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Individual Supramolecular Host−Guest Interactions Studied by Dynamic Single Molecule Force Spectroscopy

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Abstract: Supramolecular host−guest interactions in aqueous environment were studied by dynamic single molecule force spectroscopy. The unbinding between ferrocene moieties immobilized on atomic force microscopy tips and β-cyclodextrin receptors in highly ordered self-assembled monolayers on Au(111) was studied. The rupture force of individual ferrocene−β-cyclodextrin complexes was found to be 56 ± 10 pN. The value of this unbinding force was independent of the unloading rate. This was attributed to the fast (de)complexation kinetics of the host−guest complex.

Introduction

With the advent of scanning probe techniques, such as atomic force microscopy (AFM),[1] real space nanometer scale structural characterization and measurements of forces with piconewton resolution have become possible with the same instrument.[2] Consequently, there is an increasing interest to measure individual molecular interactions using force microscopy.[3,4] Examples reported in the literature include interactions between, e.g., cell binding proteoglycans,[3d] complementary strands of DNA,[3c] or biotin−(strept)avidin.[3a,3b,4] These studies on the lock and key principle at the level of individual molecules focused primarily on biological receptors with relatively high molar masses and thus sizes, and *very* slow unbinding kinetics. Evans c.s. showed that for the latter systems the most probable unbinding forces are highly dependent on the (un)loading rate of the experiment.[5] In this paper we report on our findings for a low molecular weight supramolecular system with very fast (un)binding kinetics[6] for which we found an unloading rate independent host−guest complex rupture force of 56 ± 10 pN.

Experimental Section

Synthesis of β-CD(OH)$_{14}$ was carried out by a 7-fold DDC coupling of heptakis(6-deoxy-6-amino)-β-cyclodextrin[7,8,9] with 12-(thiododecyl)-undecanoic acid. Monolayers were prepared at 60 °C, which is essential for obtaining highly ordered monolayers.[10] The characterization of monolayers of the corresponding β-CD derivatives on gold was previously published.[9] The substitution pattern of the adsorbate has negligible influence on the structural properties. Characterization of the β-CD-(OH)$_{14}$ monolayer by wettability studies showed efficient wetting by water, indicating that the cavities are exposed at the surface of the monolayer.[9,10b]

AFM and Tip Modification. Triangular shaped silicon nitride cantilevers and silicon nitride tips (Digital Instruments (DI), Santa Barbara, CA) were coated with 50−70 nm of gold in a Balzers SCD

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(4) Other experimental work on force spectroscopy has been carried out by optical tweezers (Kuo, S. C.; Sheetz, M. P. *Science* **1993**, *260*, 232−234.) and biomembrane force probe techniques (see ref 5).

(5) For unbinding kinetics which are slow compared to the rate of pulling force, a pronounced rate dependence of the unbinding forces has been predicted theoretically: (a) Evans, E.; Ritchie, K. *Biophys. J.* **1997**, *72*, 1541−1555. These predictions were recently supported by experimental data: (b) Merkel, R.; Nassoy, P.; Leung, A.; Ritchie, K.; Evans, E. *Nature* **1999**, *397*, 50−53.

(6) The *equilibrium* rate constant of decomplexation, k_d, for our system is estimated to be 10^5-10^6 s^{-1} based upon the known stability constant of ferrocenecarboxylic acid in β-CD (2400 M^{-1}: Godínez, L. A.; Schwartz, L.; Criss, C. M.; Kaifer, A. E. *J. Phys. Chem. B* **1997**, *101*, 3376−3380) and the assumption of diffusion-limited complexation [(complexation rate constant, $k_c = 10^8$ (M s)$^{-1}$].[a] Thus, binding processes occur on a time scale of μs, while the time resolution of the AFM is limited to the order of 0.1 to 1.0 ms and slower. (The time resolution of the AFM experiment is given by the pixel resolution of the AFM data (512 pixels) and the scan rate of the piezo transducer which is typically ≤1.0 to 10.0 Hz.) On the contrary, the association and dissociation rate constants of for example the avidin−biotin complex are 7×10^7 (M s)$^{-1}$ and 9×10^{-8} s^{-1}, respectively, resulting in binding processes with a time scale of months.[b] (a) Szejtli, J.; Osa, T. *Comprehensive Supramolecular Chemistry*, Vol 3, *Cyclodextrins*; Elsevier Science Publishers: Oxford 1996. (b) Green, N. M.; Toms, E. J. *Biochem. J.* **1973**, *133*, 687.

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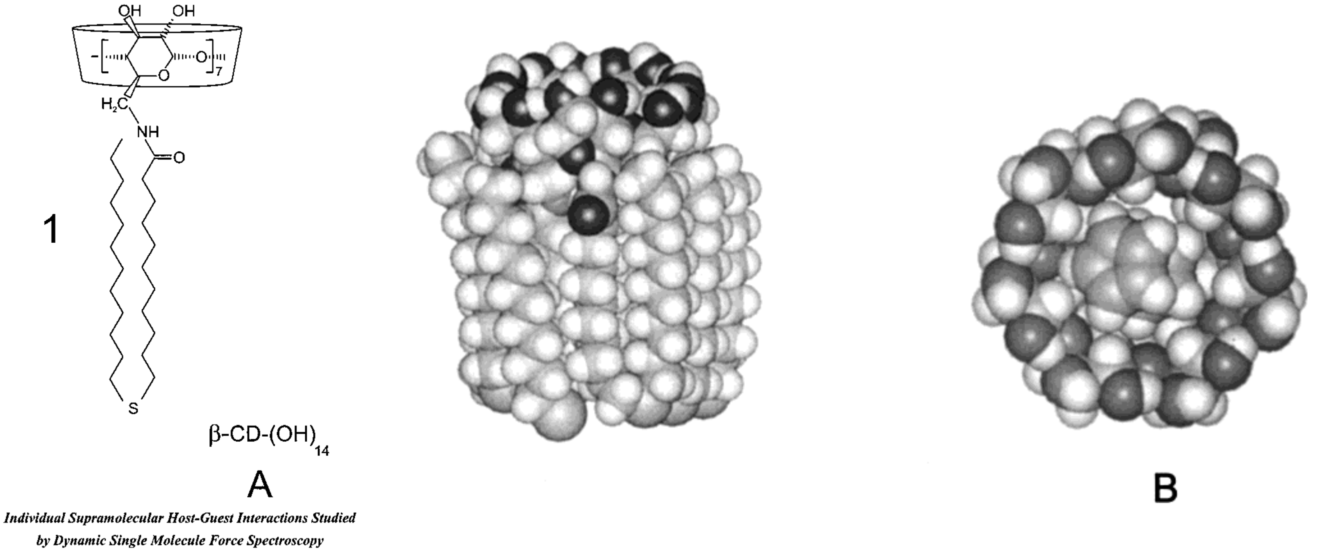

Figure 1. (A) β-Cyclodextrin CD-(OH)$_{14}$ heptasulfide receptor adsorbate **1** and space filling representation. (B) Schematic representation of a β-cyclodextrin−ferrocene complex.

040 sputtering machine at an argon pressure of 0.1 mbar or alternatively with ca. 2 nm Ti and ca. 75 nm Au in high vacuum (Balzers). The tips were functionalized as described previously[11] in solutions with a 99:1 mixture of 2-hydroxy-ethanethiol and 6-ferrocenyl-hexanethiol or 2-hydroxy-ethanethiol in ethanol:chloroform (1:2). The AFM measurements were carried out with a NanoScope III multimode AFM (DI) utilizing a liquid cell (DI). The piezo positioner was calibrated in the *z*-direction by measuring step heights of Au(111). The cantilever spring constants were calibrated as described by Tortonese and Kirk.[12] Force−distance curves (300 to 400) were recorded in pure water and 100 μM aqueous 1,8-ANS at different positions on the sample surface with a maximum external load of <1.0 nN. The unloading rate was varied between 2.00×10^2 pN/s and 2.34×10^5 pN/s. The corresponding reference measurements were carried out in pure water. The quantitative analysis of the observed individual pull-off events was performed for 100 force curves of a given set. Each individually resolved pull-off event with a rupture force of >10 pN was included in the analysis. An autocorrelation function of the histograms of the pull-off forces was calculated according to the following:

$$G(x) = \frac{1}{N_x}\sum_{k=1}^{N_x} \Delta f(k)\, \Delta f(k + x) \tag{1}$$

with $\Delta f(k) = f(k) - 1/N \sum_{j=1}^{N} f(j)$.

Results and Discussion

We have studied the complexation of ferrocene moieties in β-cyclodextrin (β-CD) heptasulfide receptors (Figure 1A and B). β-CD, which is a cyclic heptamer of glucose, is well-known to bind apolar guests, such as ferrocene, adamantane, or 1-anilinonaphthalene-8-sulfonic acid (1,8-ANS), in aqueous environment.[13] For the immobilization in self-assembled monolayers (SAMs) on gold the receptor was equipped with seven sulfide units.

The structure of SAMs of β-cyclodextrin derivative **1** was elucidated with a large number of techniques, such as electrochemistry, wettability studies, X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), and AFM. The results confirm the formation of highly ordered monolayers in which the β-cyclodextrin cavities are exposed at the surface of the monolayer. The ordering of the cavities in SAMs of β-CD-(OMe)$_{14}$ was unveiled by AFM. In Figure 2A,B a quasi-hexagonal lattice with a periodicity of 2.1 nm can be recognized. This value as a next neighbor distance corresponds well to the size of the adsorbate molecules. In these SAMs the alkane segments fill the space underneath the headgroup leading to highly ordered monolayers which expose the cavities to external guests in a readily accessible manner.[14]

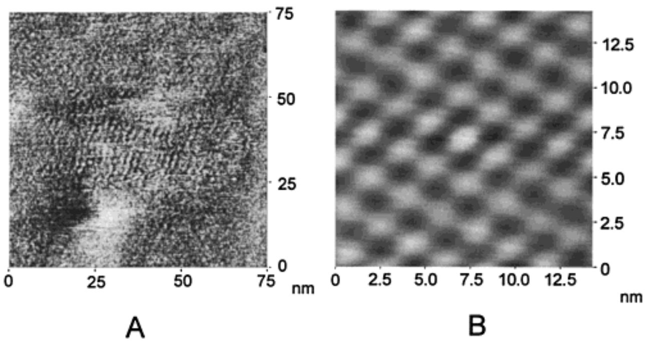

Figure 2. (A) Unfiltered friction mode AFM image of a β-CD-(OMe)$_{14}$ SAM. (B) Autocorrelation filtered AFM height image of a β-CD-(OMe)$_{14}$ SAM. Both AFM images were acquired by contact mode AFM in water.

The complexation behavior of SAMs of β-CD **1** was probed by dynamic force spectroscopy. Gold-coated AFM tips were modified with a mixed SAM of 2-hydroxy-ethanethiol and 6-ferrocenyl-hexanethiol.[15] These mixed SAMs contained 1−2% of 6-ferrocenyl-hexanethiol as shown by integration of the ferrocene signal in separate cyclic voltammetric experiments on standard evaporated gold specimens.[16] The ferrocene moieties in this mixed monolayer have a long spacer compared to 2-hydroxy-ethanethiol which renders them flexible and in a solution-like state.

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[14] For details on our concept see: Thoden van Velzen, E. U.; Engbersen, J. F. J.; de Lange, P. J.; Mahy, J. W.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1995**, *117*, 6853−6862.

[15] 6-Ferrocenyl-hexanethiol was synthesized according to a literature procedure (Creager, S. E.; Rowe, G. K. *J. Electroanal. Chem.* **1994**, *370*, 203−211). 1,8-ANS, 2-hydroxy-ethanethiol, and hexadecanethiol were purchased from Aldrich and used as received.

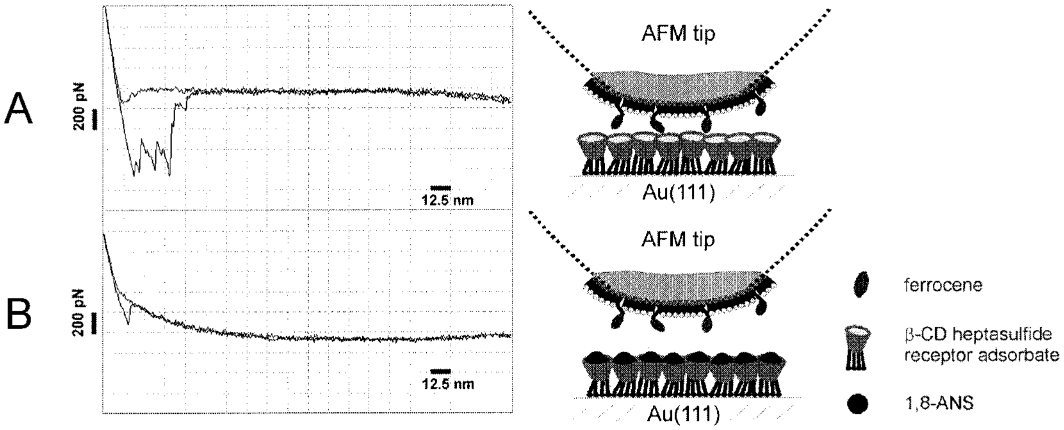

Figure 3. Force−distance curves recorded for the interaction between mixed hydroxyl-ferrocene tips and β-CD **1** in water prior to addition of 1,8-ANS (A, top) and after the addition of 1,8-ANS (B, bottom). The unloading rate was 5.00×10^4 pN/s. The interaction between β-CD cavities and ferrocene moieties in the absence and in the presence of 1,8-ANS is depicted schematically on the right.

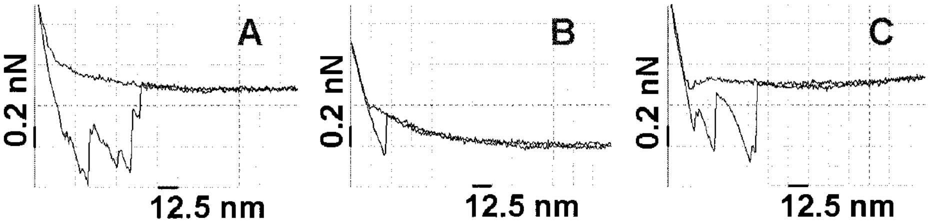

Figure 4. Force−distance curves recorded for the interaction between mixed hydroxyl-ferrocene tip and β-CD **1** in water prior to addition of 1,8-ANS (A), after the addition of 1,8-ANS (B), and after a subsequent flush with water (C).

Force−distance curves measured with these functionalized tips on SAMs of β-CD **1** on atomically flat Au(111) in ultrapure water always showed multiple pull-off events as depicted in Figure 3A. After replacing the water by an aqueous solution of the external guest 1,8-ANS (100 μM),[17] the critical pull-off forces were smaller and the number of pull-off events was drastically reduced. In about 50% of the force−distance curves measured in 1,8-ANS solutions only one pull-off event was observed (Figure 3B). The other 50% of the force−distance curves exhibited up to three pull-offs indicating that there are some residual β-CD-(OH)$_{14}$ cavities free for complexation of ferrocene. After washing away the 1,8-ANS with water, the multiple pull-offs were observed again (Figure 4). These observations are attributed to reversible blocking of the β-CD-(OH)$_{14}$ cavities by the external 1,8-ANS guest molecules. Electrochemical impedance spectroscopy results of the β-CD-(OH)$_{14}$ monolayer fully support this interpretation.[9]

Separation distances larger than the length of the adsorbate molecules can be rationalized by taking the granular morphology of the gold coating of the film into account (grain diameter ca. 25−50 nm). In case of several gold grains in the contact area a slight torsion of the cantilever is possible. The tip stays in contact with the surface at *all times* during the multiple individual pull-off events until the final pull-off occurs, and may lose contact at different grains at a time. This results in the observed "virtual" separation distances. It should be noted that the piezo travel to the final point of snap-off is always smaller than the maximum grain size observed by high-resolution SEM.

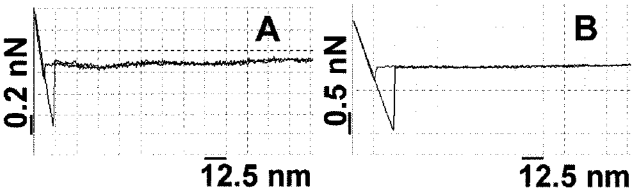

Figure 5. Force-distance curves of control experiments carried out on pure β-CD SAMs on Au(111) using 2-hydroxy-ethanethiol functionalized tips (A, no guest) and on 2-hydroxy-ethanethiol SAMs on Au(111) using the mixed hydroxyl-ferrocene-modified tips (B, no host).

AFM control experiments were carried out with 2-hydroxy-ethanethiol modified tips (no guest) on β-CD **1** SAMs and with mixed 2-hydroxy-ethanethiol and 6-ferrocenyl-hexanethiol modified tips on 2-hydroxy-ethanethiol SAMs (no host). The corresponding force−distance curves showed single pull-offs indicating the absence of specific interactions (Figure 5).[18]

The occurrence of multiple individual pull-off events during retraction of the sample from the tip is attributed to the rupture of host−guest complexes at the retracting contact area between

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(16) The redox signal of the ferrocene unit was integrated in cyclic voltammograms measured in 0.1 M NaClO$_4$ for monolayers of different ratios of the two adsorbates in the adsorption solution (1−100% 6-ferrocenyl-hexanethiol).

(17) Electrochemical impedance spectroscopy (EIS) measurements indicated a Langmuir type of adsorption of 1,8-ANS to the β-CD monolayer ($K = 289000$ M^{-1}, and $\theta_{100\ \mu\mathrm{M}} = 0.97$).[9] Permethylation of cyclodextrin, as in β-CD-(OMe)$_{14}$, breaks the hydrogen bond array between neighboring glucose units and therefore results in collapse of the cavity. No complexation could be detected for the β-CD-(OMe)$_{14}$ monolayer. This blank experiment, using the β-CD-(OMe)$_{14}$ monolayer, proves furthermore that the observed interaction for the β-CD-(OH)$_{14}$ monolayer is due to host−guest interactions and is not a result of an aspecific interaction, for example, with the alkyl chains. (a) For a fundamental discussion on EIS see: Flink, S.; Boukamp, B. A.; van den Berg, A.; van Veggel, F. C. J. M.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1998**, *120*, 4652−4657.

(18) The magnitude of the pull-off forces in the control experiments is characteristic for interactions measured in water. In additional control experiments with hexadecanethiol modified tips (no guest) on β-CD-(OH)$_{14}$ SAMs and with mixed 2-hydroxy-ethanethiol and 6-ferrocenyl-hexanethiol modified tips on hexadecanethiol SAMs (no host), specific interactions were also absent. Furthermore, tips modified with 100% 6-ferrocenyl-hexanethiol also showed no specific interactions with β-CD-(OH)$_{14}$ SAMs, proving the importance of accessibility for the ferrocene units. (a) Noy, A.; Frisbie, C. D.; Rozsnyai, L. F.; Wrighton, M. S.; Lieber, C. M. *J. Am. Chem. Soc.* **1995**, *117*, 7943−7951. (b) Sinniah, S. K.; Steel, A. B.; Miller, C. J.; Reutt-Robey, J. E. *J. Am. Chem. Soc.* **1996**, *118*, 8925−8931.

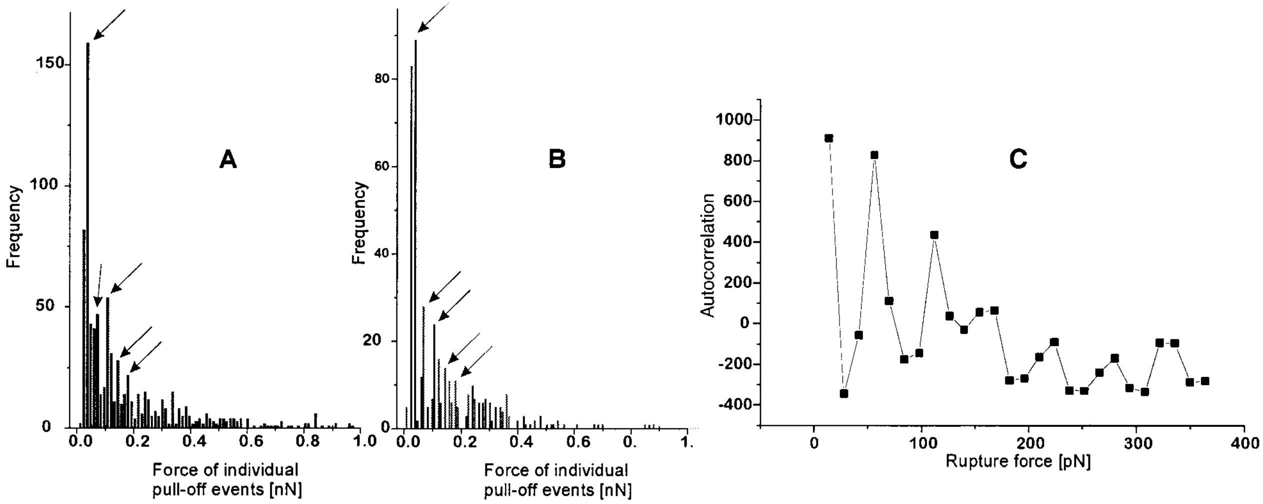

Figure 6. Histogram of individual pull-off forces measured in AFM force distance curves between a SAM of β-CD **1** and a mixed hydroxyl-ferrocene tip in water (A) and after the presence of 1,8-ANS and a thorough flush with water (B) (arrows indicate force maxima). The autocorrelation analysis of the data obtained in three independent experiments is shown in C.

probe and surface. The analysis of the individual pull-offs revealed a quantized adhesion force. In the histograms shown in Figure 6 A,B, distinct maxima at equidistant force are observed, as was confirmed by autocorrelation analysis (Figure 6C).

These maxima, which are multiples of 56 ± 10 pN,[19] are not altered after blocking of the cavities with 1,8-ANS and subsequent wash-out by water (Figure 6B).[20,21] Furthermore, the number of individual pull-offs observed in the AFM experiments (10−20) corresponds well to the number of ferrocene-terminated molecules on the contact area between SAM and the AFM tip (14−28).[22] We attribute the occurrence of multiple pull-offs to slightly different distances of the ferrocenes to the β-CD **1** surface, due, for example, to curvature of the AFM tip and the aforementioned granular nature of the gold on the tip.[3b,23]

In a theoretical analysis of the influence of the unloading rate on the rupture force by Evans and Ritchie,[5a] the reformation of the broken bonds is not considered. Therefore, at very low unloading rates, they found the spontaneous dissociation of the complex to be the most frequent rupture process, which was accompanied by a rupture force approaching zero upon further decrease of the unloading rate.[5b,24] In practice, the detection of the AFM tip retraction upon bond rupture is limited to a time scale of 10^{-3} to 10^{-4} s. In our experiments, the complexation and decomplexation are fast (time scale of 10^{-6} s) compared to the force spectroscopy measurements. In rate-dependent measurements we did not observed any pronounced dependence of the host−guest complex rupture force on the unloading rate for over 3 orders of magnitude (Figure 7).

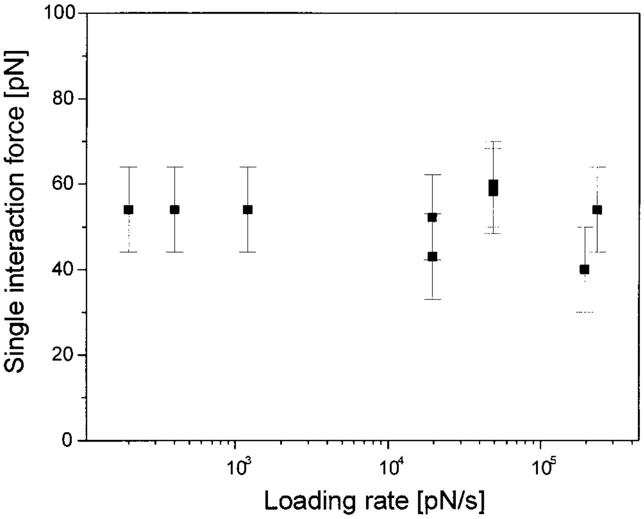

Figure 7. The quantized rupture force as a function of the unloading rate of the AFM experiment.

If one takes the total number of interactions per retraction cycle and the curvature and inhomogeneity of the tip into account (vide supra), the observed rate-independence of the rapture force can be understood. As long as a force smaller than the rupture force is exerted on a host−guest couple, spontaneous complexation and decomplexation takes place. The exertion of force is translated into strain energy in the spacer of the guest on the AFM tip and the alkyl chains of the β-CD adsorbate in the bound state. On the time scale of the host−guest binding kinetics, the tip remains at a fixed position and there is rapid, spontaneous decomplexation. Recomplexation remains possible as long as the strain energy in the bound state is overcome by the energy release upon complexation. Due to the slow relative displacement of the AFM tip, retraction of the tip is only possible when the strain energy becomes larger than the binding energy, and therefore such events occur at given force values.

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[19] The error of the individual host−guest complex rupture force ΔF was calculated based on Gauss error analysis and is primarily caused by the error in determining the cantilever spring constant k: $\Delta F = [((\mathrm{d}F/\mathrm{d}k)\,\Delta k)^2 + ((\mathrm{d}F/\mathrm{d}z)\,\Delta z)^2]^{1/2}$.

[20] The number of pull-offs was reduced by 20−30% for each cycle, which may be attributed to irreversible damage of the β-CD SAM and/or the tip.

[21] The autocorrelation function of the histogram of the second cycle, after washing the 1,8-ANS away, again proved a quantized rupture force of 56 pN. Furthermore, the few pull-offs sometimes observed in the AFM measurements in the presence of 1,8-ANS are also multiples of 56 pN.

[22] The contact area of the AFM tip was estimated based on the Johnson−Kendall−Roberts theory of contact: Johnson, K. L.; Kendall, K.; Roberts, A. D. *Proc. R. Soc. London A* **1971**, *324*, 301−313. An external load of 1.0 nN, a surface energy per unit area ($W = 10^{-3}$ N/m), and a tip radius (R = 100 nm) were experimentally determined. The following parameters for the SAMs were assumed: Young's modulus $E = 0.5$ GPa, Poisson's ratio $\nu = 0.3$. The number of ferrocene-terminated molecules in the contact area between the tip and the mixed SAMs can be estimated to be ~14−28 molecules, based on 1−2% of ferrocene-terminated thiol in the SAM (see ref 12).

[23] In high resolution scanning electron microscopy (SEM) images of modified tips the granular nature of the gold coating is readily seen (Schönherr, H. Ph.D. Thesis, University of Twente, 1999).

[24] In the ultrafast unloading regime only viscous frictional drag retards the separation (see ref 5).

On the basis of the comparison of the equilibrium binding kinetics and, in particular, the unloading rate independence observed in our experiments, we conclude that the ferrocene−β-CD interactions are probed under thermodynamic control. Although within a class of very similar host−guest complexes the rupture forces may scale linearly with the binding enthalpy,[3b] conversion of rupture force to binding enthalpy is generally not possible since the experimentally detected force is the derivative at a single (inflection) point of the potential energy curve while the binding enthalpy results from integration over the path of unbinding.

Conclusion

In conclusion, we have shown that the interaction between ferrocene moieties immobilized in mixed SAMs on AFM tips and highly ordered SAMs of β-CD heptasulfide receptor adsorbate **1** on Au(111) can be resolved by dynamic force spectroscopy. The analysis of the individual pull-off events revealed an unloading rate-independent quantized rupture force of 56 ± 10 pN. Our results show that AFM measurements can be used to obtain characteristic rupture forces for host−guest complexes, even and in particular under conditions where the kinetics of binding is rapid on the AFM time scale. This opens exciting new opportunities for molecular level studies of interactions in supramolecular chemistry.

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