Recognition Imaging with a DNA Aptamer

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ABSTRACT We have used a DNA-aptamer tethered to an atomic force microscope probe to carry out recognition imaging of IgE molecules attached to a mica substrate. The recognition was efficient (~90%) and specific, being blocked by injection of IgE molecules in solution, and not being interfered with by high concentrations of a second protein. The signal/noise ratio of the recognition signal was better than that obtained with antibodies, despite the fact that the average force required to break the aptamer-protein bonds was somewhat smaller.

INTRODUCTION

The atomic force microscope (AFM) is capable of imaging at molecular resolution in water, (1) an attribute that has been exploited for biological imaging (2). The technique is severely limited when complex samples are imaged, because it is nearly impossible to tell the difference between proteins of even rather different molecular weight from the topographical image alone. Recognition imaging is a technique that gives the AFM chemical sensitivity. With an antibody tethered to an oscillating AFM force sensing probe, binding of the antibody to its antigen is indicated by small changes in the pattern of oscillation as the probe is scanned over the surface to form an image in the normal constant-amplitude mode (3,4). A map of these changes, superimposed on the topographic image, shows where the target proteins are located in the image. Promising as this technique is, we have found it to be limited by the properties of the antibodies. The surface concentration under the probe is very high, so even small affinities for a cross reaction give significant recognition signals (Bash et al., in preparation). We have therefore explored the use of another kind of recognition molecule, the DNA aptamer. These are small stem-loop single stranded DNA molecules generated via ''systematic evolution of ligands by exponential enrichment'' (SELEX) (5,6). Though not yet anywhere nearly as available as antibodies, an aptamer sequence, once identified, is easy to use. Aptamers consists of a single strand of DNA, so they are easy to synthesize and store. They are easily folded by thermal annealing in an appropriate buffer and they are also easily attached to an AFM probe using commercially available DNA that is chemically modified at one end. In contrast, the present process for attaching antibodies to the probe relies on modification of available lysines (7), a procedure that carries the risk of altering the variable region of the antibody.

Aptamers may be more specific than antibodies (though this is not well documented). They also have a high affinity for some small molecules. This holds out the prospect that they might enable recognition imaging of minor chemical modifications important as components of an epigenetic code.

In this study, we describe the use of a DNA aptamer as ligand for recognition imaging. We show that it is highly specific in the presence of large amounts of exogenous protein.

METHODS

We chose the well-studied aptamer to Human IgE (8) because this has been shown to produce significant specific adhesion in AFM force curves (9). AFM probes were aminated and functionalized with a heterobifunctional polyethylene glycol; Mal-d$(PEG)_{12}$-NHS ester (from Quanta Biodesign, Powell, OH) leaving the thiol-reactive maleimide at the end of the PEG. The thiolated molecule 5′-GGGGCACGTTTATCCGTCCCTAGTGGCGTGC-CCC/3ThioMC3-D/-3′ (from Integrated DNA Technologies, Coralville, IA) was purified by polyacrlamide gel electrophoresis followed by ethanol precipitation, then resuspended and attached to the PEG linker to form the construct shown in Fig. 1 *a*. All other aspects of the procedure are as previously described for antibody attachment (4).

Glutaraldehyde-modified mica substrates were prepared as described elsewhere (10) and 70 μL of a 0.01-μM solution of IgE (Athens Research, Athens, GA) in MPBS buffer (PBS buffer with 1 mM Mg^{2+}; (8)) was left on the substrate for 40 min. After rinsing, the sample was placed under MPBS buffer and imaged immediately using a microscope equipped for recognition imaging (PicoPlus with PicoTREC from Molecular Imaging, Tempe, AZ). Images were taken at a scanning speed of ~2 microns per second with an oscillation amplitude of ~5 nm. The resolution of the recognition technique is limited by the tether length to ~5 nm (halfwidth at halfheight of the observed spot).

RESULTS

A typical topographic image is shown in Fig. 1 *b* with the simultaneously acquired recognition image shown in Fig. 1 *c*. The dark spots in the recognition image mark regions where the aptamer bound, and these are coincident with the location of IgE molecules, as can be seen by comparing a cross-sectional trace across the images (*e*, topography; *f*, recognition). The signal/noise (ratio of the recognition signal amplitude to the RMS noise amplitude) in the recognition signal is strikingly better than previously reported for antibodies (Fig. 1 *f* of Stroh et al. (4)). The aptamer was blocked by flowing 70 μL of a 0.01-μM solution of IgE in MPBS into the liquid cell of the microscope. When the same region of the substrate was

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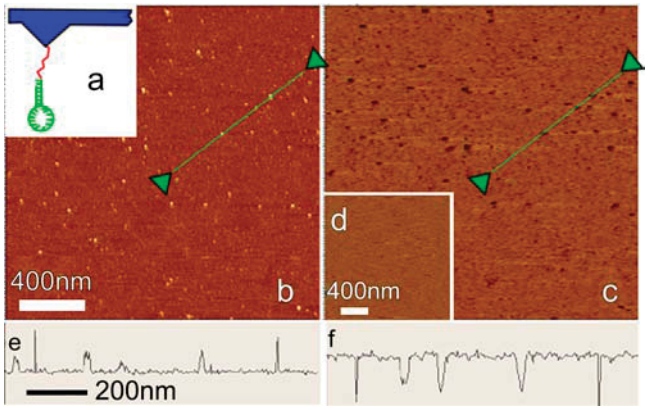

FIGURE 1 IgE imaged with a DNA aptamer. The aptamer (*green*) is tethered to the AFM probe via a PEG linker (*red*) (*a*). *b* shows topography and *c* is the simultaneously acquired recognition signal. *d* is after blocking by injecting a solution of IgE. Traces between the green arrows are shown for the topography in *e* (maximum height is 4 nm) and for the recognition in *f* (maximum signal is 0.75 V).

reimaged (Fig. 1 *d*), the recognition signal was abolished, indicating that the interaction was specific.

We used a custom image analysis program to quantify the recognition further. Another recognition image of a field of IgE molecules is shown in Fig. 2 *a*. The distribution of pixel intensities both away from, and including a recognition spot are shown in Fig. 2 *b*. There is a clear separation between the recognition signal level and the background signal (*red line* on Fig. 2 *b*) and this level was used to determine legitimate spots manually for each candidate spot (which were then marked by circles). The markers are transferred onto the topographic image (Fig. 2 *c*) so that recognized features may be identified. The use of this procedure requires careful leveling of the background. It is enhanced by a 3 × 3 median filter that removes noise spikes on individual pixels.

The number of protein-like features recognized in Fig. 2 *a* is 76, out of 84 total features in the topographic image that have a size that indicates they are IgE molecules. This 90% recognition level is typical for pure preparations of IgE, indicating that the IgE is commonly oriented with its recognition site exposed. To test for selectivity in the presence of an interfering protein,

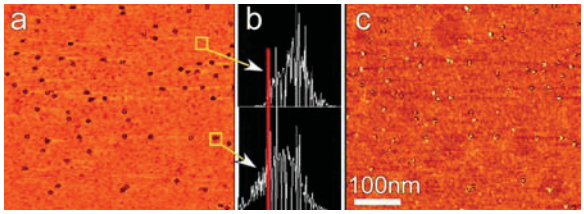

FIGURE 2 Quantifying the recognition: *a* is a recognition image of a field of IgE molecules. *b* shows histograms of the pixel intensity distribution for (*upper*) an area without a recognition spot and (*lower*) an area with a recognition spot. The vertical red line denotes the threshold set to mark the recognition events by small circles around the corresponding features in the topographic image in *c*. Images were 3 × 3 median filtered. The scale for both images is shown on *c*.

we imaged surfaces treated with either a mixture of thrombin and IgE (60:1 molar ratio) or with just pure thrombin (with similar overall coverage to that shown in Fig. 1). There were no recognition events on the surface functionalized with only thrombin. The mixed surface gave 23 recognition events out of ~300 spots that could have been either thrombin or IgE. An example of an image from this series of experiments is given in Fig. 3. This 13:1 ratio is somewhat greater that the molar ratio of the two proteins in the solution used, but IgE may adsorb onto the surface preferentially.

The improved signal/noise in the recognition signal (relative to that obtained with antibodies) might be expected to reflect a relatively stronger binding of the aptamer as indicated by the force-curve data of Jiang et al. (9). This previous study used aptamers that were linked directly to the AFM tip, and we have found that the adhesion properties can be different when the ligand is suspended using a PEG linker (as in this study). Importantly, the characteristics of the pull-off curve allow unambiguous identification of single molecule data owing to the characteristic stretching of the PEG (7).

A histogram of the distribution of pull-off forces is shown in Fig. 4. The median pull-off force is a little smaller than that obtained with antibodies (4,11) and smaller than the value reported by Jiang et al. (9). Whatever the origin of the discrepancy, Jiang et al. report only a small difference between the pull-off force for the aptamer (160 pN) and that for the antibody (140 pN). It seems unlikely therefore that the enhanced recognition signal could be accounted for simply by better binding of the aptamer.

DISCUSSION AND CONCLUSIONS

In summary, we have shown that a DNA aptamer may be attached to an AFM probe and used to generate recognition signals that are efficient (>90%) and specific, recognizing even a small amount of the target protein in a sample composed predominantly of another protein. This opens a new avenue

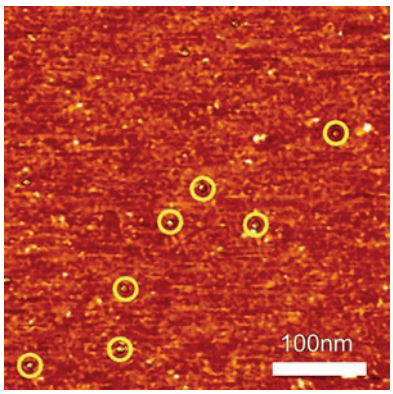

FIGURE 3 Topographic image of a mixed film IgE and thrombin with the location of recognition events marked by an IgE aptamer marked by yellow circles. The large reduction of the frequency of recognition events compared to the pure IgE samples indicates that the presence of excess thrombin is not causing spurious recognition events.

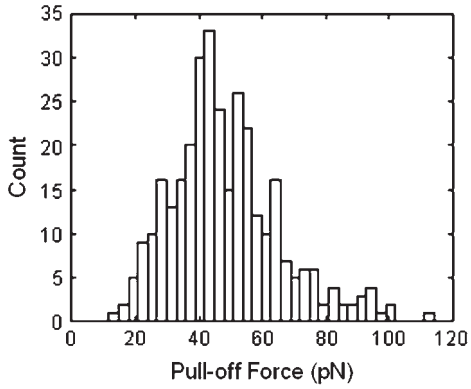

FIGURE 4 Histogram of pull-off forces for the aptamer binding IgE molecules. The average pull-off force is 49 ± 18 pN at a pulling rate of 7.8 nN/s.

for recognition imaging. Chemically simpler than antibodies, DNA aptamers might permit mapping of even quite small differences in the composition of proteins. Finally, we note that although the aptamer does not appear to bind to its target more strongly than an antibody (in this case) it gives a better signal, suggesting that nonspecific adhesion is lower.

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