Structural Characterization of Self-Assembled Monolayers of Neoglycoconjugates Using Atomic Force Microscopy

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Thiolated self-assembled monolayers of carbohydrates may serve as useful polyvalent tools to mimic the organized presentation of such molecules at the cell surface. SAMs presenting the disaccharide maltose as a neoglycoconjugate were produced, and the structure was studied by high resolution atomic force microscopy. The molecules form highly ordered structures on a gold (111) surface, with lattice parameters determined by the linker moiety rather than the headgroup.

Introduction

Carbohydrates play a key role in many normal and pathogenic processes such as embryogenesis, inflammation, tumor progression, metastasis, and so forth.[1] The interaction of these carbohydrates with their corresponding receptors is calcium-dependent, highly specific, and very weak.[2] This weak affinity is overcome in nature by a multivalent presentation of the carbohydrate conjugates (glycoproteins and glycolipids) at the cell surface, increasing the binding affinities of these antigens.[3] Glycoproteins present several copies of carbohydrate antigens along the protein backbone, while the multivalence of carbohydrates in glycolipids is reached by the aggregation of these molecules in islands known as glycolipid rafts.[4] To study and intervene in biological processes where carbohydrates are involved requires having accessible carbohydrate-based model systems providing the necessary multivalency.

Self-assembled monolayers (SAMS) on gold that present biological molecules at the interface have been shown to be useful multivalent systems for studying biological recognition.[5,6]

To unravel and understand the role of these carbohydrate antigens in biological processes we have prepared SAMs of neoglycoconjugates mimicking the presentation of glycolipids at the cell membrane. With these two-dimensional model systems we have determined the adhesion forces[7] and the binding affinities[8] between carbohydrate molecules.

The neoglycoconjugates used have a chain with a terminal thiol or thiolate to be covalently linked to a gold surface. The use of an aliphatic carbon chain as a linker leads to a well-packed SAM on the gold surface. This fact has been proved for several SAMs of alkanethiols.[9−13] Despite the increasing interest in biofunctional surfaces for biomedical and biotechnological applications,[14−16] and although a number of reports of formation of SAMs presenting oligosaccharides have been published,[8,17,18] these have almost all been concerned with the reactivity or biological activity of such monolayers. To our best knowledge, only one previous example[19] of neoglycoconjugate SAMs has been described, and it was characterized by reflection absorption infrared spectroscopy. This work showed evidence for intermolecular hydrogen bonding in such films, suggesting a densely packed arrangement of the molecules, which appeared to be oriented mostly perpendicular to the substrate surface, but no structural data on the geometry of packing were reported.

One of the techniques used to obtain structural information of surfaces at atomic resolution is scanning probe microscopy. Low-current scanning tunneling microscopy (STM) has been successfully used to obtain molecular resolution structural information from SAMs on gold surfaces, but when these SAMs are constituted by relatively long, isolating molecules as is the case of these neoglycoconjugates, atomic force microscopy (AFM) is a

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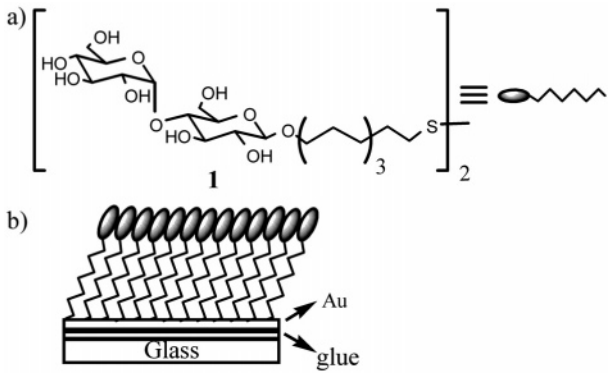

Figure 1. Chemical structure of neoglycoconjugate **1** (a) and schematic of the SAM formation (b).

more suitable technique.[20] In this letter, we present the structural information of a SAM generated using 11,11′-dithiobis[undecanyl(α-D-glucopyranosyl)(1→4)-β-D-glucopyranoside] (**1**) as a neoglycoconjugate on a very flat gold surface. The results obtained in this study indicate that these molecules present a well-defined order despite the presence of the relatively bulky disaccharide substituent, as was found in the case of other SAMs using different headgroups but the same type of linker.[21]

Experimental Section

The neoglycoconjugate 11,11′-dithiobis[undecanyl(α-D-glucopyranosyl)(1→4)-β-D-gluco-pyranoside] (**1**; see Figure 1a) was synthesized as described previously.[22] Briefly, the perbenzoylated disaccharide maltose was deprotected on its anomeric position and the corresponding trichloroacetimidate derivative was prepared. Then, glycosylation of this maltose derivative with the corresponding linker, prepared from commercially available 11-bromoundecanol, was performed using trimethylsilyl triflate as the promoter. Finally, the hydroxyl groups were deprotected in basic media to give neoglycoconjugate **1** in good yield.

Atomically flat gold surfaces were prepared by a variation on the template-stripped gold (TSG) method of Hegner et al.[23] The 150-nm-thick gold layers were deposited by molecular beam epitaxy on freshly cleaved ruby muscovite mica sheets. The mica sheets (8 mm × 8 mm) were glued gold face down onto small pieces of microscope glass. The mica was then mechanically stripped using a razor blade, thus, revealing a very flat (111) gold surface. To avoid surface contamination before SAM formation, the gold surfaces were immediately immersed in an aqueous 1 mM neoglycoconjugate solution to generate the SAM. After 2 h of immersion, the surfaces were rinsed with ultrapure water and blown dry with nitrogen. A schematic of this SAM on a gold surface is represented in Figure 1b.

Surface observations were conducted with an atomic force microscope rather than a scanning tunneling microscope because of the high length of the molecules resulting in a low conductivity. A multimode atomic force microscope from Veeco was used in contact mode in air. Silicon cantilevers with integrated silicon tips with spring constants ranging from 0.07 to 0.4 $N \cdot m^{-1}$ were employed. Images were acquired either in constant height mode (deflection mode) or in friction force mode, with repulsive forces of the order of 5 nN. Calibration of the piezo was confirmed by measurement of the lattice constant on mica and highly ordered pyrolitic graphite (with a STM head). Fast Fourier transform and autocorrelation were performed on all pictures to reveal and measure the periodic arrangement of molecules.

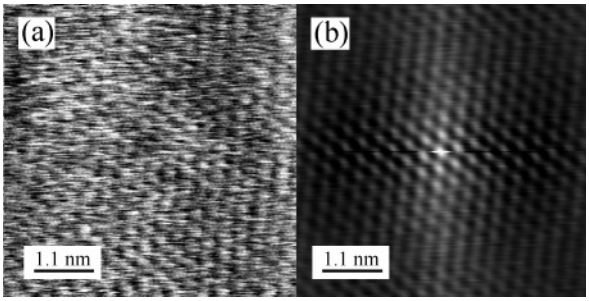

Figure 2. AFM constant height mode image (unfiltered) of the gold surface prepared by the TSG method (a) and corresponding autocorrelation function (b).

The XPS measurements were performed on a CAMECA-RIBER apparatus, with a MAC2 analyzer. A Mg anode was used as source operating. For each sample, a survey scan was performed, followed by a narrow scan of the C(1s) region. The binding energies are lined up with respect to the Au(4f) peak at 84.0 eV.

Results and Discussion

The structure of the SAM may be determined by STM only if an atomically flat surface was used as the substrate. Therefore, gold surfaces have been prepared according to the TSG method (see experimental section) and then observed by AFM to ensure that atomically flat (111) surfaces had really been obtained. Figure 2a shows a representative 5.3 × 5.3 nm^2 AFM image of a TSG surface. The typical hexagonal atomic lattice of the (111) gold surface can be distinguished on this image. The autocorrelation image presented in Figure 2b emphasizes this hexagonal lattice and also reveals a nonlinear drift along the slow scan axis (vertical in Figure 2) leading to a small deformation of the hexagonal cell. This also caused the spots to become less clear at the top and bottom of the autocorrelation image. This shows good short-range but poor long-range ordering in the vertical direction, due to the drift. A 0.30-nm lattice parameter has been measured, in good agreement with the theoretical one (measurements were made in directions close to the fast scan direction, which is much less prone to such distortion).

Once the flat gold surface was obtained, a SAM of neoglycoconjugate **1** was formed. For this aim, the gold surface was immersed into an aqueous 1 mM solution of the corresponding neoglycoconjugate for at least 2 h and the surface was cleaned by washing with pure water and dried.

Then, X-ray photoelectron spectroscopy (XPS) has been carried out for chemical analysis of both pure TSG and SAM surfaces. Figure 3a,b presents survey spectra recorded from 0 to 800 eV for pure gold and the SAM respectively, with an inset of the narrow scan in the C(1s) region. The characteristic Au(4f), Au(4d), and Au(4p) binding energy peaks dominate in both spectra. The presence of O(1s), N(1s), and C(1s) on the pure gold sample (Figure 3a) indicates surface contamination. However, on Figure 3b, several features reveal the presence of the neoglycoconjugate molecules on the surface. First, in the case of the SAM, a narrow scan shows that the C(1s) peak presents a shoulder that was not observed on the gold spectrum. This C(1s) peak can be deconvoluted into two peaks near 285 and 288 eV. They correspond respectively to the energy shift of $-CH_2-$ and $-CH_2-O-$ carbon groups that are present in the neoglycoconjugate molecules. Second, a strong attenuation of the substrate photoelectrons is observed in the SAM sample, which is consistent with the presence of an organic layer on the gold surface.

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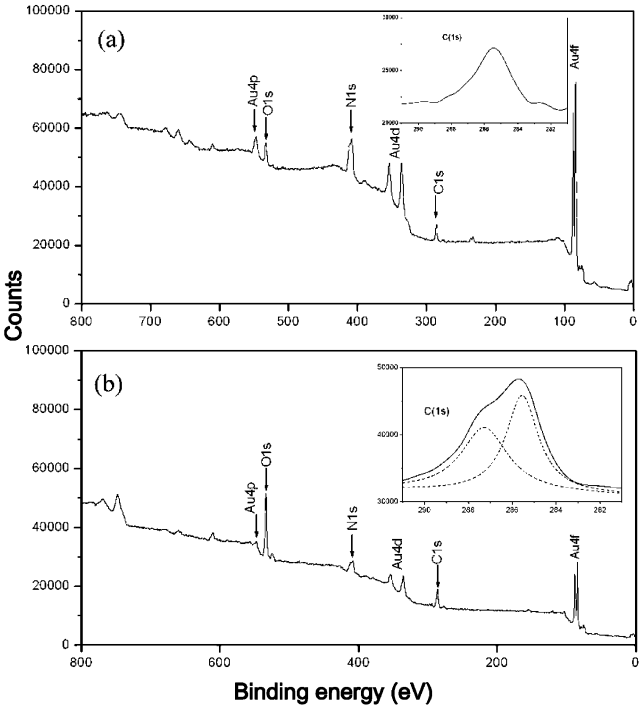

Figure 3. XPS survey spectra of respectively gold (a) and the SAM of neoglycoconjugate **1** (b). The insets correspond to a narrow scan in the C(1s) region.

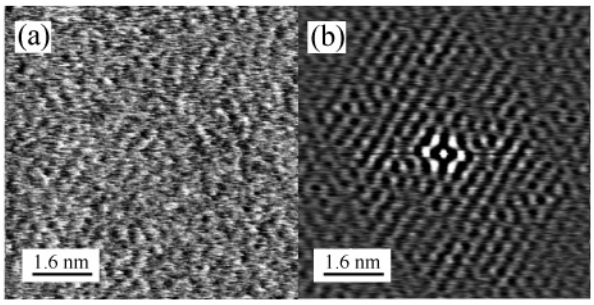

Figure 4. AFM image of the SAM of neoglycoconjugate **1** (a) and corresponding autocorrelation function (b).

These features were not found in the gold sample. Also, XPS spectra do not reveal any evidence of remaining mica neither on the gold surface nor on the SAM. Therefore, the analysis by XPS clearly demonstrated the presence of the neoglycoconjugate molecules on the gold surface for the functionalized sample.

Having assessed the quality of the substrate and the presence of the neoglycoconjugate molecules on the surface, the next step was to analyze the SAM structure at the molecular level, looking for periodic organization. For this aim, we have used AFM to observe the surface structure. Figure 4a presents an 8×8 nm^2 image of the SAM. To verify the integrity of the film upon scanning with the atomic force microscope tip, zooming out has been regularly perfomed, and no modification has been observed on the previously scanned area. The high level of noise observed on the image can be attributed to the length of the molecules (3 nm). Though strongly attached to the surface through the covalent S−Au bond, they may deform and rotate around the sulfur bond under the force exerted by the atomic force microscope tip. This effect may have been reinforced by capillary forces between the tip and the surface, as the images were performed in air.

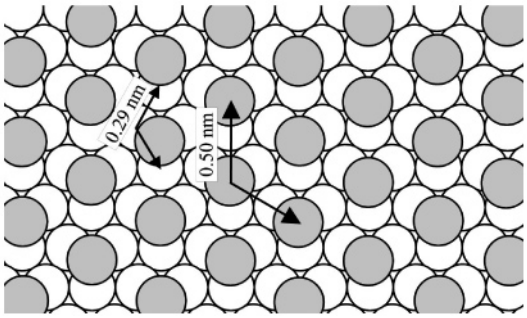

Figure 5. Schematic of the $\sqrt{3} \times \sqrt{3}R30°$ sulfur superstructure on the (111) gold surface. Filled circles correspond to sulfur atoms.

However, a periodic structure may be distinguished on the image. To investigate this structure, the autocorrelation function has been calculated. The autocorrelation function presented on Figure 4b emphasized the long-range periodicity of the AFM image, thus, revealing that molecules are organized in a hexagonal array, with a 0.49 ± 0.05 nm spacing between nearest neighbors.

The hexagon is deformed due to drift in the slow scan axis direction. This deformation was reproducible for different scan angles. The lattice parameter measured for the SAM is higher than that of gold and so ensures that, even for high tip−surface forces, the periodic contrast is due to attached molecules and not to gold atoms of the substrate. This value is consistent with the 0.497-nm nearest neighbor distance of a superstructure which is a well-known structure for alkanethiolate monolayers on (111) gold surfaces (Figure 5).

Conclusions

In summary, neoglycoconjugate **1** has been deposited on an atomically flat (111) gold surface to form a SAM. Chemical analysis performed by XPS demonstrated the presence of the organic molecules on the gold substrate. AFM study of the surface showed a long-range periodic organization of the molecules, according to a hexagonal lattice. The 0.49 ± 0.05 nm nearest neighbor distance was found to be consistent with a $\sqrt{3} \times \sqrt{3}R30°$ superstructure, often observed for other alkanethiolate SAMs on (111) gold. Therefore, the present study demonstrates that the presence of a carbohydrate head at the end of an alkanethiol does not prevent the well-established formation of an alkanethiol SAM. Furthermore, carbohydrates are specific ligands of a great group of proteins called lectins. However, the hydrophilic nature of these biomolecules avoids the nonspecific binding of other proteins. This result, through a better understanding of the molecule organization in the SAMs of neoglycoconjugates, may be useful for further studies involving this type of system, which may be of great utility as a model for the multivalent presentation of such carbohydrate epitopes present on cell surfaces in nature.

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