

CHARACTERIZATION OF SUPRAMOLECULAR ASSEMBLIES OF MALTOSE NEOGLYCOCONJUGATES AND GLYCONANOPARTICLES BY NON-CONTACT AFM

M. Fuss, M. Luna, D. Alcántara, J.M. de la Fuente, F. Briones and S. Penadés

Introduction

Since the field of Glyconanotechnology was introduced by our group in 2001, different kinds of nanostructures have been reported ranging from gold nanoparticles to self-assembled monolayers. These innovative nanostructured biomaterials have demonstrated very interesting biological and biomedical applications. As an example, we previously reported the preparation of water soluble Au-Fe_xO_y nanoparticles functionalized with a maltose neoglycoconjugate. In this work, we have been able to obtain information about the organic coverage of these gold nanoparticles by means of non-contact dynamic AFM. This method, based on long-range attractive interactions between tip and sample, is particularly indicated for compliant materials since there is no damage to the sample due to the impact or friction.

Methods

For AFM analysis, 10 μl of a solution of maltose-protected AuFe nanoparticles or neoglycoconjugate (70 μg/ml) was deposited on freshly cleaved graphite. These samples were then left to dry at about 6°C during 24 hours, allowing a slow evaporation of the water. AFM imaging took place at normal ambient conditions (~30% RH), except for the study at varying RH.

Images shown were obtained using a commercial AFM operated in non-contact amplitude modulation mode. The cantilevers had a nominal force constant of 0.7-2 N/m and a resonance frequency of about 71 kHz. Small peak-to-peak oscillation amplitudes (~5 nm) were used during imaging.

Results and Discussion

In a previous paper, our group reported the synthesis of Au-Fe_xO_y nanoparticles functionalized with biologically relevant carbohydrates covalently linked to the inorganic cluster. The so prepared glyconanoparticles have less than 2 nm diameter core and formed stable dispersions over several months. TEM micrographs show very small Au-Fe_xO_y nanoparticles perfectly aligned and encapsulated in what seems organic material (fig. 1). The existence of glyconanoparticle aggregates with an organic covering on a Au substrate was confirmed by non-contact AFM. Micelle formation in water is probably caused by the amphiphilic nature of the neoglycoconjugate, containing a hydrophilic head (sugar) and a lipophilic spacer (alkane chain).

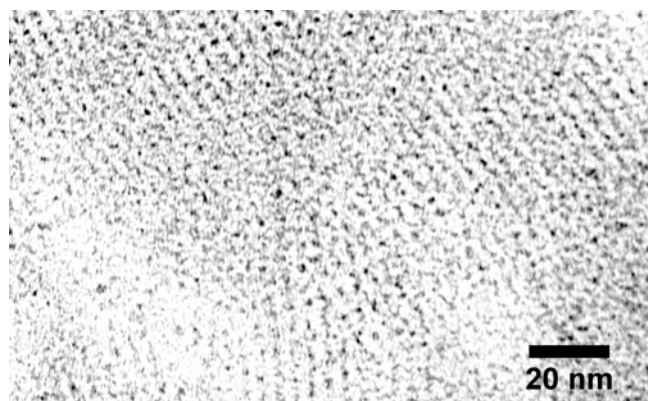


Fig. 1: TEM image showing maltose-protected gold nanoparticles perfectly aligned. The neoglycoconjugate forms micelles with a tubular shape and gold cores are found encapsulated in the organic material.

In the AFM characterization carried out on highly-oriented pyrolytic graphite, we found that maltose glyconanoparticle aggregates were no longer present. Instead, multilayers of organic material of reproducible height were observed. From profiles like the one shown in figure 2, a step height of 4.2nm was determined. Since the same structure and height was found in a control sample prepared with a solution of the neoglycoconjugate without metallic nanocores, the multilayers are believed to originate from excess maltoC₁₁SH molecules. In figure 3, a model of the multilayer formation is proposed.

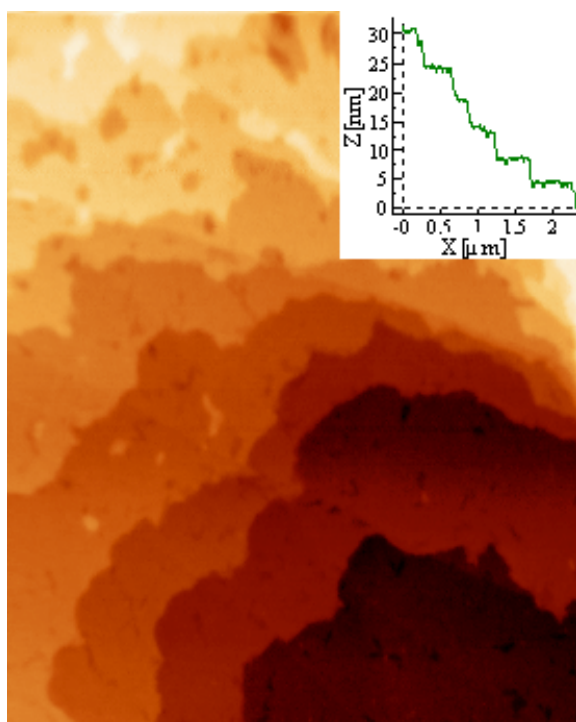


Fig. 2: In non-contact AFM mode, more than ten layers of (maltoC₁₁S-)₂ molecules were formed on graphite (2.4 x 3 μm² AFM image) from the solution containing the glyconanoparticles. The inset shows a profile through eight layers. The height of the graphite terrace visible was used for z-calibration.

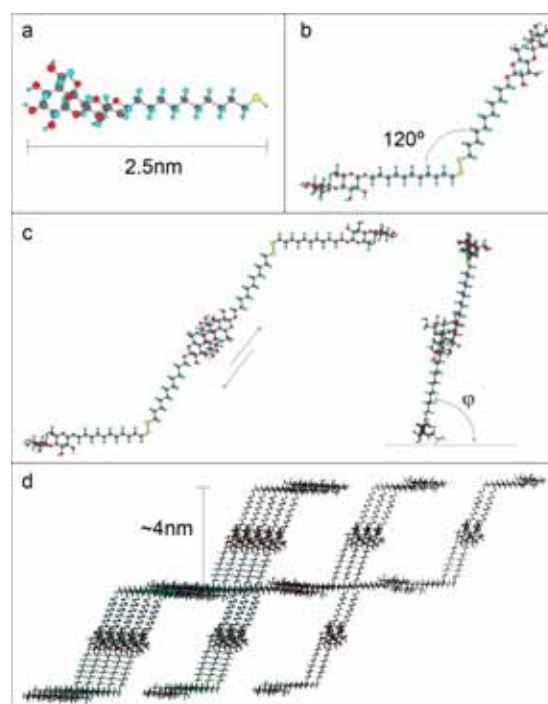


Fig. 3: Multilayer formation on graphite: (a) Excess maltoC₁₁SH molecule in aqueous solution. (b) Formation of disulfide bonds. (c) Pairs of maltose molecules form weak hydrogen bonds. The lower alkane chain adsorbs hydrophobically to the HOPG substrate. (d) Formation of organic multilayers driven by hydrophobic forces between alkane chains. The vertical step height is about 4 nm but depends on the location of hydrogen bonds (overlap of the two sugars) and the angle φ as illustrated in (c).

Again, the amphiphilic nature of the organic molecule studied seems to play a major role. The hydrophobic interaction between alkane chains and graphite, helped by weak hydrogen bond formation between maltose molecules, can facilitate the self-assembly of the neoglycoconjugate.

Owing to the weak interaction forces holding the molecules together (hydrophobic and van der Waals forces and hydrogen bonds), the organic layers cannot be probed in contact mode under normal conditions. A further consequence is the high mobility of the (maltoC₁₁S-) layers observed at high relative humidities (80-90%): a rearrangement takes place which joins small islands to form larger ones, covers holes, and finally smoothes all surface features.

Conclusions

In this work, we report the characterization of maltose glyconanoparticles by non-contact AFM. These maltose glyconanoparticles, due to the amphiphilic character of the maltose neoglycoconjugate, adopt different arrangements when adsorbed on hydrophilic (Au) or hydrophobic (graphite) surfaces. On HOPG the tubular arrangement of the maltose conjugate aggregates is decomposed to form multilayers of the maltose neoglycoconjugate by itself. We propose a model for the formation of these multilayers based on the hydrophobic interactions between the molecules and with the substrate surface.