Atomic Force Microscopy detection of antibody-antigen complexes

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Introduction

Ours idea is to use the Atomic Force Microscopy (AFM) as label-free revealing system [1]. This approach allows detecting the formation of antigen-antibody immune complexes without need of a fluorochrome labelled probe. In the process of detection of the antigen-antibody complexes by AFM the substrate functionalization method plays a central role, since the AFM is able to visualize single molecules only if the substrate roughness does not interfere with the measure.

There are several functionalization methods that allow to the antibodies to retain their orientation and/or function [2]. However, most of them are not suitable for AFM imaging, because they produce substrates presenting high roughness. Indeed, the functionalization of a suitable substrate for AFM imaging should create a monolayer of molecules that allows to the antibodies to retain their orientation and functionality without enhances the substrate roughness.

The heights of the antibodies detected by AFM varies from 4 to 6,5 nm, thus in order to permit unambiguous identification of the antibody by AFM the substrate roughness must be very low. Here a new functionalization procedure that uses a smooth solid substrate (e.g. mica, glass and silicon) and a short peptide as antibody linkers, which has a high affinity to the antibody Fc portion, is described. This procedure allows the production of surfaces with a very low roughness (below 1 nm) able to bind properly the antibodies, thus keeping their bioactivity [3, 4].

Materials and Methods

APTES-mica surface was obtained by placing the mica in a vacuum glass dessicator, which contained 50 μL of APTES 98% (Sigma-Aldrich), for 1h. To create the monolayer of antibody binding peptide on APTES-mica surface, 2 mg/mL peptide in DMF (Sigma-Aldrich) were treated with HATU and DIPEA (Sigma-Aldrich) to activate the carboxylic groups. The solution was then deposited on the APTES-mica substrate. Five solutions with different concentrations of anti-IL10 antibody (Biosource -Invitrogen, Carlsbad, CA, USA) were deposited on the treated mica solid substrate by means of a Piezoarray non-contact microarraying system (Perkin Elmer, Waltham, MA). The AFM analysis was performed using a Dimension 3100 Veeco AFM (Santa Barbara, CA, USA) with a hybrid XYZ head, using Olympus OMCL-AC160 tips with nominal apical radius <7 nm (Olympus corporate, Tokyo).

Results and Conclusions

A review of the literature has shown that particular peptides could have antibody binding activity, so we focused our attention to some small peptides, which have the ability to recognize the Fc immunoglobulin portion [5]. Using peptides sequences with high affinity for the Fc region of antibody as model system, we have identified and synthetized a new class of peptides suitable for antibodies AFM investigation. We have synthesized and tested different peptides. These peptides are able to bind with high affinity the antibody Fc portion and as antibody we have used the anti-IL10 monoclonal antibody. Indeed these peptides do not induce remarkable roughness on the substrate surface, reducing the AFM background signal, have high affinity for the antibody Fc portion and are able to link the antibody to the substrate with the proper orientation. The AFM analysis of the substrates, obtained with the peptides, shown that all of them produced surfaces with very low roughness, below 1 nm. This is due to very likely to the self-assembling peptides properties [6]. Therefore, five solutions with different concentrations of anti-IL10

antibody were deposited onto two solid supports of mica, previously coated with a layer of peptide. One support was used in order to verify the procedure by means of an immunofluorescence assay (data not shown) and the second one was used to perform AFM measurements. AFM analyses were carried on the same specimen area after the immobilization of the antibody and after the treatment with the antigen solution.

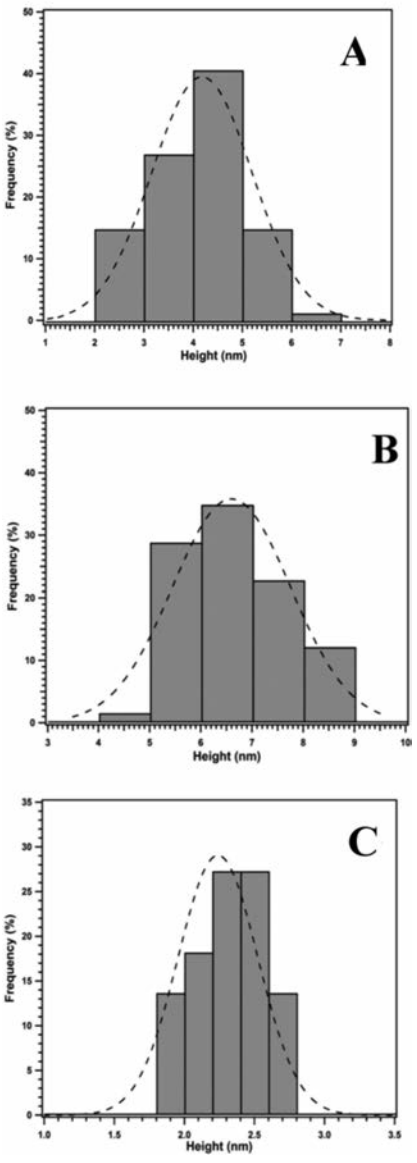

Figure 1. A) Antibody height histogram, the height of antibodies is 4.2±0.3 nm. B) Antibody-antigen complexes height histogram, the height of antibody-antigen complexes is 6.6 ± 0.3 nm C) Antigen height histogram, the height of antigens is 2.3 ± 0.1 nm.

The results of the AFM analysis are shown in Figure 1. The difference between the height of the antibody and the height of the antibody-antigen complex fits very well the height of the antigen.

In conclusion we have the proof of principle that it is possible to detect the formation of antibody-antigen complexes using the AFM as label-free technique. This result was achieved taking advantage of small peptides with a peculiar and well defined aminoacidic sequence, which confer them high affinity for antibody Fc portion. In addition we have obtained an ultra-flat surface that allows us to use the AFM as label-free detection.

References

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