Single-Molecule Studies

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Pulling a Single Polymer Molecule off a Substrate Reveals the Binding Thermodynamics of Cosolutes**

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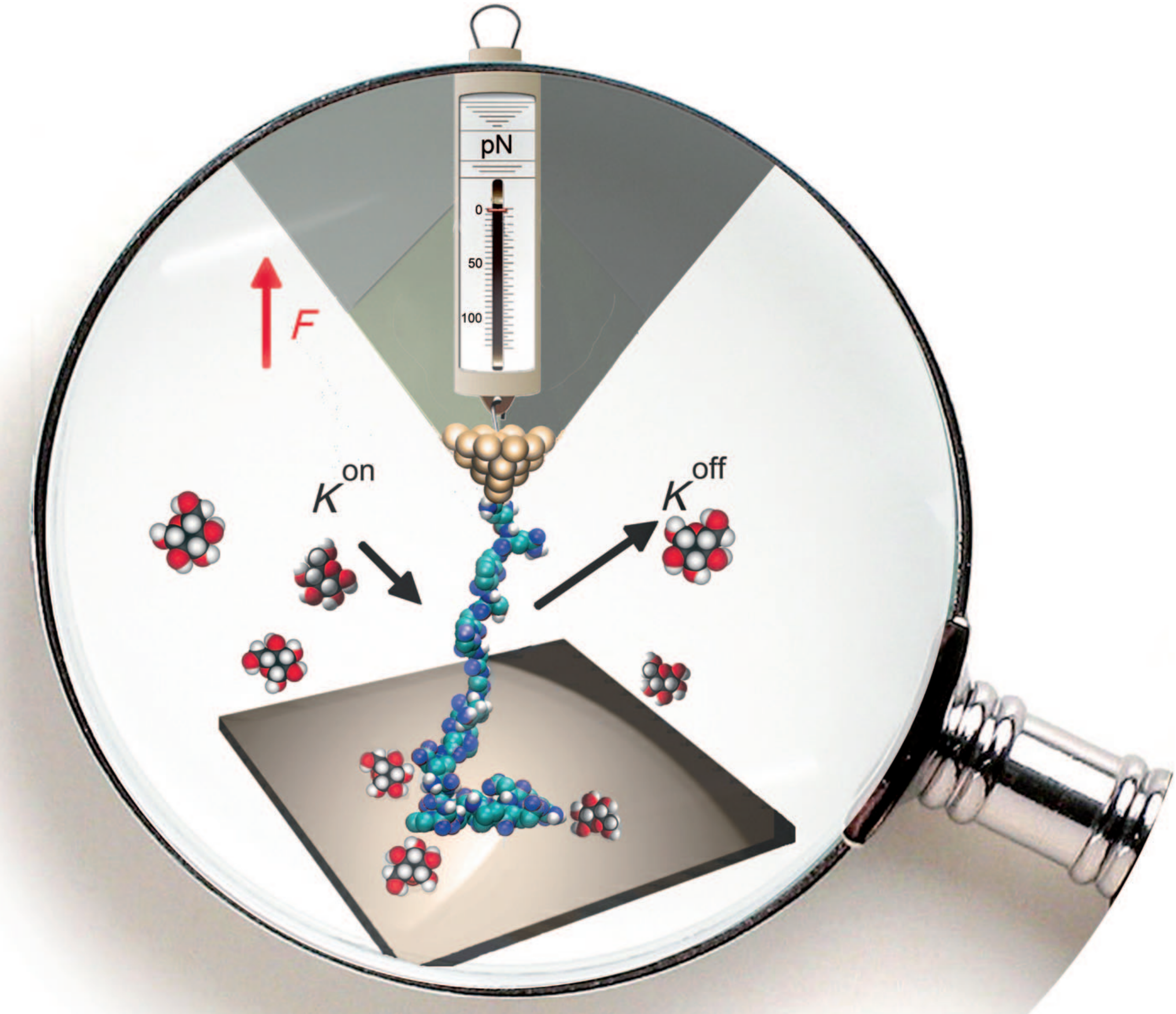

Cosolutes, such as ions, ligands, or small biomolecules, can cause a protein to fold into its biologically functional native form, to associate, to adhere or desorb from an interface, or even change its mechanical properties.[1–5] At the same time, the interaction of cosolutes with macromolecules, such as proteins, lipids and DNA, are themselves modified by changes in the environment, in particular by binding to surfaces, for example the cell membrane or the surface of histones and microtubules.[6,7] We combine single molecule atomic force microscopy with thermodynamic modeling and thereby extract the binding parameters of cosolutes onto a macromolecule in solution as well as in its surface adsorbed state. Specifically, we obtain values for the adsorption site lengths (inverse maximal line densities) and the association constants. The considerable effect of substrates on cosolute binding is demonstrated with glucose as cosolute, poly(allylamine) as polymer, and surgical stainless steel or oxidized diamond as substrates.

The thermodynamics of ligand binding onto a polymer was recently discussed[8] in terms of a one-dimensional Gibbs adsorption isotherm. In this variant of the classical Gibbs equation,[9] the surface tension is replaced by the line tension τ and the surface excess per area is replaced by the line excess per length Γ. The relationship between Γ and τ for a single polymer under fixed mechanical force F_{Des} is given by Equation (1)[8]

$$\Gamma = \frac{\theta}{d} = -\left.\frac{\partial \tau}{\partial \mu}\right|_{F_{\mathrm{Des}}} \tag{1}$$

where μ is the ligand chemical potential. To obtain a simple picture for the adsorption process, we split Γ into the dimensionless fraction of binding sites occupied by ligands, θ, and the adsorption site length d.[9] Combining Equation (1) with the Langmuir isotherm $\theta = aK/(1+aK)$,[10] we obtain Equation (2) for the ligand-binding-induced line-tension increment

$$\tau = -d^{-1} k_{\mathrm{B}} T \ln(1 + aK) \tag{2}$$

where $a = \exp(\mu/k_{\mathrm{B}} T)$ is the ligand activity, k_{B} the Boltzmann constant, T the temperature, and K the association constant. We define a and K to be dimensionless in terms of mole fractions (for details, see the Supporting Information). Note that the line tension τ is the free energy to elongate the polymer contour by a unit length and is thus distinct from the mechanical force F_{Des}.

In the single-molecule desorption experiments, the surface-adsorbed and desorbed polymer sections are in mechanical equilibrium.[11–13] Therefore, a distinction must be made between the surface-adsorbed polymer state, characterized by ligand association constant K_{s} and adsorption site length d_{s}, and the bulk polymer state, characterized by K_{b} and d_{b}. This gives rise to the ligand activity-dependent contribution to the line tension difference $\Delta\tau$ between bulk and surface states [Eq. (3)].

$$\frac{\Delta\tau}{k_{\mathrm{B}} T} = d_{\mathrm{s}}^{-1} \ln(1 + aK_{\mathrm{s}}) - d_{\mathrm{b}}^{-1} \ln(1 + aK_{\mathrm{b}}) \tag{3}$$

This line tension difference is the main contribution to the ligand-activity-dependent desorption force, that is, $F_{\mathrm{Des}}(a) = F_{\mathrm{Des}}(a{=}0) + \Delta\tau$. Fitting Equation (3) to the measured forces as a function of a allows the four parameters d_{b}, d_{s}, K_{b} and K_{s} to be determined, especially if results for different surfaces are fitted simultaneously with the additional constraint that K_{b} and d_{b} are independent of surface type.

In the following we demonstrate that the suggested method is indeed capable of quantifying the binding of cosolutes to a polymer in both states: 1) adsorbed at the substrate surface and 2) stretched in solution (Figure 1). As molecular sensor we use a single poly(allylamine) (PAAm) molecule attached to an AFM cantilever tip (see the Supporting Information). Covalent attachment of the polymer by a long flexible linker of poly(ethylene glycol) (PEG) to the AFM tip provides the long-term stability[11] needed to measure for hours with one and the same probe molecule on the different substrate materials in solutions of D-glucose at different concentrations ranging from 0 to 1 M. Moreover, the

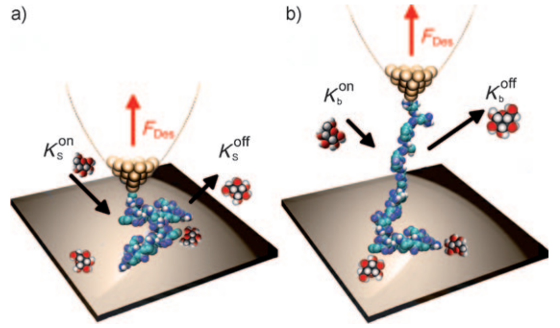

Figure 1. Glucose binding to a polymer on and off the substrate. The on- and off-rates of the cosolute glucose onto a polymer adsorbed to a substrate (K_{s}; picture a) and in solution (K_{b}; picture b) are depicted. The equilibrium association constants $K = K^{\mathrm{on}}/K^{\mathrm{off}}$ and the length of a glucose binding site d can be determined from the measured desorption forces F_{Des} as a function of the cosolute activity.

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temperature was varied to determine its influence on the binding thermodynamics of glucose (see the Supporting Information, Figure S1).

The tip with the polymer was brought into contact with the solid surface and was allowed to adsorb for one second. The following desorption of the PAAm molecule upon retraction of the cantilever with a constant velocity of 1 $\mu m\,s^{-1}$ was performed under equilibrium conditions as indicated by the observed velocity-independent force plateaus[14] (Figure 2).

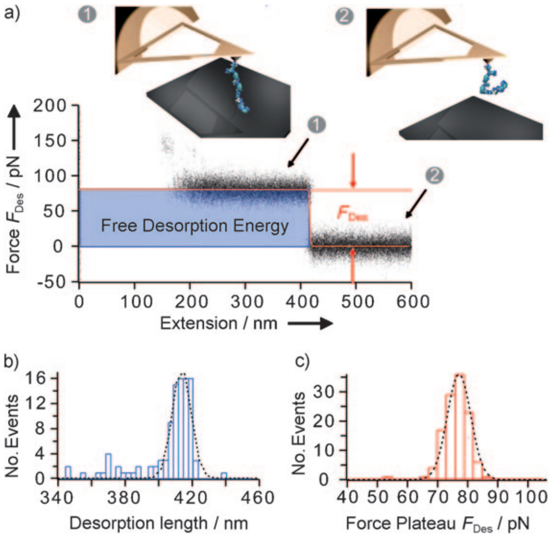

Figure 2. Equilibrium desorption of PAAm from oxidized diamond in 10 mм glucose solution. a) Superposition of 20 typical force–extension curves obtained with one and the same PAAm molecule under equilibrium conditions, as indicated by the (velocity-independent) force plateau. b,c) Detachment length distributions (b) and force distributions (c), comprising 100 approach–retract cycles with the same polymer, compared with Gaussian fits (broken lines).

Force-extension traces were recorded at different surface positions from the deflection-piezopath signal as described elsewhere.[15] (For a typical complete force-extension trace, see the Supporting Information, Figure S2.)

The plateau forces obtained on diamond increased with increasing glucose concentration, whereas the values on steel show a non-monotonic behavior (Figure 3 a). Key to our fitting strategy leading to the fits in Figure 3 is that identical fit parameters are employed in the bulk state; that is, the two data sets in Figure 3 are fitted simultaneously. This procedure makes the fit parameters quite unique. The resulting lengths of a glucose binding site along the PAAm molecule, d_b and d_s, and the corresponding association constants are summarized in Table 1.

The dimensionless bulk association constant $K_b = \frac{K_b^{on}}{K_b^{off}} = 1300$ for steel corresponds to $K_b = 32\ L\,mol^{-1}$ in molar units or to a dissociation constant in molar units of $K_b^{diss} = \frac{K_b^{off}}{K_b^{on}} = 31\ mmol\,L^{-1}$.

For the diamond substrate, K_s is about 8 % higher than K_b at 31 °C and more then 20 % higher at 46 °C, meaning that glucose molecules are associated more strongly to the polymer in the surface-adsorbed state. Furthermore, the adsorption site length in the surface adsorbed state d_s is smaller than d_b, which means that more glucose can bind to the polymer on the surface. The latter finding is quite unexpected: from a naive geometric point of view, less polymer accessibility would be expected for glucose at the substrate surface, which points to some non-trivial cooperativity between glucose adhesion at the surface and at the polymer. Both parameters enhance glucose–polymer binding on the surface and thus increase the desorption force with rising glucose concentration.

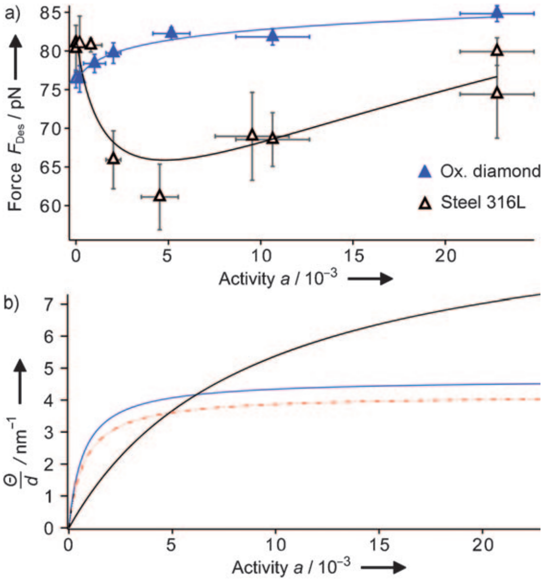

Figure 3. Surface differential glucose binding to PAAm. a) Desorption forces of PAAm with varying glucose activity on surgical stainless steel (△) and oxidized diamond (▲) at 31 °C. The solid lines are simultaneous fits of both data sets according to Equation (3). b) The line density of associated glucose molecules at the polymer in the vicinity of the steel surface (black, ——) exceeds that in solution (red, –··–··) at a glucose concentration of about 0.2 м. For comparison, the number of binding glucose is also depicted for the polymer adsorbed at the oxidized diamond surface (blue, ——).

Table 1: Binding parameters of D-glucose at PAAm on surgical stainless steel (316L) and oxidized diamond (Ox. diamond) at different temperatures.[a]

	Steel 316L	Ox. diamond	Ox. diamond
T [°C]	31	31	46
K_b	1300	1300	1400
d_b [Å]	2.40	2.40	2.68
ΔG_b [kJ mol^{-1}]	18.13	18.13	18.32
K_s	114	1404	1789
d_s [Å]	0.99	2.15	2.55
ΔG_s [kJ mol^{-1}]	11.98	18.33	18.94

[a] ΔG is the Gibbs free energy of binding to a single binding site, which is related to the temperature T and the gas constant R by $\Delta G = RT\ln K$. If K is used in molar units, then substract 9.4 kJ mol^{-1} from the ΔG values.

The non-monotonic force curve for steel can be rationalized by a competition between two factors: although the glucose–polymer association is stronger in the bulk phase, the adsorption length is smaller at the surface. As a result, up to a concentration of 0.2 M ($a \approx 5 \times 10^{-3}$), more glucose binds to the bulk rather than to the adsorbed polymer state; that is, the number of associated glucose molecules increases upon the forced desorption of the polymer and thus the desorption force decreases with increasing glucose concentration. For concentrations higher than 0.2 M, the situation is reversed (Figure 3b). The dramatically different surface parameters found on steel and diamond surfaces, and in particular the very small adsorption site length on steel compared to diamond or bulk, most likely reflect different affinities for glucose to the bare substrate materials.

Ligand adsorption has been studied in bulk by such experimental techniques as calorimetry,[16] densiometry,[17] and equilibrium sedimentation.[18] The most common method is isothermal titration calorimetry (ITC), which requires elevated concentrations and thus soluble polymers to avoid self-assembly and aggregation.[16] The single-molecule AFM approach presented herein is a complementary technique suitable even for insoluble polymers. A convincing strategy to probe ligand adsorption onto polymers in contact with surfaces has so far been missing.

In conclusion, we have demonstrated a new single-molecule sensor based on atomic force microscopy in conjunction with thermodynamic modeling. The adsorption site lengths and association constants of cosolutes binding to a solvated macromolecule and to the same molecule adsorbed at a solid substrate have been determined. Our analysis with PAAm and glucose reveals that surfaces have a significant effect on the binding affinity of small molecules to polymers, with significant variation between different surfaces. Our technique can be applied to arbitrary combinations of polymers, cosolutes, and a wide range of interfaces ranging from solid–liquid, liquid–air, or soft interfaces, such as lipid bilayers, cell membranes, or histone-covered substrates. This combined experimental/theoretical approach is thus widely applicable for the study of cosolute interaction with macromolecules in bulk and in the vicinity of interfaces. Finally, a difference in K_s and K_b or d_s and d_b can be used to manipulate polymer adsorption by the change of cosolute concentration, which thus offers attractive and convenient prospects for the control of macromolecular assembly at surfaces.

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