Imaging Antibody Molecules at Room Temperature by Contact Mode Atomic Force Microscope

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Monoclonal antibodies (IgG and IgM) have been observed by atomic force microscopy (AFM) at room temperature depositing from solution onto a freshly cleaved highly oriented pyrolytic graphite (HOPG) surface.

In recent years, monoclonal antibodies have attracted much attention in the field of chemistry, because they are homogeneous and have been extensively studied as catalytic antibodies and sensors. Although the importance of monoclonal antibodies has been recognized, it is difficult to obtain their molecular images. Recently, atomic force microscopy (AFM) has been successfully applied to a variety of chemical and biological materials.[1-3] However, since organic molecules, especially, biological macromolecules are flexible and soft, it is difficult to observe their structures directly by AFM at room temperature.[4] More recently, to overcome this difficulty, Au-coating or flow cell methods,[5] and cryo-AFM in which the head is operated in liquid nitrogen vapor under a pressure higher than atmospheric pressure, have been invented for observing molecular image of macromolecules.[6] This method provides us opportunities to observe some proteins and DNAs, such as α-macroglobulin and plasmid DNA. However, it is important to observe the molecular images of the macromolecules under conditions close to their natural environments; namely, at room temperature and in an ambient atmosphere. We report here direct observation of antibody molecules by contact mode AFM at room temperature. It is generally believed that the tapping mode AFM is more conducive to the observation of organic molecules such as proteins and DNA. However, we found at least in this case that the contact mode is more favorable than the tapping mode AFM to observe antibodies and their supramolecular structures.

For our AFM experiments, we used a monoclonal antibody for tetrakis-carboxyphenylporphyrin (IgG)[7] and that for tetrakis-phenylporphyrin (IgM). A 2 μl of buffer solution of antibody (0.5 mg/ml in 0.1 M phosphate borate buffer (pH 9.0)) was dropped on the surface of freshly cleaved highly oriented pyrolytic graphite (HOPG). The sample was allowed to stand one day in a desiccator with $CaCl_2$. All measurements were taken on a multimode Nanoscope IIIa (Digital Instruments, Santa Barbara, CA). We used the contact mode AFM with a SiN_4 tip. All the images in this paper were obtained with cantilevers of a spring constant 0.03 N/m. The line scan speed was 2 Hz with 512 pixels per line. All scales were calibrated against a standard sample and rechecked with graphite.

Figure 1 shows images of anti-tetracarboxyphenyl-porphyrin antibodies (IgG, molecular weight is 150,000). Characteristic T or Y shape molecules[8] can be seen. The overall lateral dimension is 40-50 nm, which is in good agreement with the expected values.[9] Although these molecules are monoclonal antibodies, each antibody (IgG) molecular image is not identical. This is because each antibody molecule takes somewhat different shape due to the flexibility of its hinge region. The angles

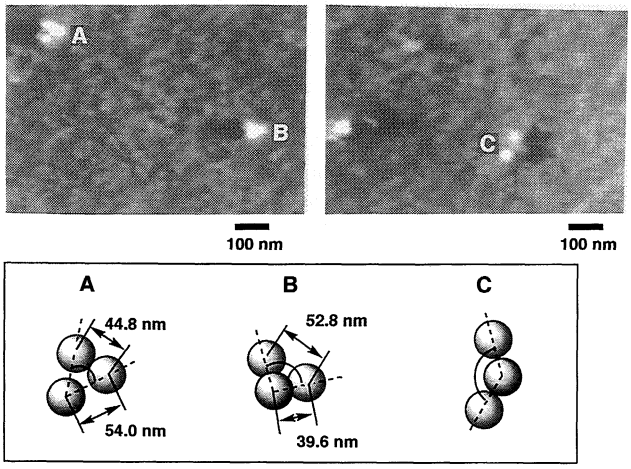

Figure 1. Images of the anti-tetracarboxyphenylporphyrin antibody 03-1 (IgG2b) by contact mode AFM at room temperature.

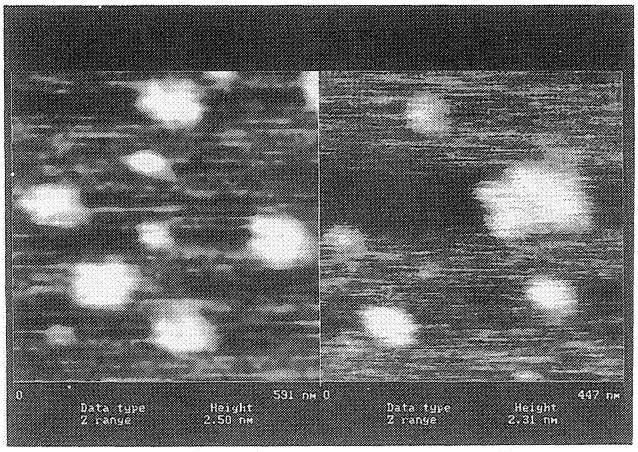

Figure 2. The contact mode image of anti-tetraphenylporphyrin monoclonal antibody (IgM).

between the Fab fractions of each immunoglobulin are different. Fab fractions and an Fc fraction can be differentiated. Figure 2 shows the contact image of monoclonal IgM (molecular weight is 900,000), which is a pentamer of IgG linked by additional peptides. Two kinds of images are evident, although the object is a single species: one is a flat pentamer and one has a much higher center. The hight of the smaller images (1.2-1.8 nm) is higher than that of a pentamer (0.8 nm). The ratio of the two kinds of images is always statiscally one to two (pentamers to smaller images). The results are consistent with those obtained by the cryo-AFM measurement. Figure 3 shows a schematic representation of IgM which shows two kinds of images, a flat pentamer and one that with a higher center, based on our results and those of cryo-AFM.

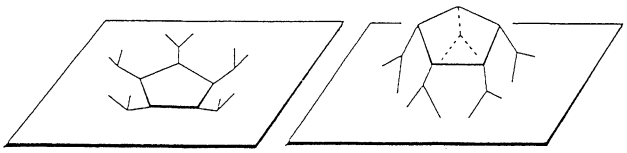

Figure 3. Schematic representations of images for IgM. (a) A flat pentamer and (b) images with higher center.

A total of 2 μl of protein solution was dropped on the graphite base. Low concentrations of proteins (0.5 mg/ml) and high concentrations of salts in buffer (0.1 mM phosphate borate buffer) should be used to minimize aggregation of proteins. We tested some substrates for the use as a base and found that the substrates should have strong interactions with proteins to protect from high disturbance of the AFM tip. We found that highly oriented pyrolytic graphite (HOPG) is the most suitable substrate for the observation of the antibody molecules, although graphite is not frequently used for AFM.

Moreover, we found that the contact mode AFM is able to be applied to observe biological macromolecules at room temperature, although it has been recognized that the contact mode AFM is difficult to use for the soft molecules because the high pressure in the contact area may damage or deform the samples by sharp tips required for high resolution. However, in our case, the contact mode AFM can be used when graphite is used as substrate, which binds proteins tightly.

AFM may be employed in sensitive immunoassay detection without any kind of labeling and is also of possible use as a sensor to detect specific interactions between antigens (chemical compounds) and antibodies.

In conclusion we have succeeded in observing antibody molecules at room temperature by contact mode AFM using highly oriented pyrolytic graphite (HOPG) as a base and high concentrations of salts to prevent coaggregation of immunoglobulins. We are now observing supramolecular assemblies between antibody molecules and artificial antigens (synthetic chemical compounds) using AFM.

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