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Molecular resolution imaging of polyglucose by scanning tunneling microscopy

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We have obtained atomic resolution images of poly-α-D-glucose by scanning tunneling microscopy. The oxygen atoms near the scanning tip were imaged, but the carbon and hydrogen atoms were not visible. The measured inter-atomic distances are consistent with the molecular structure of poly-α-D-glucose deduced from chemical and X-ray diffraction studies. The results also demonstrate that is is feasible to image surface atomic structures of a relatively thick non-conducting specimen, suggesting that the technique may be applied to the study of other macromolecules of biological importance.

Scanning tunneling microscopy (STM); Glycogen; Glucose structure

1. INTRODUCTION

Scanning tunneling microscopy (STM) has been used to study ultra-microscopic structures of biological macromolecules adsorbed on a conducting surface, and is normally performed at room temperature with the specimen either immersed in water or exposed in air [1–3]. High resolution images of many such macromolecules at near native conditions have been successfully obtained, despite the lack of any clear theoretical understanding of contrast formation by these structures. Imaging of short DNA molecules (both single-strand and double-strand helices) [4–11], amino acids [12] and other organic compounds [13–15] have demonstrated atomic resolution. Images at lower resolutions have also been obtained for larger molecules, such as phosphorylase kinase and phosphorylase *b* [16], microtubules [17], glycogen particles [18], and others. In this report, molecular resolution images of poly-α-D-glucose obtained by STM from glycogen molecules are presented, thus further extending the family within reach by the STM for ultrastructural studies. Our results show that oxygen atoms are clearly visible in the STM image when they are at the surface of the molecule, demonstrating the feasibility of atomic resolution imaging of the surface of a relatively thick non-conducting specimen at reasonable signal-to-noise ratio.

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2. MATERIALS AND METHODS

Purified glycogen particles from rabbit liver, purchased from Sigma Chemicals (Sigma type III), were dissolved in a 10 mM NaCl solution, as suggested in [18]. The appropriate glycogen concentration for STM imaging was determined by preliminary electron microscopy. Droplets of several different concentrations of glycogen solution were applied to freshly cleaved highly oriented pyrolytic graphite (HOPG) surfaces (for STM) and thin carbon films on copper grids (for electron microscopy). After a few minutes, the residual liquid was removed by a piece of optical tissue paper, leaving a thin layer of the solution on the surface to dry in air. The specimens prepared on carbon films were first rotary shadowed with Pt and observed in a transmission electron microscope. We found that 0.5 mg/ml concentration was suitable for STM imaging: glycogen particles were mostly separated without forming large clusters.

The STM images were recorded with a Nanoscope II (Digital Instruments Inc., Santa Barbara, CA). The STM was operated in the height mode, where the distance between the tip and the graphite surface is fixed. The surface topology was obtained through a conversion of tunneling current variation to distance (the calculations were automatically performed by the software). The typical bias voltages and set point currents were, respectively, about 100–200 mV and 0.2–0.5 nA. To obtain high resolution images, the scanning offset has to be kept rather small, for we found that if the *xy* offset of the D head scanner is more than 300 nm, the noise of the offset voltage is high enough to prevent atomic resolution: the graphite atomic image can no longer be obtained. Therefore, the specimen area of interest must be carefully moved to the center. During the first scan, a rather large height value was used to avoid crashing the tip. After some features were observed, the height was gradually reduced to improve both resolution and signal-to-noise ratio. The high resolution image of poly-α-D-glucose presented in Fig. 2 was obtained at a set height value of 10.0 nm.

3. RESULTS AND DISCUSSION

In the STM, we have observed intact glycogen particles [19] close to the dimensions determined from earlier electron microscopic studies [20,21]. Some of

them have the previously reported 'laminated' structures [18]. In addition, we also found large clusters formed by 200–400 Å long molecular chains. In Fig. 1, one of these clusters is shown. The structure is clearly visible even at this low resolution: most of the chains are aligned with each other in parallel. In order to reveal the atomic structures of these chains, the area marked in Fig. 1 was scanned at high resolution. The results are shown in Fig. 2, where (A) is the original data and (B) is the image after low pass filtering. The molecular structure shown is very stable and the same image can be obtained repeatedly without apparent structural or positional change (except the drifting of the scanner). It is noted that the helix structure of the α-1,4 linkage, about 6 units per turn in glycogen [22] is not seen, may be due to the drying process. A portion of Fig. 2, presented as a surface plot in Fig. 3, shows clearly that the three atoms visible in each unit are not at the same height. We interpret the observed chain structure to be a fraction of a glycogen molecule broken down during chemical isolation and purification, and formed by repeated α-D-glucose units. Detailed measurements also revealed that the chain itself has height variance along its direction. When the contrast was enhanced to show the darker area, a complicated molecular network beneath the chain could also be seen in the region of Fig. 2. Direct height measurement on Fig. 2 yields an apparent specimen thickness of about 50 Å. However, the atomic resolution obtained requires a very small gap between the chain structure and the tip, indicating the actual specimen thickness to be near 100 Å since the tip height was set at 100 Å. This discrepancy in height measurement has been reported by many others investigating different organic materials [11,16,18].

Since the molecular formula of α-D-glucose has been established from chemical studies, we have constructed a molecular model assuming α(1–4) links for comparison with the observed data (see Fig. 4). As can be seen, there are remarkable similarities between the oxygen atoms in the model and the observed image. It ap-

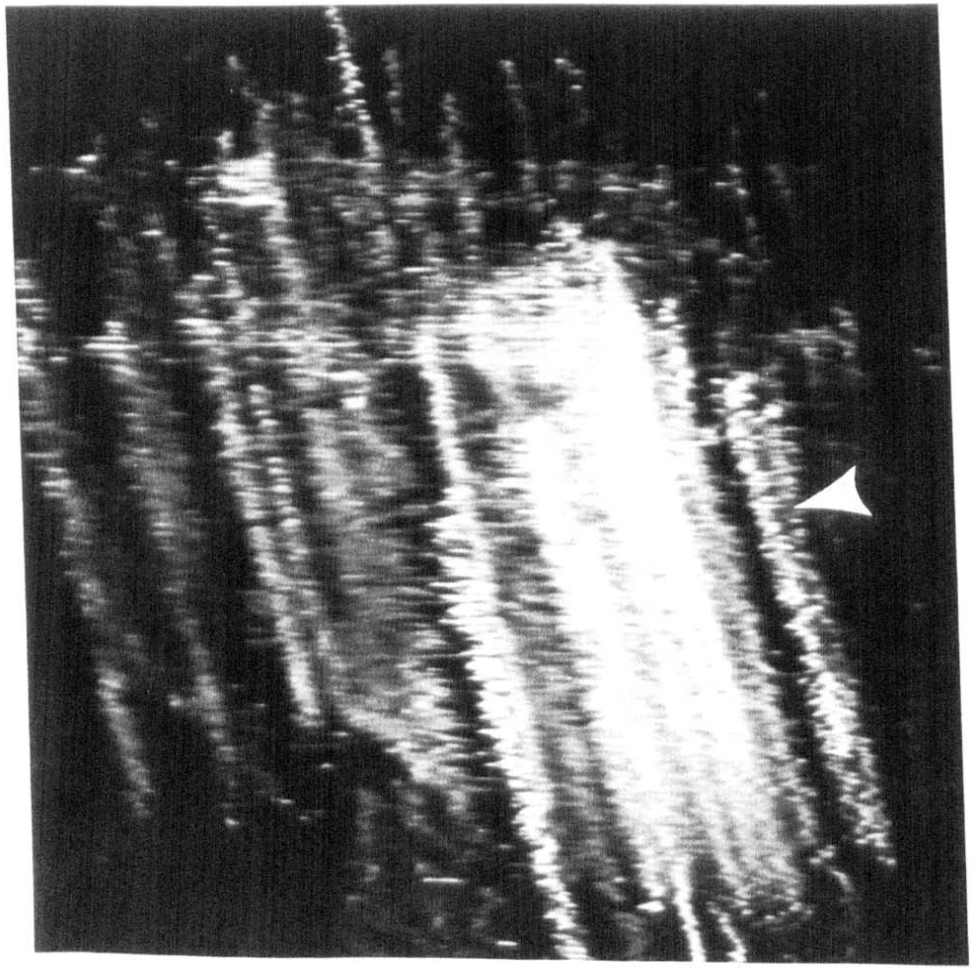
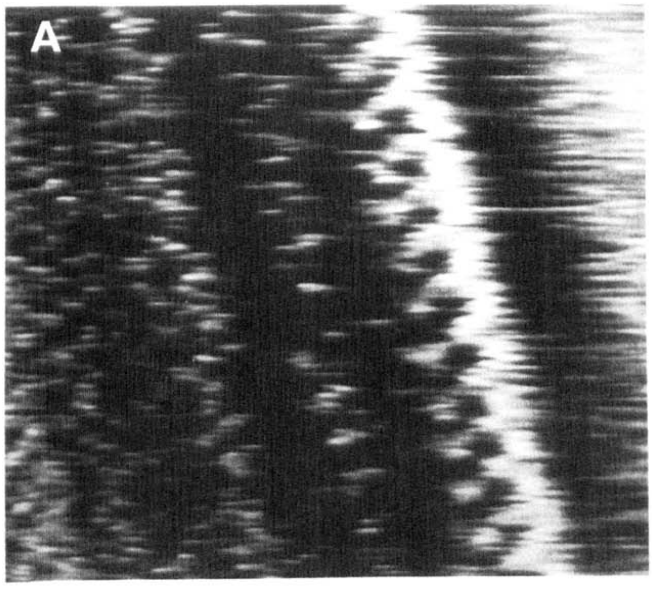


Fig. 1. One poly-α-D-glucose cluster viewed by STM at low resolution. Image size is 50 nm × 50 nm. Even at this resolution, the chain structures are clearly visible. The images are shown directly from the original data without any filtering. The area marked by the arrow is shown in Fig. 2 at high resolution.



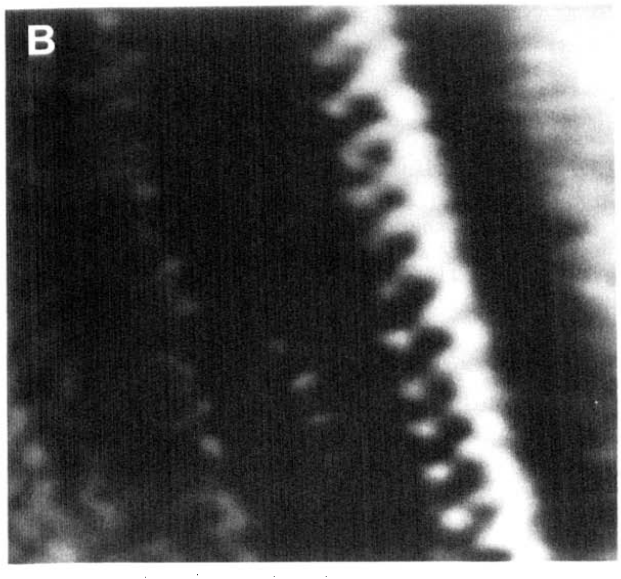


Fig. 2. The image of the marked area in Fig. 1 is shown at high resolution. The shown image is a portion of a larger scan size of 10 nm × 10 nm at a bias voltage of 180 mV, a set point current of 0.49 nA, and a height setting of 10.0 nm. (A) is the original data and (B) is the image after low pass filtering. Image size: 5 nm × 5 nm.

pears that the carbon atoms and the oxygen atoms at lower positions (which are further away from the tip) are not visible in the image. The invisibility of saturated carbons is consistent with previous observations [13] on alkylcyanobiphenyl molecules in STM, and we do not have the explanation on why only oxygen atoms are visible. Although the exact distances between oxygen atoms in poly-α-D-glucose are not available, the interatomic distances in individual glucose molecules have been measured to a high resolution by X-ray crystallography [23]. Based on these measurements and

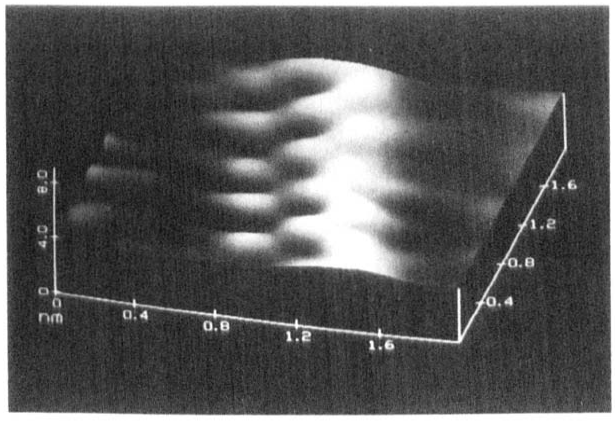


Fig. 3. A portion of Fig. 2B is shown as a surface plot. The observed structure is not flat either within each unit or along the chain. Image size: 2 nm × 2 nm.

assuming that the basic structure of glucose remains unchanged when the polymer is formed, we calculated the distances between the 3 visible oxygen atoms indicated in Fig. 4. These calculations and the results measured directly from Fig. 2 (without any correction) are shown in Table I. They are in excellent agreement: the largest deviation is only 3.6%, well within the experimental error. This result indicates a rather accurate height measurement at this scale. It should be noted that by direct structural study, we can definitely show the difference between α-D- and β-D-glucose chains. As shown in Fig. 5, the positions of O6 in each unit are obviously different for the α-α, α-β, and β-β configurations. Fig. 2 clearly shows that the chain is made only by α-D-glucose. Therefore, we directly confirm the structure deduced from chemical studies.

The accurate height measurement on the atomic scale between the oxygen atoms seems inconsistent with the reduced total specimen thickness measured according to a tunneling current/distance conversion by the software. However, this apparent inconsistency can be explained by a simple theory assuming that the average bulk specimen thickness is determined by the dielectric effect, while the atomic resolution at the surface is determined by a direct wavefunction overlapping between the tip and the specimen. Therefore, the contrast mechanisms are completely different. Simple calculations indicate that in such a situation, the height measurement of the molecule at atomic scale should be accurate, as long as overlap of the wavefunction is sufficient (close distance), a condition identifiable by the observed atomic resolution. This result is not affected by the details of the bulk material beneath the molecule [24].

In conclusion, we have demonstrated that atomic resolution images of poly-α-D-glucose molecules at the surface of a relatively thick nonconducting specimen can be directly observed in STM. Despite the discrepan-

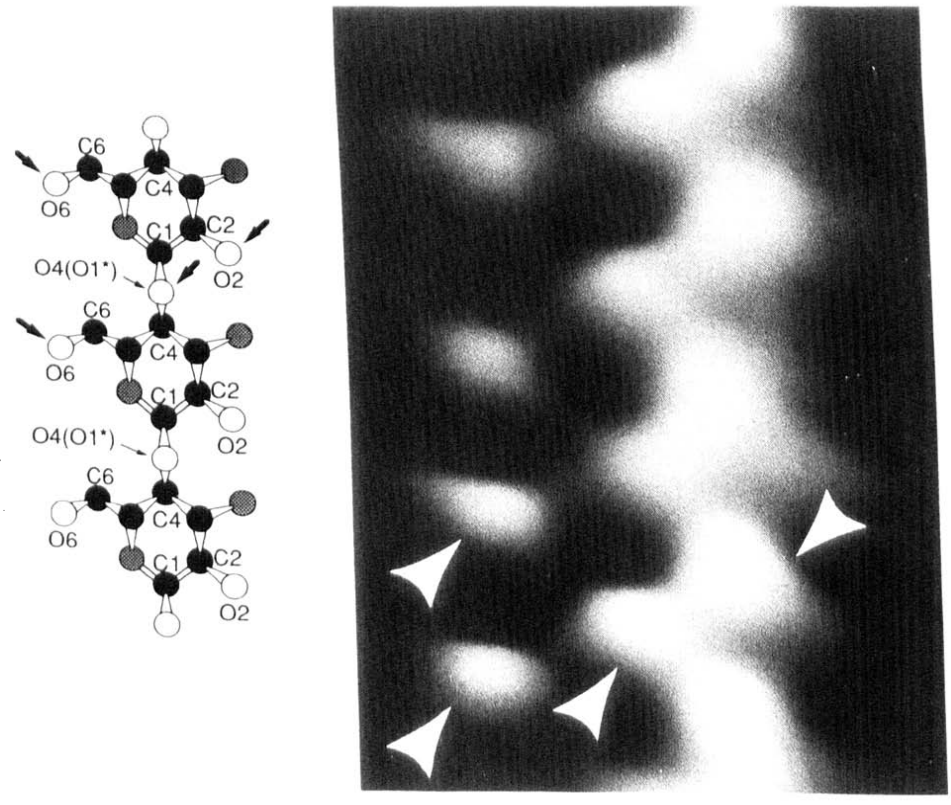


Fig. 4. Comparison between the observed image and the structure deduced from chemical studies. (Left) The three-dimensional molecular model constructed from X-ray diffraction and chemical data. Open circles represent oxygen atoms at higher positions, thus, seen in the image; shaded circles are those oxygen atoms at lower positions and solid circles are carbon atoms. (Right) 4 units of Fig. 2 are shown. Notice the easily recognizable similarity between the oxygen atoms and the observed image (marked with arrows). The oxygen atoms at lower positions and the carbon atoms are not visible in the image.

cy in bulk height meausurements, the atomic scale measurements are quite accurate when compared with X-ray data. Our results show that oxygen atoms in an organic compound can be visualized by STM, and confirm the poly-α-D-glucose structure deduced from chemical studies. We would like to point out that, until now, atomic resolution images of macromolecules, such as DNA and other organic compounds, have only been obtained from adsorbed mono-layer molecules, where the distance between the tip and the conducting surface is very small, while in our case the atomic structure was obtained at the surface of a multilayer molecular adsorbate on a conducting surface. Our results suggest that, in some cases, STM can be directly used to study the surface atomic structure of large biomolecular complexes without extensive specimen manipulation.

Table I

Inter-atomic distances of oxygen atoms in poly-α-D-glucose

Inter-oxygen atoms	STM (Å) (± SD)	X-ray (Å)	% Deviation
O6-O6*	4.34 (±0.37)	4.50	3.6
O6-O4(O1*)	4.22 (±0.52)	4.12	2.4
O6-O2*	6.44 (±0.58)	6.54	1.5

*indicates that the oxygen atom is from a neighboring unit. See Fig. 4 for details. SD is the standard deviation.

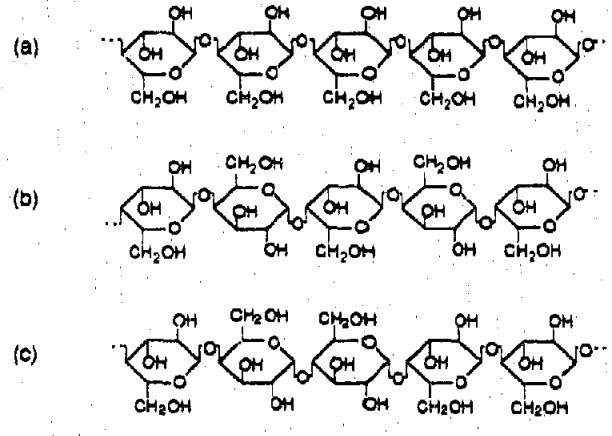


Fig. 5. Symbolic molecular structures of polyglucose formed by: (a) α,α; (b) α,β; (c) β,β. Notice the difference in appearance of the O6 atoms in the chain structure. Compared with Fig. 2, only the α,α configuration is observed.

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