An Application of Atomic Force Microscopy to Measure the Spring Constant of Single Antigen-antibody Complex

L. A. Chtcheglova*, G. T. Shubeita, S. K. Sekatskii and G. Dietler

*Institut de Physique de la Matière Condensée, University of Lausanne,
CH-1015 Lausanne, Switzerland
e-mail address corresponding author: Lilia.Chtcheglova@ipmc.unil.ch
fax: +41 (0)21 692 3635

Aim of our study is to use Atomic Force Microscopy (AFM) to measure the spring constant for single bovine serum albumin (BSA) – polyclonal antibody to BSA (Ab-BSA) complex ($k_{complex}$) as a function of pulling off force and complex extension. By the combination of AFM operated in the force-spectroscopy mode with the small dithering of the AFM tip we are able to detect the change of the dithering amplitude upon the pulling off process and to derive the value of $k_{complex}$. At the moment of single specific bond rupture the spring constant value of 0.017 ± 0.003 N/m is determined.

1. Introduction

Over the last decade, the AFM has been widely extended to measure interaction forces at the molecular level (Force Spectroscopy) [1]. By biological functionalization of tip and sample it becomes possible to investigate molecular forces, like forces between a receptor and a ligand including antigen-antibody (Ag-Ab) interactions [2].

Recently, Schindler et al. [3] proposed to use in addition a small modulation of the tip-sample distance at a selected optimal driving frequency ω. Recording the amplitude of the tip vibrations at this driving frequency leaded to an improved molecular recognition fidelity. This method opens also the possibility to measure the dynamical properties of single molecules. Here we developed this approach for the measurement of the spring constant of a stretched BSA – Ab-BSA complex. A complete study of specific unbinding forces between BSA and poly- and two monoclonal antibodies to BSA were earlier accomplished in our laboratory.

2. Materials and Methods

Standard Si_3N_4 cantilevers with a nominal spring constant of 0.06 N/m (Digital Instruments (DI), Santa Barbara, CA) and with range of resonant frequency in liquid from 8.5 to 10 kHz were functionalized with polyclonal rabbit anti-bovine serum albumin antibody (Ab-BSA). Before biological functionalizion of substrates (mica) with BSA molecules chemical coating of mica was done by silanization using 3-aminopropyltriethoxysilane (APTES). BSA and antibodies were purched from Sigma and used without further purification. All experiments were performed in a phosphate buffer solution (PBS) (50 mM, 150 mM NaCl, pH 7.4).

Experimental setup is presented in self-exploratory Fig. 1. A commercial scanning probe microscope (Nanoscope IIIa; DI) operated in "force-volume" mode was used. A small sine wave modulation voltage U_{mod} with a peak-to-peak amplitude of 20-80 mV at frequency of $\omega=2\pi*3.2$ kHz was additionally applied on the piezocrystal (normally used in tapping mode option). This modulation was equivalent to application of a driving force F_{ω} onto the cantilever, and caused a tip dithering with the amplitude of 0.5-2 nm. This resulted in the modulation of the photodiode current at the same frequency ω. The signal from the photodiode was measured by a SR750 digital lock in amplifier. Therefore, force-distance cycles and amplitude-distance cycles were recorded simultaneously. We have not detected a dependence of the results on the dithering amplitude. The smallest reasonable loading rate value of 0.7 nN/s was used.

3. Results and Discussion

Fig. 2A shows our typical experimental data. The recording of the data starts when the tip and sample are in the non-contact region (interval A-B). When the tip is in contact with the surface (B-C approaching, C-D retracting), the cantilever deflection is a linear function of the displacement of the sample.
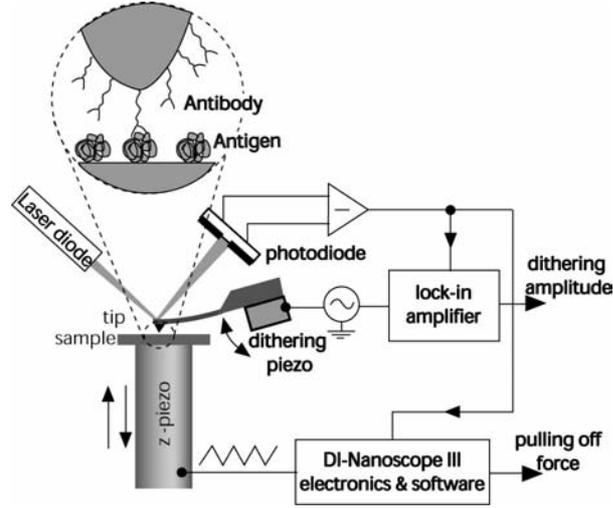
Figure 1. *The experimental setup.*

In point D, the tip loses the contact from the sample. Interval D-E represents further retraction of the tip from the surface and finally point E corresponds to the breaking of bonds (unbinding force) between functional groups of the tip and surface.

Below we estimate a value of the spring constant of a tip-sample complex $k_{complex}$ basing on the following considerations. In liquids, for small amplitudes of vibrations a process of excitation of tip vibrations can be described as forced vibrations of a nonlinear oscillator [4]. Let δz be a variable describing a tip movement away from its equilibrium position z_0 . The position of z_0 is determined by a quasi-static pulling off force F_{pul}. One can write the following differential equation for δz:

$$m_{eff}\,\ddot{\delta z} + \gamma\,\dot{\delta z} + k_{eff}\,\delta z = F_{\omega}\cos(\omega t) \tag{1}$$

Parameters m_{eff} and γ characterize the tip vibrations in liquid and $k_{eff} = k + k_{complex}$, where k is the spring constant of the cantilever. The solution of (1) is well known: $\delta z = A\cos(\omega t + \varphi)$ with an amplitude A

$$A = \frac{F_{\omega}}{\sqrt{(m_{eff}\omega^2 - k)^2 + \gamma^2\omega^2}} \tag{2}$$

and a phase shift φ

$$\sin\varphi = \frac{\gamma\omega}{\sqrt{(m_{eff}\omega^2 - k)^2 + \gamma^2\omega^2}} \tag{3}$$

If $\omega << \omega_0$ (where $\omega_0 = \sqrt{k/m_{eff}}$ and for the small quality factor $Q \geq 2$ $\omega_0 \approx \omega_{res}$, where ω_{res} – resonance frequency and $\omega_{res} = \omega_0\sqrt{1-(1/4Q^2)}$) the equation for an amplitude can be further simplify and gives

$$A = F_{\omega}/k$$

From measurements of A done with a lock in amplifier for following cycles: tip vibrations without antigen-antibody interaction, tip vibrations with such a bond at different pulling off forces F_{pul} and tip vibrations after rupture of Ag-Ab bond, we calculate the reciprocal spring constants k^{-1}, $(k+k_{complex})^{-1}$ and k^{-1} respectively. The value of k is known that makes possible to determine $k_{complex}$ of single antigen-antibody complex as a function of z and F_{pul} .

From our recorded data we distinguish the following cases (see Fig. 2):
single bond rupture with unbinding force of about 130 pN (Fig. 2A), double bond rupture (Fig. 2B, 295 pN), the forces greater than 1nN are believed to be the breaking of multiple glutaraldehyde-amine bonds (data not shown) and "no specific event" (Fig. 2C). The lower curve in Fig. 2C imitates well a single bond rupture behavior but the upper curve does not shows

peculiarities (there is no descending tendency of k during the pulling off process). Consequently, Fig. 2C does not represent specific antigen-antibody interactions.
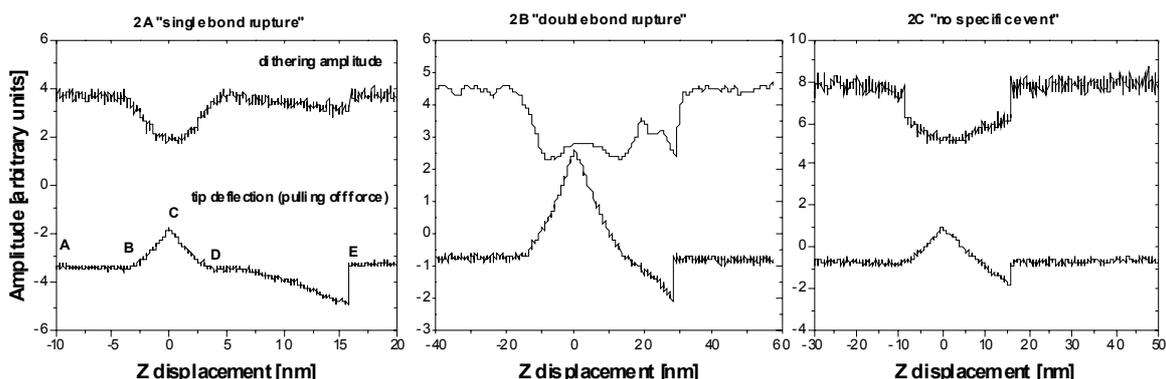
Figure 2. *Lock in amplifier signal (or dithering amplitude) and pulling off force as function of sample displacement z.*

Fig. 3 shows z-dependencies of complex spring constant $k_{complex}(z)$ (Fig. 3A) and of pulling off force $F_{pul}(z)$ (Fig 3B) for single-bond rupture. We have observed that the spring constant of single (and double) BSA - Ab-BSA complex always increases with the increasing of the pulling off force and reaches its maximum at a moment of bond rupture. We obtain a good correlation between the value of specific interaction force and the estimated complex spring constant. At the moment of single Ag-Ab bond rupture the average value of 0.017 ± 0.003 N/m for $k_{complex}$ is calculated.
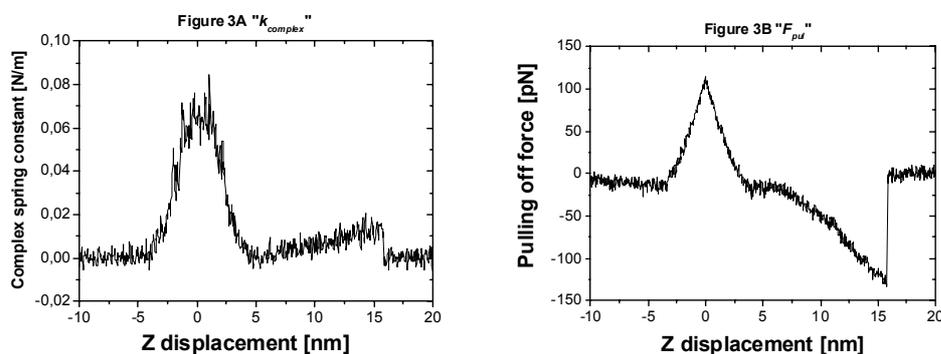
Figure 3. *Complex spring constant and pulling off force for single BSA-Ab-BSA complex as function of sample displacement z.*

This study gives us the important complementary information about the process of antigen-antibody binding which is not yet well understood. We are strongly confident that the technique described above reveals an importance of the measurement of the spring constant for the analysis of the tip-sample interaction. We are convinced that the spring constant of tip-sample complex *plays* essential role as a unique characteristic comparable with a pulling off force F.

Acknowledgements

[1] Ducker, W. A., Senden, T. J., Pashley, R. M. Nature, 353 (1991) 239.
[2] Hinterdorfer, P., Baumgartner, W., Gruber, H. J., Schilcher, K., Schindler, H. Proc. Natl. Acad. Sci. USA, 93 (1996) 3477.
[3] Shindler, D. Badt, P. Hinterdorfer, F. Kienberger, A. Raab, S. Wielert-Badt, and V. Ph. Pastushenko, Ultramicroscopy, 82 (2000) 227.
[4] D. Sarid, in *Scanning Force Microscopy,* Oxford University Press, London, 1991.