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Probing molecular interaction between concanavalin A and mannose ligands by means of SFM

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Abstract Recently, the scanning force microscope (SFM) has been widely used for direct monitoring of specific interactions between biologically active molecules. Such studies have employed the SFM liquid-cell setup, which allows measurements to be made in the native environment with force resolution down to a tenth of a picoNewton. In this study, the ligand–receptor strength of monoclonal anti-human prostatic acid phosphatase and prostatic acid phosphatase, representing an antigen–antibody system with a single type of interaction, was determined. Then, the interaction force occurring between concanavalin A and the carbohydrate component of the glycoproteins arylsulfatase A and carboxypeptidase Y was measured. High mannose-type glycans were sought on the human prostate carcinoma cell surface. Application of an analysis based on the Poisson distribution of the number of bonds formed in all these measured systems allowed the strength of the molecular interaction to be calculated. The values of the force acting between two single molecules were 530 ± 25, 790 ± 32, and 940 ± 39 pN between prostatic acid phosphatase and monoclonal anti-human prostatic acid phosphatase, between concanavalin A and arylsulfatase A, and between concanavalin A and carboxypeptidase Y, respectively. The value calculated from data collected for the force between concanavalin A and mannose-containing ligands present on the surface of human prostate carcinoma cells was smaller, 116 ± 17 pN. The different values of the binding force between concanavalin A and mannose-containing ligands were attributed to the structural changes of the carbohydrate components.

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Introduction

The scanning force microscope (SFM) is a powerful tool for studying not only surface topography (Rotsch et al. 1997) and cell elasticity (Lekka et al. 1999a), but also the adhesion forces acting between surfaces (Florin et al. 1994). Among its many advantages, two seem crucial. Firstly, this technique offers the possibility of direct measurement of discrete intermolecular forces with resolution down to tenths of picoNewtons, with very high spatial resolution. Secondly, the measurements can be performed in a liquid environment, providing natural conditions for the investigated molecules (Capella and Dietler 1999; Evans 2001).

In biology and biochemistry, scanning force microscopy offers a unique opportunity to measure forces occurring between molecules in their native states, such as receptors embedded in the cell membrane and acting with ligands (Lee et al. 2000; Grange et al. 2001; Werten et al. 2002). These involve many weak noncovalent bonds (i.e., electrostatic, van der Waals, and/or hydrogen bonds) or hydrophobic interactions between geometrically complementary molecules. These interactions are highly specific and can be very strong (Leckband 2000; Yuan et al. 2000; Evans 2001). Recently, many groups have exploited this technique to detect specific molecular recognition events between different types of receptor–ligand molecules. The first and the most often studied interaction measured by SFM was that of the biotin–avidin complex (Florin et al. 1994; Yuan et al. 2000). Also measured were the specific interactions of several other systems such as those between the antibody single-chain Fv fragment molecule and a fluoresceinated cantilever (Ros et al. 1998), or between a human serum albumin (HSA) and a polyclonal anti-HSA antibody (Hinterdorfer et al. 1996). Usually, the

preparation procedure involves attaching one type of molecule to the cantilever, and the complementary one to a substrate. When the SFM tip contacts the substrate the ligand–receptor pair forms. The adhesion (binding) force of the ligand–receptor pair can be determined from the force value required to separate the tip from the substrate. The technique has also been used to investigate the interaction between complementary strands of DNA oligomers (Grange et al. 2001). Interest is increasingly directed towards the application of such a unique technique to study molecular interaction in vitro, that is, to measure forces between a functionalized SFM tip and the surface of living cells, for example, the specific interaction between a concanavalin A-covered tip and mannan polymers on the surface of living yeast (*Saccharomyces cerevisiae*) cells (Gad et al. 1997). Another study used the interaction between an SFM tip covered with lectin (*Helix pomatia*) and *N*-acetylgalactosamine-terminated glycolipids to distinguish group A from group 0 red blood cells (Grandbois et al. 2000).

There are many examples of specific ligand–receptor interactions, including those arising between complementary DNA strands, enzyme and substrate, antigen and antibody, or lectin and carbohydrate. The latter interaction mediates crucial biological processes such as those involved in cell signaling, organogenesis, fertilization and inflammation (Lis and Sharon 1998). Carbohydrate–protein interactions have been shown to facilitate the initial attachment of pathogens to host cells and the growth and metastasis of malignant cancer cells. Understanding the mechanism of action of carbohydrate chains can be crucial for drug design and cancer treatment (Lis and Sharon 1998; Lityńska et al. 2001).

Lectins are molecules that recognize different types of carbohydrate chains. They are very useful probes in studying the carbohydrates of cell surfaces, based on their specificity in recognizing oligosaccharide structure. There are lectins recognizing mannose (or glucose), fucose, sialic acid, *N*-acetylglucosamine, *N*-acetylgalactosamine/galactose and other complex carbohydrates (Lis and Sharon 1998). Concanavalin A (ConA) from *Canavalia ensiformis* is a well-known lectin with specificity for α-D-mannosyl and α-D-glucosyl residues.

Our paper presents the results of investigations of lectin–carbohydrate interactions using glycoproteins [arylsulfatase A (ASA) and carboxypeptidase Y (CaY)] and living cells of human prostate carcinoma (PC-3) expressing concanavalin A-specific ligands (i.e., high mannose-type glycans) on their surface. For comparison a model protein system representing antigen–antibody interaction was studied. The interacting molecules were monoclonal anti-human prostatic acid phosphatase (aPAP) and prostatic acid phosphatase (PAP). The strength of ligand–receptor interaction was determined and specific and nonspecific force interactions were distinguished quantitatively based on SFM adhesion measurements and an analysis based on Poisson statistics. To confirm that the observed force originated from specific interactions between lectin and its mannose ligands, these interactions were blocked by adding either mannose or concanavalin A to the solution.

Materials and methods

Materials

Adhesion force was studied in the following systems:

1. PAP–aPAP, where PAP was prostatic acid phosphatase isolated according to the method of vanEtten (vanEtten and Saini 1978) and aPAP was monoclonal anti-prostatic acid phosphatase from mouse ascites fluid, clone PAP-12 (Sigma).
2. ConA–ASA, where ConA was the lectin concanavalin A from *Canavalia ensifomis* (ICN Biomedicals) and ASA was the glycoprotein arylsulfatase A purified from human placenta (Laidler and Litynska 1997).
3. ConA–CaY, where CaY was carboxypeptidase Y (DIG Glycan Differentation Kit, Roche).
4. ConA–PC-3, where PC-3 was a human prostate carcinoma cell line from metastasis to bone [CRL-1435; American Type Culture Collection (ATCC)].All protein solutions (PAP, aPAP, ConA, ASA, CaY) were prepared as 0.1 mg/ml concentrations in 10 mM phosphate-buffered saline (PBS, pH 7.4, Sigma-Aldrich).

PC-3 cells were cultured on Petri dishes in RPMI 1640 medium (supplemented with 10% fetal calf serum, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/l glucose and 1.5 g/l $NaHCO_3$), pH 7.4 at 37 °C in 95% air/5% CO_2 atmosphere.

Immobilization of proteins either on an SFM tip made of silicon nitride or on glass coverslips (Menzel Gläser; 24×24 mm^2, pre-treated with 0.01% v/v poly-L-lysine water solution in order to improve cell attachment to the glass substrate) was done as follows:

Immobilization of PAP or ConA on silicon nitride tips The immobilization procedure followed the protocols described by Luckham and Smith (1998) with slight modifications. Standard silicon nitride tips (MLCT-AUHW; Atos) were cleaned by immersing them in acetone for 5 min and then irradiating with UV light for 30 min. Next, they were put into 4% (v/v) 3-aminopropyltrieth-oxysilane (APTES; Sigma-Aldrich) toluene solution for 2 h. Afterwards, the silanized surfaces were washed once in ethanol and three times in PBS, and then immersed in 2.5% (v/v) solution of glutaraldehyde (Fluka) in PBS for 30 min. Next they were washed in PBS buffer and then functionalized by putting them into PAP (0.1 mg/ml) or ConA (0.2 mg/ml) PBS solution for 1 h.

Immobilization of aPAP, CaY, ASA on glass surface The glass coverslips were treated in the same way as described above. After rinsing in PBS buffer they were put into 0.1 mg/ml solution of aPAP, CaY or ASA, in PBS, pH 7.4, for 1 h.

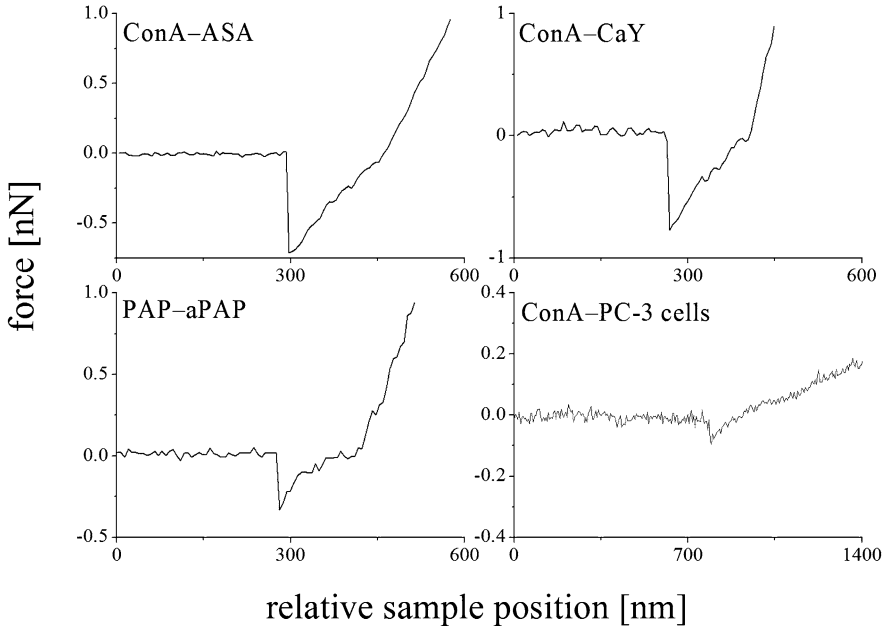

Fig. 1 Force versus relative sample position curves recorded for all investigated molecular pairs: *PAP–aPAP*, *ConA–ASA*, *ConA–CaY*, and *ConA–PC-3*. The force scale was obtained by multiplying the cantilever deflection by the spring constant value. The studies used an SFM setup in which only the sample could be moved. Therefore, *zero* on the *x*-axis means the starting point (the position of the contact point is not fixed)

SFM measurements The prepared glass coverslips and silicon nitride tips were used immediately for measurements lasting no longer than 3 h. The measurements for PAP–aPAP pairs were performed in PBS solution (pH 7.4) and for ConA–ASA, ConA–CaY and ConA–PC-3 pairs in TBS (50 mM TRIS-HCl, pH 7.4, 0.15 M NaCl, Sigma) containing 1 mM of Ca^{2+}, Mg^{2+} and Mn^{2+} ions. To show that the SFM-measured force between molecules originated from specific interaction, ligands lying on the surface were blocked by either ConA (1 mg/ml) or mannose (0.3 mM).

The measurements of molecular interaction were performed in contact mode using a home-built SFM described in detail elsewhere (Lekka et al. 1999b, 2001). To provide close to natural environmental conditions, a home-built Plexiglas liquid cell without sealing was used. Commercial silicon nitride cantilevers with spring constants of 0.01 or 0.03 N/m (Atos, Germany) and a nominal tip radius of about 50 nm were used as probes. Their spring constants were checked by monitoring the thermally excited resonant frequencies of the cantilever, and the tip radii were determined using the TGT01 silicon standard (NMDT, Russia). Their values were about 60 nm for an untreated silicon nitride tip. The deflection of the cantilever, proportional to the force acting between the end of the tip and the investigated surface, was recorded using laser photodiode technique. By monitoring the deflection as a function of the relative sample position one can obtain the relationship of the force to the distance between the probing tip and the surface (i.e., the force curve), which is composed of the two parts recorded during either the approach of the AFM tip to the surface or its withdrawal from the surface. The latter part of the force curve contains the information about the interaction force between molecules. Figure 1 shows the typical force curves taken for all investigated pairs; all curves were collected for the same velocity value of 3.5 μm/s. The total number of recorded force curves is presented in Table 1. All measurements (PAP–aPAP, ConA–CaY, ConA–ASA and ConA–PC-3) were repeated using freshly prepared SFM tips and new samples.

Table 1 Comparison of the parameters calculated for PAP–aPAP, ConA–CaY, ConA–ASA and ConA–PC-3 pairs of interacting molecules. Correlation factor *R* describes the quality of the linear regression. The values of the nonspecific force were determined as described in the text

Interacting pair	PAP–aPAP	ConA–PC-3	ConA–ASA	ConA–CaY
F for single ligand–receptor pair (pN)	530 ± 25	116 ± 17	790 ± 32	940 ± 39
F_0 if F_0 is constant (pN)	406 ± 60	147 ± 65	63 ± 30	123 ± 54
F_0 if F_0 is not constant (pN)[a]	180 ± 130	60 ± 30	240 ± 160	370 ± 110
R	0.991	0.876	0.996	0.993
Adhesion probability (%)	79	10[b]	72	60
Events attributed to specific interaction (%)	59	86	48	30

[a]The mean value of the nonspecific force and its standard deviation obtained for all measured sequences

[b]The low number of observed adhesion events for the ConA–PC-3 pair can be attributed to the lower surface density of mannose-containing ligands present in the cell membrane

Results

In this work, the adhesion force can be defined as the force needed to separate the SFM tip from the surface, and its value can be determined from the measured pull-off force (Florin et al. 1994). However, due to experimental errors, the average force value must be estimated from a histogram corresponding to a large number of curves of force versus distance. The bin size of the histogram should be chosen by estimating the theoretical force detection limit dictated by the thermal vibrations of the free cantilever (Gittes and Schmidt 1998). A more practical limit estimation (especially for measurements done in liquid solution) can be obtained from an analysis of the amplitude of fluctuations around the base line (the region of the force curve with no detectable interaction). In our study, its value was within the range of 20–30 pN.

The adhesion probability was defined as the ratio between the number of force curves showing the adhesion event and the total number of curves (see Table 1). The obtained histograms were normalized by the total number of recorded curves (i.e., multiplied by adhesion probability). For example, in the case of the PAP–aPAP pair, adhesion was present in 79% of the curves. The smallest adhesion probability was found for ConA–PC-3, where only ~10% of the curves showed the adhesion event. Such a low number of observed adhesion events can be attributed to the lower surface density of mannose-containing ligands present on the cell membrane. None of the obtained frequency distributions exhibited an observable periodicity.

The strength of a bond in a single pair of molecules was calculated according to the method proposed by Williams and coworkers (Williams et al. 1996; Lo et al. 1999, 2001). Briefly, the basis for this approach is the assumption that the observed adhesion force is the sum of a finite number of discrete, independent, randomly formed chemical bonds of similar strength, and that the number of these bonds is governed by Poisson statistics. The mean adhesion force, μ, is a product of the strength of the single-bond force, F, and the mean number of bonds, m, active during the contact of the tip with the surface ($\mu = \mathrm{mF} + F_0$), where F_0 is the value of nonspecific force. For the Poisson distribution, the variance of the bond number is equal to its mean value. Thus, the relationship between the variance and the mean adhesion force is linear ($\sigma_\mu{}^2 = F\mu - FF_0$); its slope determines the single-bond force, F, while the intercept with the y axis describes the additional nonspecific force, F_0.

As presented in Fig. 2, the relationship between the mean value of the adhesion force obtained for a given sequence of measured force curves and its variance was linear for all studied systems. Table 1 gives the determined values of bond strength and of the nonspecific force. The results showed stronger force values in case of the protein systems (PAP–aPAP, ConA–ASA, ConA–CaY) when the three types of molecules were immobilized on glass substrate than when ConA–mannose

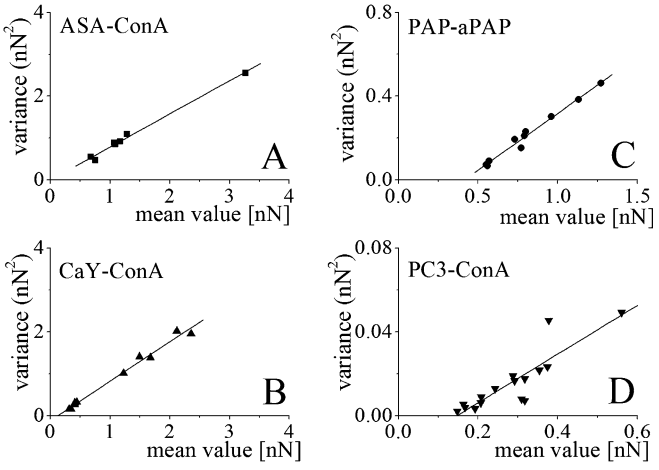

Fig. 2 Variance as a function of the mean value determined for ConA–ASA pairs (**A**), ConA interacting with CaY (**B**), PAP and aPAP pairs (**C**) and mannose ligands on a PC-3 cell surface (**D**). The *slope of the lines* gives the values of the force required to separate the two molecules, i.e., to break a single ligand–receptor bond. Events showing no adhesion (recorded frequently for ConA–PC-3) were omitted for clarity

ligands were present on the surface of PC-3 cells (530 ± 25, 790 ± 32 and 940 ± 39 pN, versus 116 ± 17 pN).

Poisson distribution analysis also produces the value of the nonspecific force occurring in liquid solutions. If the nonspecific force is present, it influences the y-axis intercept value of the linear relationship of the adhesion force and its variance. In all investigated systems the nonspecific force was present and was assumed to be constant. Its value was 406 ± 60 pN for PAP–aPAP, 63 ± 30 pN for ConA–ASA, 123 ± 54 pN for ConA–CaY, and 147 ± 65 pN for ConA–PC-3. These numbers are comparable to (and sometimes even larger than) the bond force values. For example, the nonspecific force was ~77% of the specific force for the PAP–aPAP complex and ~127% for ConA–PC-3 cells. This may indicate the strong significance of this type of interaction occurring in the presence of other molecules, a charge on the cell surface, etc. Both forces (specific and nonspecific) are large, and when added together, they produce a high value for the adhesion force (even ~1,000 pN) if the adhesion force is taken to be the sum of specific and nonspecific forces. Therefore, the simple assumption of a constant nonspecific force value seems unrealistic, and the model should be refined by introducing some distribution of the nonspecific force, represented by $\sigma^2_{F_0}$. Then the relationship between the variance and the mean value can be rewritten as follows:

$$\sigma^2_\mu = F\mu - FF_0 + \sigma^2_{F_0} \quad (1)$$

The relationship between the variance and the mean adhesion force remains linear. The slope of the line determines the single-bond force, but its intercept with the y-axis is a two-term expression containing the non-

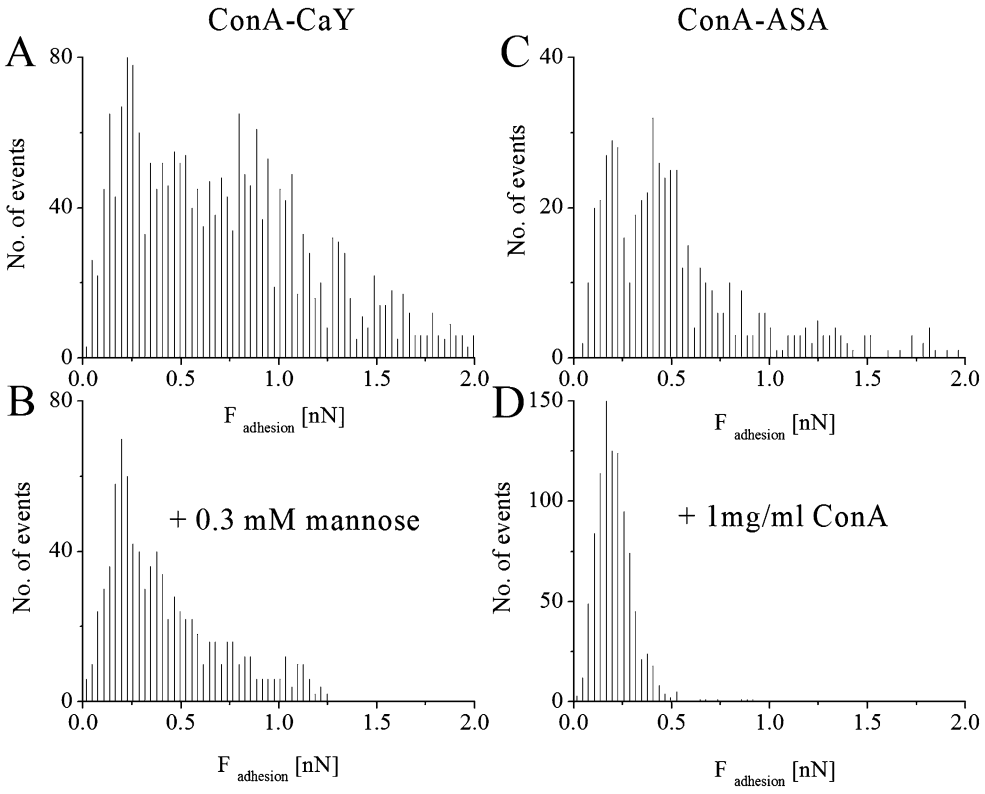

Fig. 3 Inhibition of specific interactions between ConA–CaY molecules **A** before and **B** after adding 0.3 mM mannose, and between ConA–ASA molecules **C** before and **D** after adding 1 mg/ml ConA to TBS buffer

specific force and its variance. Using Eq. (1) and substituting $\mu = mF + F_0$, the values of the nonspecific force for each measurement sequence were determined. Their average values were about 180 ± 130 pN for PAP–aPAP, 240 ± 160 pN for ConA–ASA and 370 ± 110 pN for ConA–CaY pairs, and 60 ± 30 pN for the ConA–PC-3 complex.

When the ligand–receptor complex breaking forces are measured, it is essential that control experiments be carried out to ensure that the obtained data correspond to the specific interaction. Two control measurements were made to demonstrate the specificity of the studied lectin–carbohydrate interaction. The binding sites for ConA were blocked by adding either mannose (0.3 mM) or concanavalin A (1 mg/ml) to TBS buffer (50 mM TriS-HCl, pH 7.4, 0.15 M NaCl, 1 mM of Ca^{2+}, Mg^{2+} and Mn^{2+} ions). The data obtained for the control measurements are presented in Fig. 3.

Figure 3A shows the histogram obtained before adding mannose. The frequency distribution was very broad. After adding 0.3 mM mannose, the peak at about 200 pN remained, but some higher values of the adhesion force were still measured, up to 1.2 nN (Fig. 3B). Similar results were obtained after adding 1 mg/ml concanavalin A to TBS buffer when the ConA–ASA pair was studied. The frequency distribution (Fig. 3C) before blocking showed two peaks at ~330 and ~720 pN. After blocking, only the peak at ~330 pN was observed (Fig. 3D). Therefore, the higher peak position was unambiguously attributed to the specific interaction between ConA and mannose ligands, and the lower one to the nonspecific interaction between these molecules.

Discussion

The adhesion phenomenon is crucial for living cells. It is responsible for the majority of cell interactions with other cells and/or with various proteins of the external environment (Lis and Sharon1998; Hammer 1996; BenZe'ev 1997; Pertuzelli et al. 1999). The loss or alteration of these interactions may affect cell functioning and lead to many pathological states. Investigation of the interaction mechanism is key to understanding a wide spectrum of biological processes such as inflammation or cancer metastasis.

Analysis of the parts of force curves recorded during withdrawal of the tip from the surface demonstrates the potential of SFM technique to provide information about how a molecule binds to its specific partner (Capella and Dietler 1999; Heinz and Hoh 1999). The value of the force required to separate a ligand from its receptor can be a useful parameter for describing the strength of the binding force in a ligand–receptor pair. This force is characteristic for each pair of molecules. It should be pointed out that the glutaraldehyde used as a cross-linking agent attaches protein molecules to the SFM tip as well as to the substrate by forming strong covalent bonds. During separation of the two molecules, SFM measurement of the pull-off force not only gives a measure of the adhesion force but also indicates the

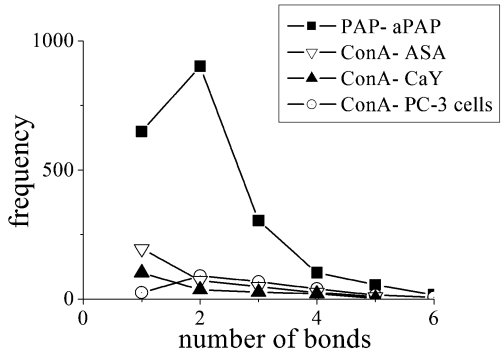

Fig. 4 Frequency distribution of the number of bonds. The maximum of the distribution gives the most probable number of bonds formed between molecules

weakest bond of the system. On the other hand, it is known that specific ligand–receptor interaction involves a large number of weak noncovalent bonds (Leckband 2000). Thus the measured adhesion force can be attributed to the force acting between molecules. However, the adhesion force measured by SFM can originate from specific interaction, nonspecific interaction or both. This makes it difficult to determine which type of force is involved in the measured adhesion force when there is no evident periodicity present in the histograms. The specificity of molecular interaction can be confirmed by blocking binding sites and in this way preventing specific ligand–receptor interaction (Hinterdorfer et al. 1996; see Fig. 2).

Single-bond force values were determined according to the Poisson statistical analysis method described above. This method makes it possible to differentiate specific and nonspecific forces and to obtain quantitative descriptions of each component. Note that it gives the value of the force acting between two single molecules and *not* the total adhesion force, which is the sum of single adhesion interactions occurring within the contact area between the SFM tip and the investigated surface. The advantage of the Poisson statistical analysis method is that it is independent of the tip shape and contact area.

The ligand–receptor bond strength investigated for monoclonal anti-human prostatic acid phosphatase and prostatic acid phosphatase (the antigen–antibody system characterised by a single type of interaction) was represented by the linear relationship between the variance and its mean force. The value for the antigen–antibody system (PAP–aPAP, 530 ± 25 pN) was larger than known from the literature for other protein–ligand pairs composed of, for example, streptavidin and biotin (207 ± 5.8 pN; Yuan et al. 2000). This is explained if we remember that the loading rate together with the binding force describes the dynamic response of the individual ligand–receptor pair to forces breaking the bond. A higher loading rate leads to an increase in the value of the force breaking the bond (Bell 1978). In our experiment, the loading rate of 16,200 pN/s (determined for a system spring constant of 3.2 mN/m) was seven times higher than the reported loading rate for the streptavidin–biotin complex (Yuan et al. 2000) of 2,300 pN/s. This fact can be used to separate the specific interaction from nonspecific forces occurring during AFM measurement, as the latter do not depend on the loading rate.

The values of the force required to break the bond between molecules immobilized on substrate were larger (790 ± 32 pN for ConA–ASA; 940 ± 39 pN for ConA–CaY) than in the case of measured interaction between ConA and mannose ligands present on a surface of PC-3 cells (116 ± 17 pN). This stronger interaction may originate from its own higher affinity and the lack of other structures that could screen the specific interaction; in the case of mannose ligands present on a cell surface, the specific interaction can be screened by the glycocalix components, which are charged in native conditions (the glycocalix viewed as the outer surface of the cell membrane covered by branched oligosaccharide chains covalently bound to glycoproteins or glycolipids).

Surprisingly, the linearity of the relationship between the mean adhesion value and the variance of the adhesion force suggests that ConA molecules interacted in only one way with the receptors present on the outer part of the PC-3 cell membrane. The lectin mainly recognizes the α–mannose unit present in the high mannose-type glycans constituting membrane glycoproteins. The obtained binding force may originate from the interaction between ConA and the minimal oligosaccharide structure required for binding that extends beyond the glycocalix. Their different values may be attributed to differences in the access of concanavalin A to its ligands in each case.

Together with the values of the specific force, the component resulting from nonspecific forces can also be obtained by the method described in this paper (see Table 1). These nonspecific forces arise mainly from electrostatic interactions due to the charge distributed on the cell surface and on molecules, both charged when immersed in aqueous solution. They are significant even though they are not directly involved in the specific binding process, and the interplay between specific and nonspecific forces controls all molecular interactions (Leckband 2000). Maximum errors (estimated as $\pm 3\sigma$ from the mean value) were used to sort the experimental data in order to evaluate the number of specific adhesion events. The remaining cases were attributed to adhesion originating from nonspecific forces occurring between protein interactions.

Next, the distribution of the number of created bonds was determined (Fig. 4). The most probable value for the bond number was obtained from the distribution peak value. It was two bonds for PAP–aPAP systems and one bond for all types of ConA interactions. The number of bonds formed depends on the density of ligands and receptors within the contact area between the SFM tip and the surface, and on the affinity of the molecules that form the bonds.

To summarize, scanning force microscopy was used to study interactions between PAP–aPAP molecules representing a single type of interaction (antigen–antibody) and between concanavalin A and high mannose-type glycans either present on the surface of PC-3 cells or constituting part of the glycoproteins representing lectin–carbohydrate interaction. In all cases the value of the binding force between a single pair of molecules was evaluated with the Poisson statistical analysis method. This method was combined with maximum error estimation in a sorting procedure performed to determine the nonspecific component of the measured adhesion interaction. The characteristic binding force was obtained for all the investigated molecular pairs. Particularly promising is the estimation of the binding force obtained between a single receptor and its corresponding ligands lying on the surface of the living cell, where the presence of different types of others molecules could interfere with the interaction. The ability to distinguish between different kinds of receptors may prove very useful in investigating cell-affinity alterations due to treatment of cells, or membrane receptor expression in different types of cancers.

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References

Bell G (1978) Models for the specific adhesion of cells to cells. Science 200:616–627

Ben-Ze'ev A (1997) Cytoskeletal and adhesion proteins as tumor suppressors. Curr Opin Cell Biol 9:99–108

Capella B, Dietler G (1999) Force–distance curves by atomic force microscopy. Surf Sci Rep 34(1–3):1–104

Evans E (2001) Probing the relation between force lifetime and chemistry in single molecular bonds. Annu Rev Biomol Struct 30:105–128

Florin E, Moy VT, Gaub H (1994) Adhesion forces between individual ligand–receptor pairs. Science 264:415–417

Gad M, Itoh A, Ikai A (1997) Mapping cell wall polysaccharides of living microbial cells using atomic force microscopy. Cell Biol Int 21(11):697–706

Gittes F, Schmidt Ch (1998) Thermal noise limitations on micromechanical experiments. Eur Biophys J 27:75–81

Grandbois M, Dettmann W, Benoit M, Gaub HE (2000) Affinity imaging of red blood cells using an atomic force microscope. J Histochem Cytochem 48(5):719–724

Grange W, Strunz T, Schumakovitch I, Güntherodt HJ, Hegner M (2001) Molecular recognition and adhesion of individual DNA strands studied by dynamic force microscopy. Single Mol 2:75–78

Hammer DA (1996) Biological adhesion at interfaces. Annu Rev Mater Sci 26:651–691

Heinz WF, Hoh J (1999) Spatially resolved force spectroscopy of biological surfaces using the atomic force microscope. Trends Biotechnol 17:143–150

Hinterdorfer P, Baumgartner W, Gruber H, Schilcher K, Schindler H (1996) Detection and localisation of individual antibody–antigen recognition events by atomic force microscopy. Proc Natl Acad Sci USA 93:3477–3481

Laidler P, Lityńska A (1997) Arylsulfatase A from human placenta possesses only high mannose-type glycans. Int J Biochem Cell Biol 29(3):475–83

Leckband D (2000) Measuring the forces that control protein interactions. Annu Rev Biophys Biomol Struct 29:1–26

Lee G, Metzger S, Natesan M, Yanavich C, Dufrêne YF (2000) Implementation of force differentiation in the immunoassay. Anal Biochem 287:261–271

Lekka M, Laidler P, Gil D, Lekki J, Stachura Z, Hrynkiewicz AZ (1999a) Elasticity of normal and cancerous human bladder cells studied by scanning force microscopy. Eur Biophys J 28(4):312–316

Lekka M, Lekki J, Marszaek M, Golonka P, Stachura Z, Cleff B, Hrynkiewicz AZ (1999b) Local elastic properties of cells studied by SFM. Appl Surf Sci 141(3–4):345–349

Lekka M, Laidler P, Ignacak J, abedź M, Lekki J, Struszczyk H, Stachura Z, Hrynkiewicz AZ (2001) The effect of chitosan on stiffness and glycolytic activity of human bladder cells BBA. Mol Cell Res 1540(2):127–136

Lis H, Sharon N (1998) Lectins: carbohydrate-specific proteins that mediate cellular recognition. Chem Rev 98(2):637–674

Lityńska A, Przybyo M, Pochec E, Hoja–ukowicz D, Cioczyk D, Laidler P, Gil D (2001) Comparison of the lectin-binding pattern in different human melanoma cell lines. Melanoma Res 11(3):205–212

Lo YS, Huefner ND, Chan WS, Stevens F, Harris JM, Beebe TP (1999) Specific interactions between biotin and avidin studied by atomic force microscopy using the Poisson statistical analysis method. Langmuir 15:1373–1382

Lo YS, Zhu Y, Beebe T (2001) Loading-rate dependence of individual ligand–receptor bond–rupture forces studied by atomic force microscopy. Langmuir 17:3741–3748

Luckham PF, Smith K (1998) Direct measurement of the recognition forces between proteins and membrane receptors. Faraday Discuss 111:307–320

Musiani S, Batelli MG (2003) Mannose receptor determination by an ELISA-like method. J Biochem Biophys Meth 55:121–125

Petruzzelli L, Takami M, Humes D (1999) Structure and function of cell adhesion molecules. Am J Med 106:467–476

Ros R, Schwesinger F, Anselmetti D, Kubon M, Schäfer R, Plückthun A, Tiefanauer L (1998) Antigen binding forces of individually addressed single-chain Fv antibody molecules. Proc Natl Acad Sci USA 95:7402–7405

Rotsch Ch, Braet F, Wisse E, Radmacher M (1997) AFM imaging and elasticity measurements on living rat liver macrophages. Cell Biol Int 21(11):685–696

vanEtten RL, Saini MS (1978) Selective purification of tartrate-inhibitable acid phosphatase: rapid and efficient purification (to homogeneity) of human and canine prostatic acid phosphatases. Clin Chem 24(9):1525–1530

Werten PJL, Rémigy HW, de Groot BL, Fodias D, Philipsen A, Stahlberg H, Grubmüller H, Engel A (2002) Progress in the analysis of membrane protein structure and function. FEBS Lett 529:65–72

Williams JM, Han T, Beebe TP (1996) Determination of single-bond forces from contact force variances in atomic force microscopy. Langmuir 12:1291–1295

Yuan Ch, Chen A, Kolb P, Moy VT (2000) Energy landscape of streptavidin–biotin complexes measured by atomic force microscopy. Biochemistry 39:10219–10223