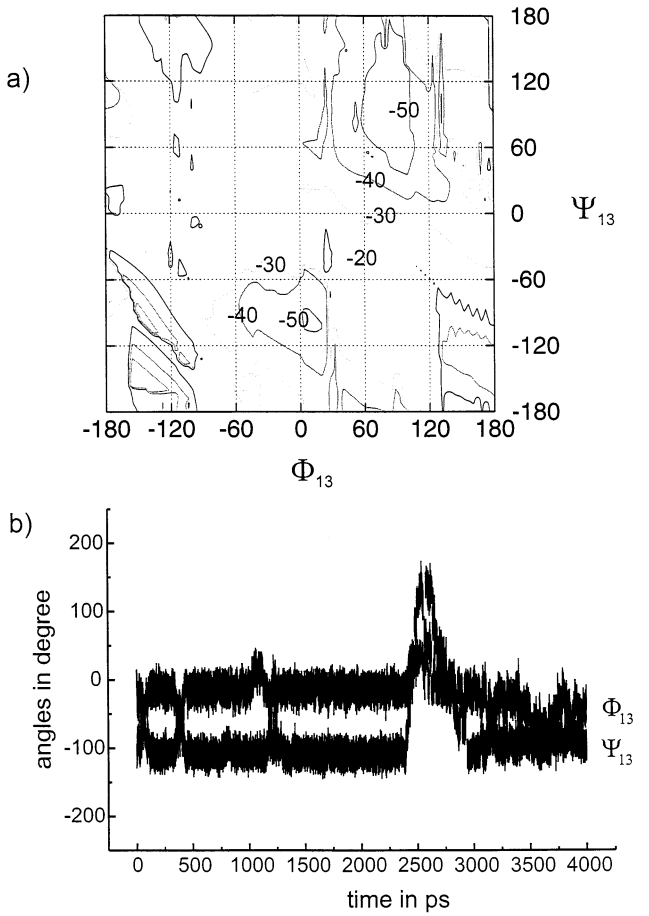

Fig. 2. Interglycosidic torsion angles in the β-(1→3) linkage. (a) Relaxed CHARMM energy map for the potential surface of the glycosidic connection in the β-(1→3) disaccharide. (b) Time dependence of the interglycosidic torsion angles ϕ_{13} and ψ_{13} in the β-(1→3) CS4 dimer.

studies [10] provide values of $\phi_{13} = -86.4°$, $\psi_{13} = 128°$ and $\phi_{14} = -79.9°$, $\psi_{14} = -128.7°$, which cannot directly be compared with the simulation data. The comparison with the data of Zsiska et al. [11], who performed calculations on CS4-related subunits composed of β-D-Gal-(1→3)-β-D-GlcUA and β-D-GlcUA-(1→4)-β-D-Gal using a Metropolis Monte Carlo algorithm and simulating the solvent implicitly by a dielectric constant of $\varepsilon = 10$, is difficult because the *N*-acetyl group was not included. Values of $\phi_{13} = 52°$, $\psi_{13} = -4°$ or $\phi_{14} = 47°$, $\psi_{14} = -17°$, respectively, were found in that study. The influence of the sulfate and carboxylate groups on the conformation of different CS4-related disaccharides was mainly investigated in Ref. [11]. Interestingly, it was shown that the charged groups, even in highly charged molecules, do not necessarily determine the three-dimensional structure of the oligosaccharides. This may justify performing the calculations on a model subunit of CS4 with charged sulfate and carboxylate groups, where the charges were compensated by Na^+ ions. By analogy with Scott et al. [8,12,13], the two-fold helical structure in an aqueous environment was found. Moreover, hydrophobic patches of nine CH units oriented to one polymer side were indicated.

Hydrogen bonding

Pair distribution functions. The average distribution of the solvent molecules around a solute can be described by pair distribution functions. To define solvation regions, it is sufficient to examine atomic pair distributions. Considering the distribution of water molecules W around a particular atom A of

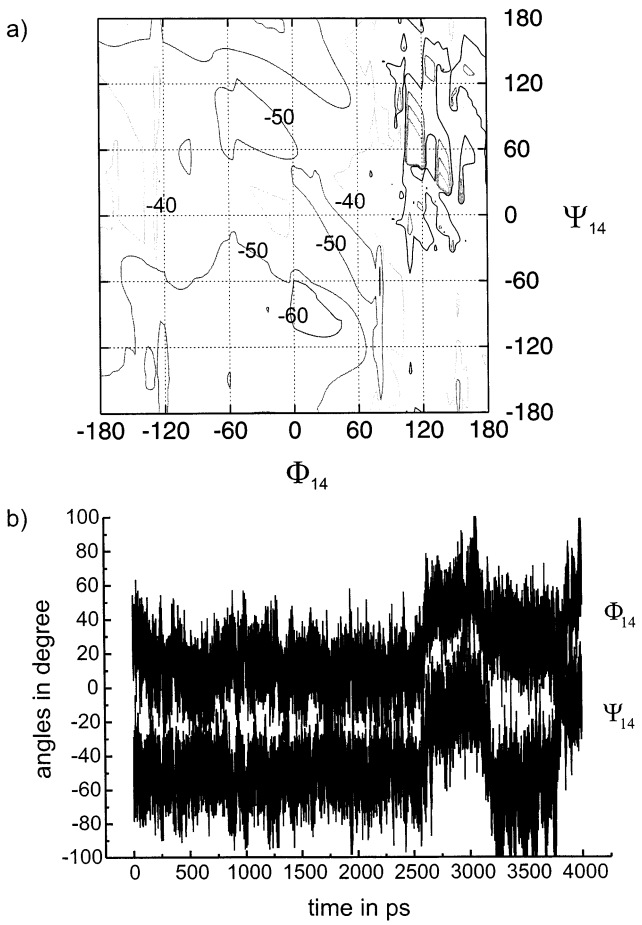

Fig. 3. Interglycosidic torsion angles in the β-(1→4) linkage. (a) Relaxed CHARMM energy map for the potential surface of the glycosidic connection in the β-(1→3) disaccharide. (b) Time dependence of the interglycosidic torsion angles ϕ_{14} and ψ_{14} in the β-(1→4) CS4 dimer.

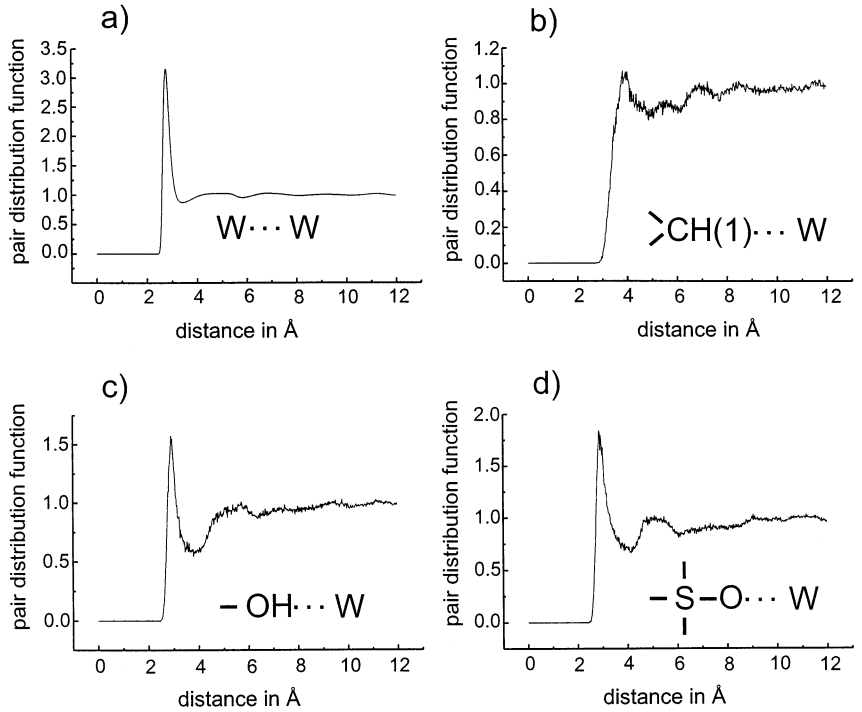

Fig. 4. Distribution of water molecules around selected chondroitin sulfate atoms. (a) Pair distribution function for bulk water. (b) Water pair distribution function around the ring CH(5) group of CS4. (c) Water pair distribution function around an OH group of CS4. (d) Water pair distribution function around a sulfate oxygen.

the CS4 molecule, the distribution function is given by

$$g_{WA}(r) = \frac{1}{4\pi\rho_W r^2}\frac{dN_{WA}(r)}{dr}$$

where $N_{WA}(r)$ is the average number of water oxygen atoms within a sphere of radius r around atom A and ρ_W is the density of water molecules in the system.

In Fig. 4, the oxygen–oxygen pair distribution function $g_{OO}(r)$ of the bulk water (Fig. 4(a)) and the distributions around different atoms of the CS4-subunit (Fig. 4(b–d)) are shown. Table 1 gives the position of the first minimum in the pair distribution functions and the number of nearest-neighbour water molecules obtained by integration up to the first minimum. Fig. 4(a) corresponds rather well to a bulk water distribution [14]. The other distributions are in good agreement with the results from D-Glc simulations by Brady [15]. Generally, the polar groups, especially the sulfate groups, associate a large amount of water molecules. Thus, the presence of sulfate groups increases considerably the hydration ability of CS4 in comparison with hyaluronic acid oligosaccharides [16].

Energy and geometry criteria for hydrogen bonds between CS4 and the surrounding water molecules. The hydration of the model tetrasaccharide was also examined on the basis of special energy and geometry criteria. Fig. 5(a) shows the distributions of the interaction energies between water molecules and CS4. There is an asymmetry in the relative distribution of CS4–water pair interaction energies in the range of negative energies. This asymmetry is due to water molecules forming hydrogen bonds to CS4. Interpreting solute atom–water interaction energies lower than

Table 1
Position of the first minimum of various water pair distribution functions and number of nearest-neighbour water molecules around different atoms and groups of a CS4 subunit

Pair distribution [a]	Minimum position (Å)	Nearest-neighbour water molecules
$g_{OO}(r)$ (bulk)	3.5	4.3
$g_{OC}(r)$	4.0	3.2
$g_{OOH}(r)$	3.8	4.5
$g_{OSO}(r)$	4.0	4.8

[a] See Fig. 4.

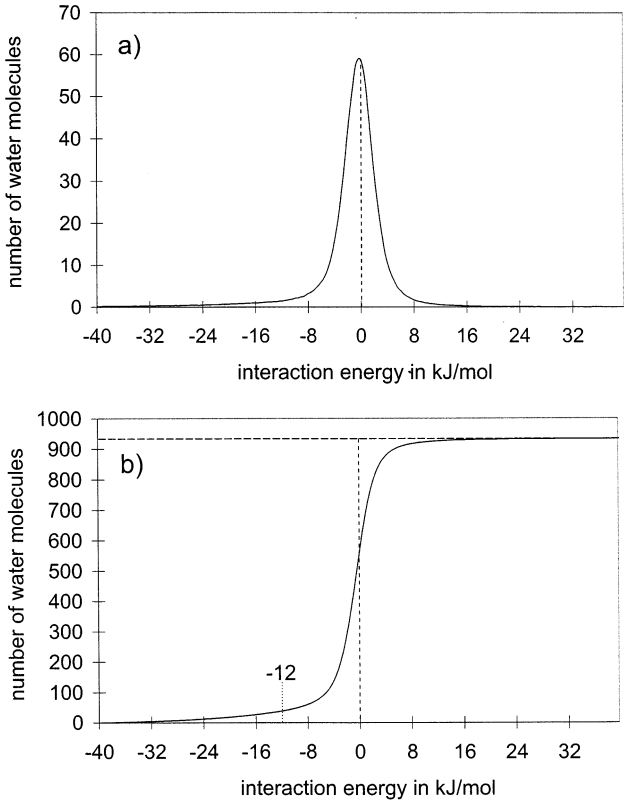

Fig. 5. Distributions of CS4–water interaction energies. The selection criterion used for the definition of bound water is indicated by dotted lines. (a) Relative distribution of CS4–water pair interaction energies. (b) Cumulative distribution of CS4–water pair interaction energies.

-12 kJ mol^{-1} as a hydrogen bond [17], the number of water molecules bound to CS4 can be calculated from the cumulative distribution of the interaction energies (Fig. 5(b)). Using this criterion, about 20 hydrogen bonds per disaccharide unit were estimated.

Further information on the hydration behaviour was obtained by the application of a geometry criterion to find hydrogen bonds between water molecules and CS4. This criterion was defined by the distance between the water oxygen atoms and the hydrogen bond acceptor atoms of CS4, which should be smaller than 3.9 Å, and assuming the distances between the water hydrogen atoms and the CS4 acceptor atoms to be smaller than 2.5 Å [18] (Fig. 6(a)). A proton donor behaviour of CS4 groups was not observed. Using this geometry criterion, the average lifetime of the hydrogen bonds can be estimated. The average number of hydrogen bonds to different atom types of the CS4 molecule and their average lifetimes are illustrated in the Fig. 6(b and c). The lifetimes of the majority of hydrogen bonds were estimated to be in the range of 0.1 and 0.4 ps. In comparison with bulk water (distance of water molecules to all CS4 atoms above 5 Å) with lifetimes of hydrogen bonds around 1 ps, these lifetimes near the CS4 surface are smaller. The relatively short times indicate a high dynamics of hydrogen bond formation and breaking. This behaviour has been described as realistic for systems of small molecules surrounded by a greater number of water molecules [19]. Near the sulfate groups the lifetimes are larger than calculated for bulk water (cf. Fig. 6(c)). For the explanation of this behaviour one has to bear in mind that the sulfate group is much more exposed to the bulk water phase than other groups of the CS4 subunit. Therefore, the lifetimes are more similar to the lifetimes of hydrogen bonds of

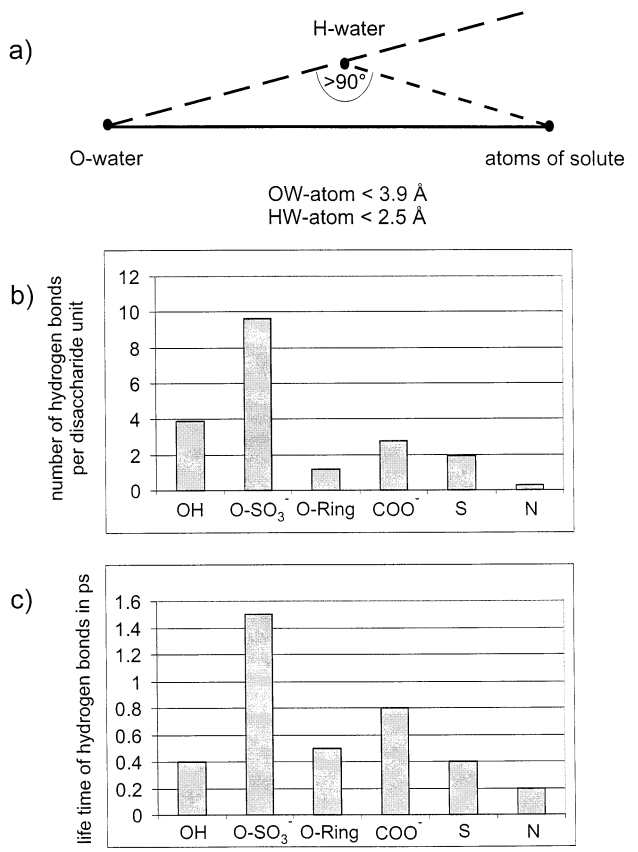

Fig. 6. Average number and lifetime of hydrogen bonds between water molecules and a CS4 disaccharide subunit. (a) Conventions for a geometrical hydrogen bond criterion. (b) Average number of hydrogen bonds between water molecules and the various atoms of a CS4 dimer subunit. (c) Average lifetime of hydrogen bonds close to various atoms of a CS4 dimer subunit.

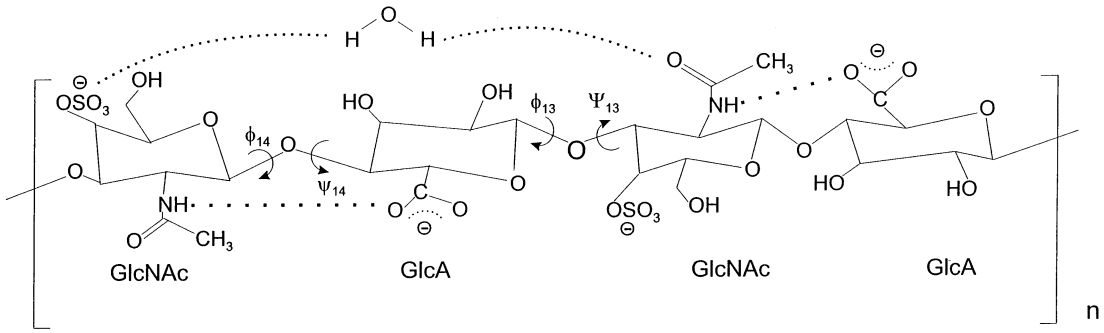

Fig. 7. Water-mediated and intramolecular hydrogen bonds in a CS4 tetramer subunit.

bulk water. It will be discussed later that the formation of water bridges between sulfate groups and *N*-acetyl groups stabilises the structure of the CS4 chain. In these water bridges the lifetime of hydrogen bonds is also increased.

On average, 19.7 hydrogen bonds per dimer unit are formed to the water environment. The comparison with experimental data is difficult because none of the experimental methods provides directly the number of hydrogen bonds. The number of non-freezable water molecules associated with chondroitin sulfate can be estimated using differential scanning calorimetry [20–22]. In good agreement with our calculations, about 20 water molecules per disaccharide were found for high water contents. In any case, the number of water molecules around CS4 is considerably higher than for other carbohydrates, when comparing with values of 8.0, 6.3 and 6.5 per disaccharide unit determined by Kawai et al. [23] for trehalose, sucrose and maltose, respectively. The high hydration capacity of CS4 seems to be of decisive importance for the realisation of its biological function.

Conformation and hydrogen bonding. In a recent study on HA [16], water molecules connecting two neighbouring sugar units by hydrogen bonds were indicated. According to Berendsen [24], water molecules with a higher binding energy compared with a water molecule in the bulk are called 'specifically bound water'. Such water molecules usually have a great importance for the conformation of the solute and also appear in CS4 to a lower extent. The detailed analysis of the CS4–water system shows a new type of interaction, where one water molecule connects non-neighbouring sugar subunits via hydrogen bonds from the sulfate group at the one to the acetamido group of the other sugar residue (Fig. 7). Such hydrogen bonds occur in 50% of all conformations in the trajectory.

Beside these water-mediated hydrogen bonds, intramolecular hydrogen bonds connecting neighbouring sugar units via the NH hydrogens of the acetamido group and the oxygen atoms of the carboxylate group of the other sugar ring were detected. These intramolecular hydrogen bonds are closed in 80% of all conformations within the simulation time.

Reorientation and translational self-diffusion of water molecules.—For the investigation of the microdynamic reorientation behaviour of the water molecules, the time correlation functions $g_1(t)$ and $g_2(t)$ were determined for the rotational motion according to the general expression:

$$g_l(t) = \lim_{t'\to\infty} \frac{1}{t'} \int_0^{t'} P_l(\vec{\mu}(\tau)\cdot\vec{\mu}(t+\tau))\mathrm{d}\tau$$
$$= \langle P_l(\vec{\mu}(0)\cdot\vec{\mu}(t))\rangle \equiv \langle P_l(\cos\theta(t))\rangle.$$

The Legendre polynomials $P_l(x)$ are of the order $l=1$ for $g_1(t)$ and $l=2$ for $g_2(t)$. They describe the average reorientation rate of the molecular dipole direction given at a time t by the unit vector $\vec{\mu}(t)$. The average values are calculated considering all pairs of configurations separated by a time delay t during the simulation of finite length t'. The equation for g_l can be simplified for $l=1$ and $l=2$ to $g_1(t) = \langle\cos\theta\rangle$ and $g_2(t) = \langle(3\cos^2\theta - 1)/2\rangle$, respectively.

The time correlation functions $g_1(t)$ and $g_2(t)$ for bulk water, water close to a CH group and water close to an OH group were

calculated. All water molecules possessing a distance between their oxygens and the atoms of CS4 larger than 5 Å are considered as bulk water; water molecules with distances smaller than 5 Å are referred to as 'structured'. The correlation functions decay to zero when the molecular orientation becomes randomised with respect to the initial value. The correlation function $g_2(t)$ typically decreases more rapidly than $g_1(t)$, as already discussed by Hertz [25]. The initially rapid decrease of the correlation functions during the first 0.05 ps represents the overall molecular oscillation with a loss of phase memory. By exponential fitting of the correlation functions, the correlation times τ_1 and τ_2 for $g_1(t)$ and $g_2(t)$, respectively, are calculated. Their individual values are given in Table 2. The water molecules close to apolar groups, e.g., to a CH group, reorientate more slowly and those nearer to polar groups faster than the bulk water molecules. Since a direct measurement of the rotational correlation time is impossible, comparison with experimental data is difficult. The rotational correlation times τ_1 correspond to measurements of the molecular dipole direction by dielectric relaxation spectroscopy; the correlation times for the reorientation of water molecules determined by proton NMR T_2 relaxation measurements correspond to the rotational correlation times τ_2. In these measurements, the reorientation of the intramolecular proton spin–spin vector fixed in the molecule is detected. Our calculated reorientation correlation times of about 2 ps were compared with estimates from proton NMR relaxation measurements carried out for different polymer solutions by Lüsse and Arnold [26]. In these experiments, reorientation times of 3 ps for free water and 5.6 and 13.9 ps in dextran sulfate solutions of 90 and 60 wt.% water content, respectively, were observed. The correlation times from the MD simulations are smaller than the experimental values, which can be explained by the higher water content of about 96 wt.%. The fast reorientation of the water molecules close to polar groups seems to be possible due to the extension of the hydrogen bond network to the polymer surface. This network is very dynamic, as indicated by the short lifetimes of the hydrogen bonds. In the vicinity of apolar groups, this hydrogen bond network is interrupted resulting in higher correlation times.

Complementary to the rotational correlation functions, the translation diffusion coefficients of water molecules in the vicinity of different groups were finally determined (Table 2). For isotropic translational diffusion, the mean square displacement of a particle is initially square-dependent on time, but this dependence becomes linear at the asymptotic limit. Using Einstein's equation $2tD = (1/3)\langle|\vec{r}_i(t) - \vec{r}_i(0)|\rangle$, the diffusion constants were calculated from the time courses $r_i(t)$. The differences in the correlation times correspond well to those found for the translational diffusion. The diffusion coefficients determined from the MD trajectories depend on the distances of the water molecules to the various atoms of CS4. Water molecules close to CS4 (2–4 Å) show a self-diffusion coefficient of 1.6×10^{-9} m^2 s^{-1}, whereas those in the next water layer possess self-diffusion coefficients of 2.0×10^{-9} m^2 s^{-1}. The diffusion of water is diminished for water near the OH groups as

Table 2
Correlation times τ_1 and τ_2 of the water reorientation and translational self-diffusion coefficients D of water motion for various regions and distances around the CS4 molecule

Range	τ_1 (ps)	τ_2 (ps)	D (10^{-9} m^2 s^{-1}) [a]	D (10^{-9} m^2 s^{-1}) [b]	D (10^{-9} m^2 s^{-1}) [c]	D (10^{-9} m^2 s^{-1}) [d]
Bulk	2.3	1.2				2.3
CH group	4.0	0.8		1.0	1.6	
OH group	3.1	0.5	0.7	1.3	2.0	

[a] For the shell 2–3 Å.
[b] For the shell 3–4 Å.
[c] For the shell 4–5 Å.
[d] Beyond 5 Å.

well as near the CH groups in CS4. The diffusion coefficient of bulk water is 2.3×10^{-9} m^2 s^{-1} in agreement with an experimental value of 2.5×10^{-9} m^2 s^{-1} at 298 K [27]. For the determination of the bulk water diffusion constants we considered all water molecules with their oxygen distances larger than 5 Å to atoms of CS4. These diffusion coefficients can easily be compared with those measured by Knauss et al. [28] in a CS4 solution employing the NMR field gradient technique, which are between 2.6×10^{-10} and 1.7×10^{-9} m^2 s^{-1} when the water content is increased from 20 to 80 wt.%. In particular, the agreement between the calculated and experimental values for the higher water contents is satisfactory. However, it has to be taken into consideration that the observation time of the diffusion process in a typical NMR experiment is in the order of milliseconds compared with nanoseconds in the MD simulation. The molecular motions 'seen' in the MD simulations are in a distance region from a few Å to about 20 Å compared with a few micrometres in PFG-NMR. The most significant effect of the presence of the biopolymer network is to slow down the water mobility. The influence of an increase of the polymer concentration on the decrease of the diffusion coefficient is, therefore, much more pronounced in the NMR experiments.

3. Methods

The definition of the torsion angles around the glycosidic linkages in Fig. 1 follows the recommendation of the IUPAC–IUB Commission of Biochemical Nomenclature [29] with

$\phi^{C}_{13} = \text{O-5}-\text{C-1}-\text{O-1}-\text{C-4}'$,

$\psi^{C}_{13} = \text{C-1}-\text{O-1}-\text{C-4}'-\text{C-5}'$

and

$\phi^{C}_{14} = \text{O-5}-\text{C-1}-\text{O-1}-\text{C-3}'$,

$\psi^{C}_{14} = \text{C-1}-\text{O-1}-\text{C-3}'-\text{C-4}'$.

Relaxed energy maps for the potential surfaces of the glycosidic linkages of the β-(1→4) and β-(1→3) disaccharides were computed in intervals of 4° for the torsion angles ϕ and ψ.

Three different empirical force fields were employed for comparison (CHARMM 24b2 adapted for carbohydrates [30,31], AMBER [37], MM3 [38–40]). Since the results were in good agreement, the MD simulations were only performed on the basis of CHARMM 24b2.

For this purpose, a tetrasaccharide subunit with β-(1→4)-β-(1→3)-β-(1→4) linkages was selected. The geometry of the subunit was built from the data in the Brookhaven Protein Data Bank [10] arising from a fibre diffraction pattern of sodium chondroitin-4-sulfate at 93% relative humidity. The initial structure was reoptimised with charges for the sulfate group recommended by Lamba et al. [32]. The optimised molecule was embedded into a cubic box of 31.4 Å size together with 946 TIP3P water molecules [33,34]. This situation corresponds to 4 wt.% CS4. To compensate the negative charges of the CS4 sulfate groups, four Na^+ ions were placed closely to these groups. After 20 ps of heating to 300 K and 50 ps equilibration, trajectories of 4 ns evolution time were recorded, which provided the data for the structural and thermodynamic analyses. The equations of motion of the system were integrated using a Verlet algorithm [35] with time steps of 1 fs. Periodic boundary conditions were employed and a cut-off distance of 14 Å was applied for the non-bonded and electrostatic interactions [36].

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