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Molecular imaging of single cellulose chains aligned on a highly oriented pyrolytic graphite surface

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Abstract—Individual cellulose macromolecules were successfully visualized on a highly oriented pyrolytic graphite (HOPG) surface by tapping-mode atomic force microscopy under ambient condition. Monomolecular-level dispersion of cellulose chains was achieved through the momentary contact of dilute cellulose/cupri-ethylenediamine (Cu-ED) solution onto the HOPG substrate. Both concentrations of cellulose and Cu-ED provided critical impacts on the topographical images. Single cellulose chains with molecular height of ca. 0.55 nm could be observed under the optimal conditions, showing rigid molecular rods with a unique morphology of hexagonal regularity. It was strongly suggested that the cellulose chains were aligned along the HOPG crystal lattice through a specific attraction, possibly due to a CH–π interaction between the axial plane of cellulose and the HOPG π-conjugated system. These phenomena would imply the potential applications of an HOPG substrate for not only nano-level imaging, but also for molecular alignment of cellulose and other structural polysaccharides.

Keywords: Cellulose; Nano-imaging; Molecular alignment; Highly oriented pyrolytic graphite; Atomic force microscopy

1. Introduction

The morphological nano-imaging of biomacromolecules at the single molecular level provides various kinds of fundamental information on biological and physicochemical aspects, resulting in a number of innovative possibilities for the development of functional biopolymer materials.[1,2] Atomic force microscopy (AFM), one of the major scanning probe microscopic analyses, is a powerful tool for molecular imaging under various analytical conditions. AFM visualization offers the conformational details of individual polymers on the nano-flat substrates at an atomic resolution, especially in the vertical direction.[3] Hence, nano-imaging via AFM has recently attracted much attention for determining the sharp distinction of real shapes of macromolecules and their assembly, being complementary to the conventional analytical methods providing the average information on polymer population, for example, light scattering, viscoelastic, spectroscopic and chromatographic characteristics.[4]

Many researchers have currently reported that moderate interaction at the polymer/substrate interface is an essential factor for successful AFM imaging without any displacement and distortion of the polymer samples.[3] Cleaved mica and highly oriented pyrolytic graphite (HOPG) are typical atomically-smooth substrates for the AFM imaging of hydrophilic and hydrophobic polymers, respectively. In the case of deoxyribonucleic acid (DNA) imaging, anionic DNA chains were successfully attached onto the negatively-charged mica surface through an electrostatic screening via cationic mediators.[5–7] On the other hand, it was reported that hydrophobic *n*-alkane-grafted polymers were regularly oriented on an HOPG surface via hydrophobic interaction, and their epitaxial crystallization was visualized by AFM analysis.[8–10] Thus, AFM imaging can provide a significant insight into the fundamental knowledge of polymer characteristics.

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Cellulose is the most abundant biomacromolecule, and forms the main constituent of the cell wall in higher plants.[11] Cellulosic polymers have been widely used in a diverse array of applications, for example, in paper, textile fibres, film, plastics, food additives, cosmetics and medical supplies.[12] Recently, the molecular features of cellulose for self-assembly and hierarchical organization have generated much interest in the area of smart materials from bio- and nano-engineering perspectives.[12,13] The three equatorially-positioned hydroxyl groups of the anhydroglucopyranose (AHG) unit in the cellulose β-(1 → 4)-D-glucan-chains trigger the spontaneous formation of a regular and strong hydrogen-bonding network, resulting in the vertical stacking of hydrophobic axial faces mainly through van der Waals attraction.[14–16] Such molecular assembling features lead to poor solvent solubility, and thus the strong molecular interaction makes the AFM observation of individual cellulose chains much more difficult. Successful molecular imaging of various water-soluble polysaccharides has been more or less achieved,[17–20] but not yet for cellulose.

Recently, we reported very clear AFM images of carboxymethylcellulose (CMC), a typical water-soluble (hydrophilic) cellulose ether derivative, by using a hydrophobic HOPG substrate.[21] The conformational changes in individual CMC chains were successfully visualized under various salt conditions, where the residual AHG units in the CMC chains possibly played an important role in the moderate CMC attachment onto the HOPG surface for clear molecular imaging. In this study, we challenged the molecular imaging of single cellulose chains by tapping-mode AFM analysis using a hydrophobic HOPG substrate. Homogeneous cellulose/cupri-ethylenediamine (Cu-ED) solution was placed in momentary contact with the HOPG surface, enabling the physical adsorption of molecular cellulose on the HOPG surface. The morphological changes in cellulose chains at a mono-molecular level were investigated by altering the Cu-ED and cellulose concentrations.

2. Results and discussion

2.1. Effect of Cu-ED concentration on cellulose visualization

In general, water-soluble polymer chains can be simply placed on a hydrophilic mica surface by applying the polymer solution dropwise, and in most cases AFM observation is possible by controlling the solution conditions.[3] However, this conventional approach was not effective for AFM imaging of CMC[21] and cellulose (data not shown), possibly due to their strong molecular assembling properties, although cellulose is homogeneously soluble in aqueous 500 mM Cu-ED solution in a complex state, as illustrated in Figure 1.[22] Hence, the hydrophobic HOPG substrates were applied to adequately immobilize the cellulose chains for AFM imaging.

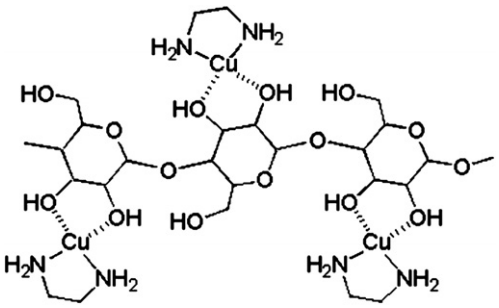

Figure 1. Schematic illustration of cellulose coordinated with the cupri-ethylenediamine complex (Cu-ED) at its C-2 and C-3 hydroxyl groups.[22]

Figure 2a shows the AFM image of the HOPG surface treated with 20 mg L^{-1} cellulose/500 mM Cu-ED solution. Long-chain cellulose with a molecular weight (MW) of ca. 2.8×10^5 g mol^{-1} was subjected to the AFM imaging. Disordered tangled cellulose chains were observed, but molecular imaging was possible to some extent under the conditions that no cellulose chains were found on the mica substrate. Nothing was also found on the HOPG substrate treated with cellulose-free 500 mM Cu-ED solution, and X-ray photoelectron spectroscopy (XPS) of the HOPG surface of Figure 2a confirmed that there was no elemental peak of Cu and N derived from Cu-ED solution (see Supplementary data). Thus, residual Cu-ED portions were little as to be neglected within the limit of XPS detection, probably indicating that most of the cellulose/Cu-ED coordination was cleaved by sufficiently rinsing with water. As in the case of the previous CMC study,[21] a successful AFM imaging could be achieved by adjusting the contact time of the cellulose droplet on the HOPG surface (within 1 s); a shorter contact time achieves better molecular dispersion. In all cases for the AFM imaging, longer contact times brought about excessive adsorption and massive aggregation of cellulose molecules and their assembly, resulting in an obscure molecular imaging because of the overall coverage of the HOPG surface (data not shown).

Lowering the Cu-ED concentration at the same cellulose content (20 mg L^{-1}) provided the different AFM images shown in Figure 2b–e. In the case of 250 mM Cu-ED concentration, a wire net-like assembly with flexible cellulose chains was observed (Fig. 2b), while the regular nano-network morphology comprised of linearly-ordered polymer segments was found in the AFM image of the HOPG surface treated with the 100 mM Cu-ED solution (Fig. 2c and d). Molecular-level cellulose chains of 0.58–1.28 nm thickness were confirmed by the vertical information of the AFM image, that is, a height profile along the white line indicated in Figure