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Citation: J. Chem. Phys. 125, 214704 (2006); doi: 10.1063/1.2397681
View online: http://dx.doi.org/10.1063/1.2397681
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Published by the American Institute of Physics.

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Understanding the nonfouling mechanism of surfaces through molecular simulations of sugar-based self-assembled monolayers

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(Received 24 July 2006; accepted 20 October 2006; published online 4 December 2006)

This paper presents a molecular simulation study of the interactions of a protein (lysozyme) with self-assembled monolayers (SAMs) of mannitol and sorbitol terminated alkanethiols in the presence of explicit water molecules and ions. The all-atom simulations were performed to calculate the force generated on the protein as a function of its distance above the SAM surfaces. The structural and dynamic properties of water molecules both above the SAM surfaces and around the SAM head groups were analyzed to provide a better understanding of the nonfouling behavior of the sugar-based SAM surfaces. Results from this work suggest that both mannitol and sorbitol SAMs generate a tightly bound, structured water layer around the SAM chains. This hydration layer creates a repulsive force on the protein when it approaches the surface, resulting in a nonfouling surface despite the presence of hydrogen-bond donor groups. This work demonstrates the importance of strong surface-water interactions for surface resistance to nonspecific protein adsorption.

INTRODUCTION

Surface resistance to nonspecific protein adsorption remains a poorly understood phenomenon and a significant hindrance to the development of biocompatible devices for many applications including medical implants and devices, biosensors, and drug delivery carriers. While there is a great interest in developing new nonfouling materials, the molecular-level mechanism of protein resistance remains poorly understood. Chapman et al. proposed four criteria that must be met for a surface to be nonfouling. The surface must (i) not be hydrophobic, (ii) present hydrogen-bond acceptors, (iii) not contain hydrogen-bond donors, and (iv) be electrically neutral over the surface. These criteria begin to establish the chemical characteristics of a nonfouling surface. There is, however, experimental evidence that these four criteria do not adequately describe the phenomena. For example, both OH-terminated oligo(ethylene glycol) (OEG) self-assembled monolayers (SAMs) and mannitol SAMs (Ref. 6) have been shown to resist nonspecific protein adsorption despite containing hydrogen-bond donors. Furthermore, in the work by Ostuni et al., sorbitol covered surfaces were shown to have high protein adsorption. Unlike mannitol SAM surfaces, sorbitol covered surfaces were formed by chemically derivatizing an interchain carboxylic anhydride group of an alkanethiol pair, in which at most one of every two SAM chains was chemically modified to present sorbitol groups. This difference in nonfouling behavior is hard to explain as mannitol and sorbitol differ only in the chirality of one hydroxyl functional group.

In this work, molecular simulations were performed to study the interactions of a protein (lysozyme) with SAM surfaces presenting either mannitol or sorbitol terminal groups in the presence of explicit water and counter ions. Due to the buried, complex interfaces between proteins and functional surfaces, it is difficult to probe some molecular-level information at the interfaces with current experimental techniques. Molecular simulation techniques are well suited for such studies of the surface-water-protein interfaces and provide a direct comparison between these two stereoiso-mers. The objectives of this work are to study the role of hydrogen-bond donors in the interactions of sugar SAM surfaces with proteins and to compare the difference between mannitol and sorbitol groups for their interactions with proteins. By pairing experimental data with simulation techniques, it may be possible to gain more insight into the mechanisms of protein resistance. It should be pointed out that while some researchers have focused on the influence of the physical characteristics of a surface covered with nonfouling groups to its nonfouling behavior, such as the packing density and molecular flexibility (or chain length) of OEG SAMs, this work strives to understand the chemical characteristics of functional groups that lead to their resistance to nonspecific protein adsorption and to understand the intrinsic nonfouling properties of these functional groups.

SIMULATION MODELS AND METHODS

Simulation models

In this work, SAM surfaces with terminal mannitol and sorbitol groups were used. The mannitol and sorbitol SAMs share the same chemical composition, Si(CH2)7−O−CH2−(CHOH)3. However, mannitol and sorbitol differ in the stereoreactivity of one OH group as shown in Fig. 1. A single chain of each molecule was constructed using the CHARMM program and was energy minimized in vacuum. The minimized single chains were then replicated into a 10 × 10 sur-
folding have been extensively studied by a wide range of techniques. The oxygen atoms have been arbitrarily numbered with the terminal oxygen as O6 and the ether linkage as O1.

The face of SAM molecules. Based on electronic diffraction and scanning tunneling microscopy studies of alkanethiols on Au(111), the sulfur atoms of each SAM were arranged in a \((3 \times 3)R30^\circ\) lattice structure with a sulfur-sulfur spacing of 0.497 nm. Since both mannitol and sorbitol head groups were found to have a molecular area of roughly 25 Å²/chain, the \((3 \times 3)R30^\circ\) structure [21.4 Å²/chain (Refs. 7 and 15)] was used to represent the SAM arrangement. The SAM chains were initially tilted \(\sim 30^\circ\) from the Z axis perpendicular to the surface.

Lysozyme is often used as a model protein for the studies of protein adsorption since its structure, dynamics, and folding have been extensively studied by a wide range of techniques. The x-ray crystal structure of lysozyme, comprised of 129 amino acids, was taken from the Protein Data Bank (entry code 7LYZ). Polar and aromatic hydrogen atoms were explicitly added to the protein. The amino acids such as histidine (HIS), arginine (ARG), and lysine (LYS) were protonated while glutamate (GLU) and aspartate (ASP) were taken as deprotonated; four disulfide bonds were added. The N terminus (NH2) and the C terminus (COO–) were assigned with charges of +1e and −1e, respectively. These assignments create a net charge of +8e on the protein at pH 7. Similar to previous work, lysozyme is used to probe surfaces for their ability to adsorb protein. Results from this work are compared with those of OEG SAMs from previous studies.

The CHARMM22 parameter set, an all-atom force field, was used to represent the protein and the sugar groups. For the SAM chains, the CHARMM2 parameters for an alpha-glucopyranose ring were modified to create a linear sugar structure with appropriate chirality. The main-chain dihedrals were linearized and the chiral centers twisted to generate the correct final structure. However, none of the harmonic constants were affected. Finally, the charge equilibration method, implemented in Insight II (Accelrys, Inc.), was used to calculate the atomic charge distributions of the sugar head groups. The modifications of the CHARMM parameters related to the sugar SAMs are available upon request from the author. Water molecules were treated with a three-point charge model (TIP3P).

The potential energy function used in these simulations includes bond, Urey-Bradley (UB), angle, dihedral, and improper terms, as well as the nonbonded van der Waals (vdw) and Coulombic interactions given by

\[
U = \sum_{\text{bonds}} k_b (b - b_0)^2 + \sum_{\text{UB}} k_{\text{UB}} (r - r_0)^2 + \sum_{\text{angles}} k_\theta (\theta - \theta_0)^2 + \sum_{\text{dihedrals}} k_\phi [1 + \cos(n\phi - \delta)] + \sum_{\text{improper}} k_x (\chi - \chi_0)^2 + \sum_{\text{vdW}} \epsilon_{ij} \left( \frac{R_{\text{min}}}{r_{ij}} \right)^{12} - 2 \left( \frac{R_{\text{min}}}{r_{ij}} \right)^{6} + \sum_{\text{Coulombic}} \frac{q_i q_j}{4 \pi \epsilon_0 r_{ij}}.
\]

The first five terms in the potential energy function describe the bonded, intramolecular interactions, in which \(k_b\), \(k_{\text{UB}}\), \(k_\theta\), \(k_\phi\), and \(k_x\) are the bond, UB, angle, dihedral, and improper force constants and \(b_0\), \(r_0\), \(\theta_0\), \(\delta\), and \(\chi_0\) are the equilibrium values for bond length, UB length, bond angle, dihedral angle, and improper angle, respectively. The last two terms in the potential energy function are the nonbonded, intermolecular, vdw, and Coulombic interactions. The vdw interactions are described with a 12-6 Lennard-Jones (LJ) potential, where \(\epsilon_{ij}\) is the LJ well depth and \(R_{\text{min}}\) is the separation distance at the LJ minimum. The electrostatic interactions are described with a Coulombic potential, where \(q\) is the partial atomic charge and \(r_{ij}\) is the distance between atoms \(i\) and \(j\). All cross LJ terms were calculated using the geometric combination rule of \(\epsilon_{ij} = \sqrt[6]{\epsilon_i \epsilon_j}\) and the arithmetic combination rule for \(R_{\text{min}} = (R_{\text{min}} + R_{\text{min}})/2\), respectively.

**Simulation methodology**

We performed molecular simulations in two steps. First, we conducted a series of Metropolis Monte Carlo (MC) simulations in the NVT canonical ensemble at \(T=300\ \text{K}\) to determine the orientation of lysozyme above the mannitol and sorbitol SAM surfaces. The system included the protein and the surface and used a continuum distance-dependent dielectric medium. The lowest-energy configuration was then used as the starting point for molecular dynamics (MD) simulations in explicit water solvent.

In the MC phase, the lysozyme was manually positioned at several separation distances ranging from 5 to 10 Å above the SAM surfaces with random orientations. During simulations, the lysozyme was moved by either uniform random displacement or rotation around an arbitrary axis with an acceptance rate of roughly 50% based on Metropolis criteria. In all MC simulations, the SAM surface was fixed in the xy plane, water was treated as an implicit solvent continuum.
ties were randomly assigned with a Maxwell-Boltzmann distribution centered at 300 K. The sulfur atoms of each SAM chain were fixed in the xy plane during all MD simulations. The center of mass of the protein molecule was harmonically constrained. Each system utilized periodic boundary conditions applied only in the x and y directions. The velocity Verlet method was used to integrate Newton’s equations. An NVT ensemble was used with a time step of 1.0 fs. The system temperature was kept constant at 300 K using the Berendsen thermostat with a time constant of 0.1 ps. All bonds involving hydrogen atoms were kept rigid using the RATTLE method with a geometric tolerance of 0.0001. The switch function was used to calculate vdw interactions between 0.8 and 1.0 nm. The force-shifting function was used for long-range electrostatic interactions with a cutoff distance of 1.2 nm. The atom-based force-shifting function technique was selected since it was demonstrated before to generate stable nanosecond trajectories for double-stranded DNA.19 The cell-based neighbor list with a cutoff range of 1.32 nm was used to reduce computational time for energy and energy-derivative calculations. The cell-based neighbor list was updated automatically if any atom in the list was moved by more than \((1.32-1.2)/2=0.06\) nm. During simulations, configurations and trajectories were saved approximately every 1.0 ps for 1 ns. Configurations for the final 100 ps were used for the analysis of structural and dynamic properties, including the force exerted on the protein from both water molecules and SAM chains. The six simulation systems varied in size from about 15 000 to 19 000 atoms. All these simulations were performed on a 16-node LINUX cluster Intel x86 (CPU 1.0 GHz) using our BIOSURF program. The program was developed in house as a generalized engine for molecular simulations of biological interfaces.7 Standard simulation techniques20–22 are used in the program.

RESULTS AND DISCUSSION

We first performed MC simulations to determine protein orientation above the SAM surface. MD simulations were then used to study the interactions among protein, water, and mannitol (or sorbitol) SAMs. This work focused on the forces exerted on the protein from the SAM and water and the structural and dynamic behaviors of water molecules near the SAM surface.

Protein orientation

MC simulations were performed first to obtain the optimal protein orientation above the sugar SAM surfaces. Multiple initial orientations were used in MD simulations. The lowest-energy orientation was the same for both surfaces, for which the hydrophilic residues ARG128 and ARG125 were the closest to the surfaces with the V-shaped, biologically active site, oriented away from the surface as shown in Fig. 2. These results are similar to those of the lysozyme orientation over OEG SAM surfaces.7 Even though the types of residues close to the surfaces are generally the same, the exact groups are slightly different. The lysozyme is used as a probe in these simulations; thus the orientation should be kept similar from simulation to simulation to allow comparison between the SAM surfaces investigated. Since the resi-

model, and the protein was modeled as a rigid molecule. Simulations were run for 50 000 MC steps and only the non-bonded vdw and Coulombic interactions between the SAM and the protein were considered. The final protein orientation with the lowest energy was taken as the initial state for future MD simulations.

Once the optimal lysozyme orientation was determined, three systems with lysozyme placed above a mannitol SAM surface at separation distances, defined by the minimum separation distance between any atom of the protein and any atom of the SAM, of 5, 10, and 20 Å and three similar systems over a sorbitol SAM surface were constructed. These systems, including the SAM terminal groups, were solvated with preequilibrated TIP3P water molecules. Counterions, one sodium and nine chlorines, were added to neutralize the total charge of the system. The amount of water was adjusted so that the ion concentrations of the systems corresponded to those under physiological conditions. Subsequently, any water molecule within 3.0 Å of the protein or SAM was removed. The whole system, including protein, water, ions, and SAM was initially energy minimized for 10 000 steps using the conjugate gradient algorithm. Following the minimization, the systems were heated to 300 K using a short MD run of 10 000 1.0 fs steps and 30 K increments; the protein backbone and SAM molecules were harmonically constrained to their initial positions. The heating process allows for the initial relaxation of the SAM surface as well as starting the hydration of both the protein and the SAM chains. After heating, additional 3000 1.0 fs MD steps were run to allow further isothermal equilibration. All initial construction, heating, and equilibration were conducted with the CHARMM program. The final frame of the equilibrium steps was used as the initial configuration for 1 ns of MD simulation. Figure 2 is representative of all six cases.

While the initial coordinates were taken from the completed heating and equilibrium simulations, the initial velocities were randomly assigned with a Maxwell-Boltzmann distribution centered at 300 K. The sulfur atoms of each SAM chain were fixed in the xy plane during all MD simulations. The center of mass of the protein molecule was harmonically constrained. Each system utilized periodic boundary conditions applied only in the x and y directions. The velocity Verlet method was used to integrate Newton’s equations. An NVT ensemble was used with a time step of 1.0 fs. The system temperature was kept constant at 300 K using the Berendsen thermostat with a time constant of 0.1 ps. All bonds involving hydrogen atoms were kept rigid using the RATTLE method with a geometric tolerance of 0.0001. The switch function was used to calculate vdw interactions between 0.8 and 1.0 nm. The force-shifting function was used for long-range electrostatic interactions with a cutoff distance of 1.2 nm. The atom-based force-shifting function technique was selected since it was demonstrated before to generate stable nanosecond trajectories for double-stranded DNA.19 The cell-based neighbor list with a cutoff range of 1.32 nm was used to reduce computational time for energy and energy-derivative calculations. The cell-based neighbor list was updated automatically if any atom in the list was moved by more than \((1.32-1.2)/2=0.06\) nm. During simulations, configurations and trajectories were saved approximately every 1.0 ps for 1 ns. Configurations for the final 100 ps were used for the analysis of structural and dynamic properties, including the force exerted on the protein from both water molecules and SAM chains. The six simulation systems varied in size from about 15 000 to 19 000 atoms. All these simulations were performed on a 16-node LINUX cluster Intel x86 (CPU 1.0 GHz) using our BIOSURF program. The program was developed in house as a generalized engine for molecular simulations of biological interfaces.7 Standard simulation techniques20–22 are used in the program.

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due types of lysozyme closest to the sugar surfaces are similar to those to the OEG surface, it is reasonable to compare results from this work with those from previous simulations of OEG SAM surfaces.

**Force on protein**

The force exerted on the protein normal to the SAM surface provides a clear distinction between the surfaces that resist protein adsorption and those that promote protein adsorption. If the force on the protein is repulsive, the surface will be nonfouling; if the force is attractive, the surface will promote protein adsorption. Figure 3 plots the force on the protein normal to the SAM surface where a positive force will repel the protein. Results show that both the mannitol and sorbitol systems generate repulsive forces on the protein. In this work, the total force calculated is the sum of the forces from the SAM surface and the water. It is interesting to note that the SAM surfaces themselves generate negligible forces on the protein. The total force is mainly attributed to the water layer near the surface. Based on the fact that significant contributions to the repulsive force on the protein come from the water layer, the structural and dynamic properties of the water layer were analyzed in order to determine the source of this repulsive force. This result is quite similar to previous work by Zheng et al. for OEG SAMs. The repulsive force of 0.9 nN obtained for both the mannitol and sorbitol SAM surfaces lies in the same range.

**Water structure and dynamics**

It is generally accepted that the ability of a surface to resist nonspecific protein adsorption is strongly tied to its ability to bind water. In this work, four key structural and dynamic properties of water were investigated: water hydrogen bonding around SAM chains, the radial distribution function of water with respect to specific SAM atoms, water residence time in layers above the SAM surface, and the water self-diffusion coefficient. In every case, the water properties were not influenced by protein-SAM separation distance. Thus, all of the data presented below come from the simulation of lysozyme at 5 Å above the SAM surface.

<table>
<thead>
<tr>
<th>Atom</th>
<th>Ave. No. of H bonds</th>
<th>Atom</th>
<th>Ave. No. of H bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>0.00</td>
<td>O1</td>
<td>0.00</td>
</tr>
<tr>
<td>O2</td>
<td>0.00</td>
<td>O2</td>
<td>0.00</td>
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<tr>
<td>O3</td>
<td>0.00</td>
<td>O3</td>
<td>0.00</td>
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<tr>
<td>O4</td>
<td>0.01</td>
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<tr>
<td>O5</td>
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<td>O5</td>
<td>0.30</td>
</tr>
<tr>
<td>O6</td>
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<td>O6</td>
<td>2.03</td>
</tr>
<tr>
<td>Total</td>
<td>2.43</td>
<td>Total</td>
<td>2.33</td>
</tr>
</tbody>
</table>

Ave./oxygen 0.41 Ave./oxygen 0.39

**Hydrogen bonding**

In this work, hydrogen bonding was defined using a geometric description with a donor-acceptor distance less than 0.35 nm and a donor-hydrogen-acceptor angle greater than 120°. As our system included multiple components capable of hydrogen bonding, two specific interactions were investigated. First, hydrogen bonding between the SAM surface and the water was examined. Here the surface was treated as both a hydrogen bond acceptor and donor. Second, water-water hydrogen bonding in the layer immediately above the SAM surface was investigated, as the number of hydrogen bonds per water molecule can help quantify water-water interactions. Water-water hydrogen bonding per water molecule is also a measure of water structure. Table I contains the average number of hydrogen bonds between the hydrating water and the individual oxygen atoms in each SAM chain. Figure 4 shows the number of water-water hydrogen bonds per water molecule in 4 Å layers throughout the structure along the z axis.

It is clear from Table I and Fig. 4 that the overall hydrogen bonding behavior is very similar for both mannitol and sorbitol surfaces. Both SAM systems bind and order nearly the same amount of water. Using the TIP3P water model, bulk water-water hydrogen bonds per water molecule have been reported to be roughly 3.5, which was also shown here. In both sugar SAM systems, water-water hydrogen bonding is lower in the first 4 Å layer above the SAM due to

![FIG. 3. Total force acting on the protein normal to the SAM surface. Results for an OEG₄ and OH-terminated SAMs from Chang et al. (Ref. 29) are shown for comparison.](image-url)

![FIG. 4. Number of water-water hydrogen bonds per water molecule in 4 Å layers through the structure. The SAM top is defined by the average z coordinate of the terminal oxygen atom (O6).](image-url)
water-SAM interactions. However, the second 4 Å water layer contains more water-water interactions, closer to 4.0 hydrogen bonds per water molecule. This is similar to that found in ice,\textsuperscript{24} suggesting a strong SAM-water interaction that leads to multilayer water structuring.

The only difference between the two systems is the hydration distribution throughout the SAM head group. Results show that the sorbitol head group binds more water molecules through interactions with the terminal OH group (O6) and less water molecules through interactions with the second OH group from the top (O5). Since the third OH group from the top (O4) is the functional group with different chiralities between mannitol and sorbitol, this OH group may cause the differences in the hydration distribution in the first two OH groups from the top. However, the overall numbers of SAM-water hydrogen bonds per SAM molecule are similar, 2.43 for mannitol and 2.33 for sorbitol. The number of hydrogen bonds per SAM chain in this work is comparable to that for nonfouling (OEG)\textsubscript{4} SAMs reported as 2.5.\textsuperscript{7} In addition, while a few water molecules form hydrogen bonds with the third OH group from the top of the chain on the mannitol surface, water molecules only interact with the top two OH groups on the sorbitol surface, despite the initial presence of water molecules throughout the SAM head groups.

**Water radial distribution function**

The radial distribution function (RDF) or $g(r)$ can provide insight into the local water structure near the SAM surface.\textsuperscript{26} The RDF of water around the terminal OH group (O6) is presented in Fig. 5(a), while the water RDF around the chiral OH group (O4) is shown in Fig. 5(b). Since SAM or protein atoms can be found in the spherical shell in RDF calculations, $(Vr)$ is modified by subtracting the volume of any nonwater atoms found in the shell. This creates more accurate RDF data as it will approach the bulk value of 1 without artificial scaling. It can be seen in Fig. 5(a) that once again, there are no significant differences between the mannitol and sorbitol surfaces. Both RDF plots show a strong first peak at 0.29 nm. This was also seen for OEG-terminated SAMs in work done by Zheng et al.\textsuperscript{7} and Tasaki.\textsuperscript{25} The strong first peak is indicative of a tightly bound layer of nearest neighbor water molecules. The RDF data also mirror the hydrogen bonding data in that the sorbitol surface appears to have more water bound to the terminal OH groups (O6) as shown by the taller first peak. The RDF plots also demonstrate potential water structuring beyond the nearest shell. Unlike the bulk water RDF shown in Fig. 5(b), both the mannitol and sorbitol SAM surfaces create water structure, as seen as peaks in the RDF curve, well into the water layers above the surface within 1 ns of simulations. The RDFs for water around the chirally different OH group (O4) in Fig. 5(b) are different but show similarities between the mannitol and sorbitol SAM surfaces.

**Self-diffusion coefficient**

The water self-diffusion coefficient $D$ was calculated in 4 Å layers above the SAM surfaces. Self-diffusion coefficients provide insight into the water dynamics and mobility near the surfaces.\textsuperscript{30} This mobility near the SAM surface helps quantify the affinity between the SAM chains and water molecules. A low self-diffusion coefficient indicates that water molecules are tightly bound. The self-diffusion coefficient is determined from the slope of the mean squared displacement (MSD) versus time via the Einstein relationship.\textsuperscript{20} In this work, the bulk TIP3P water self-diffusion coefficient was calculated in 4 Å layers through a bulk water box and was found to be $5.1 \times 10^{-5}$ cm$^2$/s which compares well with other published values of $5.06 \times 10^{-5}$ cm$^2$/s.\textsuperscript{23,26} In the water layers directly above the mannitol and sorbitol SAM surfaces, water self-diffusion coefficients are $4.0 \times 10^{-5}$ and $4.2 \times 10^{-5}$ cm$^2$/s, respectively. Again, the difference between the two surfaces is minimal. Results show that both surfaces create decreased mobility, tightly bound water layers directly above the SAM chains, suggesting the presence of strong water-surface interactions as also seen by the other properties.

**Water residence time**

One additional measure of water affinity to a surface is the fraction of water that remains in a layer of specified thickness over a time interval. This residence time is evaluated from the survival time correlation function $C_R(t)$. The $C_R(t)$ functional expression is shown below,

$$C_R(t) = \frac{1}{N_W} \sum_{i=1}^{N_W} \frac{\langle P_{Rj}(0)P_{Rj}(t) \rangle}{\langle P_{Rj}(0) \rangle^2},$$

where $P_{Rj}$ is a binary function that equals 1 if the $j$th water molecule resides in a layer of thickness $R$ for time $t$ without leaving the layer evaluated for all water molecules $N_W$ that begin in that layer.\textsuperscript{28} Figure 6 shows survival time correlation functions plotted over time for both mannitol and sorbitol surfaces.
SAM systems as well as for bulk water for comparison.

Like before, water properties were investigated in the first 4 Å above the SAM surface. By fitting an exponential decay function to the $C_R(t)$ curve, it is possible to calculate a mean residence time of water molecules in the layer as follows:

$$C_R(t) = Ae^{-t/\tau_s},$$

where $\tau_s$ is the mean residence time. After fitting the data, the mean residence time of the water above the SAM surface was calculated to be 10.5 ps for the mannitol system, 13.5 ps for sorbitol, and 4.3 ps for bulk TIP3 water. The survival time correlation function for bulk water compares well to other published data, such as the one reported by Dastidar and Mukhopadhyay. Clearly, water survival time correlation function is dependent on layer thickness; thus only relative comparisons are valid. For a given analysis in which the layer thickness is constant, the mean residence time is representative of the interaction strength between the water and the hydrated surface. These data suggest that water has a stronger interaction with both SAMs than it does with itself. Furthermore, the sorbitol SAM may interact more strongly with water immediately above the surface than the mannitol SAM, as indicated by the longer mean residence time of water above the sorbitol surface and also supported by the larger number of hydrogen bonds formed between the terminal OH group and water in the sorbitol system.

DISCUSSION

It is generally accepted that the nonfouling behavior of a surface is linked to its strong interactions with water molecules. Our simulation results show a strong repulsive force as a protein approaches the mannitol or sorbitol surface. The force mainly comes from the bound water layer near the SAM surface but not from the SAM surface itself. Our simulation results also show tightly bound water molecules around and above the mannitol or sorbitol chains. This is evidenced by increased water-water hydrogen bonding, decreased self-diffusion coefficients, and longer mean residence times than bulk water. This structured water layer forms a physical barrier to protein penetration. In this work, hydrogen bonding between the water and SAM chains is observed within the chains, suggesting water penetration. However, hydrogen bonds were not observed with all of the OH groups along the SAM chain. It is speculated that slightly decreasing the SAM packing density might open more OH groups to hydrogen bonding, thereby increasing the number of water-surface hydrogen bonds and the degree of the hydration of the SAM surface and the subsequent protein resistance. Previously, it was observed that there was an optimal surface packing density between 60%–80% for the effective resistance of OEG SAMs to protein adsorption.

Despite the concerns that hydrogen-bond donor groups could promote nonspecific protein adsorption and the experimental observation that the mannitol and sorbitol SAM surfaces behave differently, it appears from our simulations that both mannitol and sorbitol SAMs should resist nonspecific protein adsorption. The resulting forces exerted on a protein and water properties near the surfaces are nearly indistinguishable between the two sugar SAM cases. While experiments are under way to verify these simulation results, these results are not unexpected. Mannitol and sorbitol only differ in the OH orientation at one carbon that lies relatively deep within the SAM surface. Therefore, we do not expect a significant difference in their behavior. One possible explanation for the difference in experimental results for mannitol and sorbitol systems possibly lies in the chain packing density. In the mannitol experiments, pure mannitol SAMs were formed. However, in the sorbitol experiments, at most one of every two SAM chains was functionalized with a sorbitol group, leading to a surface with lower sorbitol coverage. This decrease in surface coverage could be responsible for the protein adsorption observed in the experiments. Based on the simulation results, it is predicted that SAM surfaces that are homogeneously functionalized with either mannitol or sorbitol terminal groups should resist nonspecific protein adsorption despite of the presence of hydrogen-bond donor groups.

CONCLUSIONS

In this work, restrained molecular simulations were used to probe the interactions among protein, water, and sugar-based SAM surfaces presenting mannitol or sorbitol terminal groups. Our simulations show a strong repulsive force acting on the protein as it approaches the SAM surface in both the mannitol and sorbitol systems, despite the presence of hydrogen-bond donor groups. These calculations also depict a tightly bound, structured water layer directly above both SAM surfaces. It is proposed that this layer generates strong repulsive forces on the protein above the SAM. Thus, despite containing hydrogen-bond donor OH groups and some experimental data to the contrary, these results suggest that homogeneous SAMs functionalized with either mannitol or sorbitol terminal groups should be resistant to nonspecific protein adsorption. These results suggest that the ability of a surface to resist nonspecific protein adsorption is directly linked to its ability to strongly interact with water molecules.

ACKNOWLEDGMENTS

We would like to thank the National Science Foundation for financial support under CMS-0225622 and CTS-0433753. We also acknowledge helpful discussions with Dr. Jie Zheng, Dr. Shengfu Chen, and Professor Wen-Yih Chen.
39 Y. Chang, personal communication.