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Adsorption of β-Lactoglobulin on Spherical Polyelectrolyte Brushes: Direct Proof of Counterion Release by Isothermal Titration Calorimetry

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Abstract: The thermodynamics and the driving forces of the adsorption of β-lactoglobulin on spherical polyelectrolyte brushes (SPB) are investigated by isothermal titration calorimetry (ITC). The SPB consist of a polystyrene core onto which long chains of poly(styrene sulfonate) are grafted. Adsorption isotherms are obtained from measurements by ITC. The analysis by ITC shows clearly that the adsorption process is solely driven by entropy while $\Delta H > 0$. This finding is in accordance with the proposed mechanism of counterion release: Patches of positive charges on the surface of the proteins become multivalent counterions of the polyelectrolyte chains, thereby releasing the counterions of the protein and the polyelectrolyte. A simple statistical-mechanical model fully corroborates the proposed mechanism. The present analysis shows clearly the fundamental importance of counterion release for protein adsorption on charged interfaces and charged polymeric layers.

Introduction

The investigation of the interaction of proteins with surfaces is one of the most important fields in biotechnology.[1−4] Often it is necessary to suppress the nonspecific adsorption of proteins in order to avoid phenomena like biofouling.[2,5] In many cases, however, adsorption cannot be totally suppressed. Thus, nanoparticles used in applications like in nanomedicine will immediately adsorb proteins in vivo. This "corona" of proteins will largely determine the response of the organism to the particles.[4,6−9] Hence, adsorption of proteins presents a problem closely related to the toxicology of nanoparticles.[4,6,7,9,10] Additionally, immobilization of proteins on solid supports is an important issue in modern biotechnology.[11,12] Here the adsorption should not impede the biological function nor the catalytic activity. Hence, an in-depth understanding of the interaction of proteins with surfaces is required for a wide variety of basic problems, such as nanotoxicology,[4,13] protein separation,[14] bioadhesion,[2,15] or biocatalysis.[16]

It is clear that various factors contribute to the strength of protein adsorption onto surfaces. Up to now, uncharged polymers attached to the surfaces have been used to adjust the interaction of solid surfaces with proteins. Thus, great efforts have been made to design protein-resistant surfaces by attachment of poly(ethylene oxide) chains.[2,17] Moreover, poly(*N*-isopropylacryl amide) microgels have been used to immobilize proteins on colloidal particles.[6,7,18,19] Recently, interest in protein adsorption has shifted to charged polymers. It has been shown that layers of densely grafted polyelectrolyte chains termed

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(1) *Physical Chemistry of Biological Interfaces*; Baszkin, A., Norde, W.; Eds.; Marcel Dekker, Inc.: New York, 2000.
(2) Senaratne, W.; Adruzzi, L.; Ober, C. K. *Biomacromolecules* **2005**, *6*, 2427.
(3) Weibel, D. B.; Whitesides, G. M. *Curr. Opin. Chem. Biol.* **2006**, *10*, 584.
(4) Nel, A. E.; Mädler, L.; Velegol, D.; Xia, T.; Hoek, E. M. V.; Somasundaran, P.; Klaessig, F.; Castranova, V.; Thompson, M. *Nat. Mater.* **2009**, *8*, 543.
(5) Erol, M.; Du, H.; Sukhishvili, S. *Langmuir* **2006**, *22*, 11329.
(6) Linse, S.; Cabaleiro-Lago, C.; Xue, W.-F.; Lynch, I.; Lindman, S.; Thulin, E.; Radford, S. E.; Dawson, K. A. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8691.
(7) Cabaleiro-Lago, C.; Quinlan-Pluck, F.; Lynch, I.; Lindman, S.; Minogue, A. M.; Thulin, E.; Walsh, D. M.; Dawson, K. A.; Linse, S. *J. Am. Chem. Soc.* **2008**, *130*, 15437.
(8) Lundqvist, M.; Stigler, J.; Lynch, I.; Cedervall, T.; Dawson, K. A. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 14265.
(9) Röcker, C.; Pötzl, M.; Zhang, F.; Parak, W. J.; Nienhaus, G. U. *Nat. Nanotechnol.* **2009**, *4*, 577.
(10) Giger, K.; Vanam, R. P.; Seyrek, E.; Dubin, P. L. *Biomacromolecules* **2008**, *9*, 2338.
(11) Minko, S. *Polym. Rev.* **2006**, *46*, 397.
(12) Uhlmann, P.; Houbenov, N.; Brenner, N.; Grundke, K.; Burkert, S.; Stamm, M. *Langmuir* **2007**, *23*, 57.
(13) Nel, A.; Xia, T.; Mädler, L.; Li, N. *Science* **2006**, *311*, 622.
(14) Ruckenstein, E.; Guo, W. *Biotechnol. Prog.* **2004**, *20*, 13.
(15) Siegers, C.; Biesalski, M.; Haag, R. *Chem.—Eur. J.* **2004**, *10*, 2831.
(16) Caruso, F. *Adv. Mater.* **2001**, *13*, 11.
(17) Blaettler, T. M.; Pasche, S.; Textor, M.; Griesser, H. J. *Langmuir* **2006**, *22*, 5760.
(18) Duracher, D.; Elaissari, A.; Mallet, F.; Pichot, C. *Langmuir* **2000**, *16*, 9002.
(19) Lindman, S.; Lynch, I.; Thulin, E.; Nilsson, H.; Dawson, K. A.; Linse, S. *Nano Lett.* **2007**, *4*, 914.

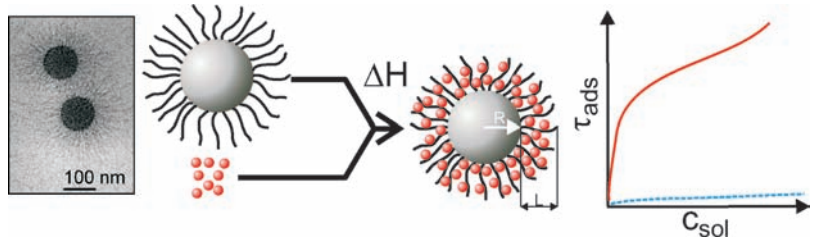

Figure 1. Scheme of the polyelectrolyte-mediated protein adsorption: The SPB shown in a cryogenic transmission electron micrograph (left-hand side) are in the osmotic limit at low ionic strength and nearly all counterions are confined within the brush layer. The polyelectrolyte chains are strongly stretched due to the high osmotic pressure inside the brush layer. These brush particles are mixed with BLG in aqueous solution of low salt concentration. The heat of mixing is determined precisely. The right-hand side shows schematically the amount of adsorbed protein per gram of SPB, τ_{ads}, versus the concentration of protein, c_{sol}, remaining free in solution. At low ionic strength the amount of adsorbed protein (red) is high whereas little adsorption takes place at high ionic strength (blue).[20]

polyelectrolyte brushes may take up large amounts of proteins when the ionic strength is low.[5,20−26] This strong protein adsorption at low ionic strength takes place on the wrong side of the isoelectric point of the protein, that is, when the brush and the protein carry the same net charge. If, however, the ionic strength in the system is high, the polyelectrolyte brushes repel proteins. This effect has been observed for planar systems[22,23] as well as for spherical systems (spherical polyelectrolyte brushes, SPB).[20] Moreover, similar effects have been observed for polyelectrolyte multilayers.[5,24−26] Hence, the "polyelectrolyte mediated protein adsorption" (PMPA)[20,27] is a phenomenon that occurs whenever a dense layer of highly charged polymers interacts with proteins in aqueous solution.

The unexpected strong adsorption onto polyelectrolyte layers of the same charge has been explained[20] in terms of the counterion release:[28−31] At low ionic strength the brush layer is in the "osmotic limit" (see e.g. refs 32−35), that is, nearly all counterions are confined within the brush layer, thus creating a high osmotic pressure. The surface of the proteins exhibits both positively and negatively charged patches. Upon entry of the protein into the brush layer the positively charged patches will act as a multivalent counterion of the negatively charged polyelectrolyte brush, thus releasing a concomitant number of counterions.[20,36,37] The release of counterions from a dense polyelectrolyte brush into a reservoir with low salt concentration leads to a marked gain of entropy.[20] This is in contrast to the adsorption of proteins on hydrophobic nanoparticles that is often driven by enthalpic forces.[6] On the other hand, at high ionic strength ("salted brush"; see e.g. ref 34) the activity of the counterions in the brush and the solution are equal and no adsorption should take place. An alternative explanation of the PMPA is related to the charge regulation (see ref 21 and further citations given there): At low ionic strength the pH within the brush layer is lower and the protein assumes a net positive charge. However, this mechanism does not lead to a significant release of counterions since it involves only the transfer of protons to the carboxyl groups of the protein.

As a consequence of this, the adsorption of proteins to a dense polyelectrolyte layer should be accompanied by a marked increase of entropy when the ionic strength is low, whereas virtually no effect should be measurable at high ionic strength. Here, we present the first direct thermodynamic study of polyelectrolyte-mediated protein adsorption. The experimental procedure is shown schematically in Figure 1: The caloric effect of mixing proteins with spherical polyelectrolyte brushes is determined with high precision. The spherical polyelectrolyte brushes used here consist of a polystyrene core onto which long chains of poly(styrene sulfonate) are grafted.[20] β-Lactoglobulin (BLG) was used as a model protein (see Supporting Information). Isothermal titration calorimetry (ITC) has been shown to be highly suited for the determination of thermodynamical quantities of protein binding onto colloidal particles.[19] Thus, the adsorption isotherm of the protein on the particles is determined by ITC, leading to the thermodynamic adsorption constant and the entropy of protein adsorption. Finally, a simple statistical−mechanical model can be used to model the observed thermodynamic data in a semiquantitative fashion.

Results and Discussion

First, we discuss the analysis of the adsorption of β-lactoglobulin onto the spherical polyelectrolyte brushes by ITC at low ionic strength. Figure 2a shows the raw data of the ITC measurement for the adsorption process of BLG onto the SPB. The heat of dilution of BLG did not need to be taken into account for the analysis because most of the protein is adsorbed on the SPB particles. Hence, only a small fraction remained in buffer solution and the heat generated through the dilution of this fraction can be neglected.

(20) Wittemann, A.; Ballauff, M. *Phys. Chem. Chem. Phys.* **2006**, *8*, 5269.
(21) de Vos, W. M.; Biesheuvel, P. M.; de Keizer, A.; Kleijn, J. M.; Cohen Stuart, M. A. *Langmuir* **2008**, *24*, 6575.
(22) Reichhart, C.; Czeslik, C. *Langmuir* **2009**, *25*, 1047.
(23) Hollmann, O.; Steitz, R.; Czeslik, C. *Phys. Chem. Chem. Phys.* **2008**, *10*, 1448.
(24) Schwinte, P.; Ball, V.; Szalontai, B.; Haikel, Y.; Voegel, J.-C.; Schaaf, P. *Biomacromolecules* **2002**, *3*, 1135.
(25) Quinn, A.; Tjipto, E.; Yu, A.; Gengenbach, T. R.; Caruso, F. *Langmuir* **2007**, *23*, 4944.
(26) Bruening, M. L.; Dotzauer, D. M.; Jain, P.; Lu, O.; Baker, G. L. *Langmuir* **2008**, *24*, 7663.
(27) Leermakers, F. A. M.; Ballauff, M.; Borisov, O. V. *Langmuir* **2007**, *23*, 3937.
(28) Record, M. T.; Anderson, C. F.; Lohman, T. M. *Q. Rev. Biophys.* **1978**, *11*, 103.
(29) Record, M. T.; Zhang, W.; Anderson, C. F. *Adv. Protein Chem.* **1998**, *51*, 281.
(30) Ball, V.; Winterhalter, M.; Schwinte, P.; Lavalle, Ph.; Voegel, J.-C.; Schaaf, P. *J. Phys. Chem. B* **2002**, *106*, 2357.
(31) Czeslik, C.; Jackler, G.; Steitz, R.; von Gruenberg, H.-H. *J. Phys. Chem. B* **2004**, *108*, 13395.
(32) Pincus, P. *Macromolecules* **1991**, *24*, 1912.
(33) Borisov, O. V.; Zhulina, E. B.; Birshtein, T. M. *Macromolecules* **1994**, *27*, 4795.
(34) Ballauff, M.; Borisov, O. *Curr. Opin. Colloid Interface Sci.* **2006**, *11*, 316.
(35) Ballauff, M. *Prog. Polym. Sci.* **2007**, *32*, 1135.
(36) Cooper, C. L.; Dubin, P. L.; Kayitmazer, A. B.; Turksen, S. *Curr. Opin. Colloid Interface Sci.* **2005**, *10*, 52.
(37) Cooper, C. L.; Goulding, A.; Kayitmazer, A. B.; Ulrich, S.; Stoll, S.; Turksen, S.; Yusa, S.-I.; Kumar, A.; Dubin, P. L. *Biomacromolecules* **2006**, *7*, 1025.

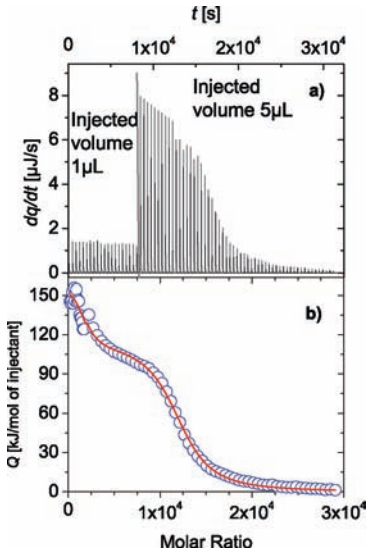

Figure 2. ITC data for the adsorption of BLG onto SPB in MES buffer at pH 6.1 and an ionic strength of 5 mM. The upper panel shows the raw data of the ITC. Furthermore, the gray line indicates the region where the volume of titrant was changed to achieve more data points for lower molar ratios. The integrated heats of each injection (circles) are shown in the lower panel together with the fit of the two sets of independent binding sites model (red line).

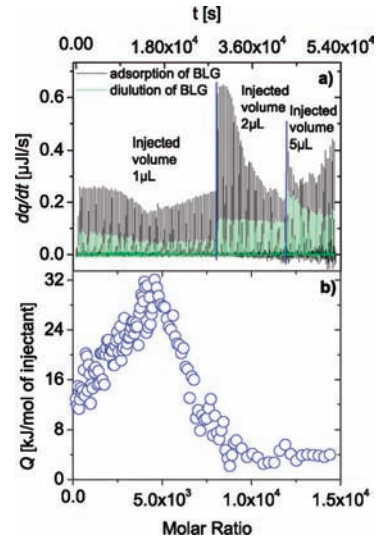

Figure 3. ITC data for the adsorption of BLG onto SPB at 30 °C in MES bufer at pH 6.1 and an ionic strength of 30 mM. The upper panel shows the raw data of the adsorption of BLG onto SPB particles (black) and the dilution of BLG by buffer (green). Furthermore, the blue lines indicate the regions where the volume of added solution was changed to achieve more data points for a better resolution. The integrated heats of each injection (circles) are shown in the lower panel after the subtraction of the protein dilution by buffer.

The signal that is observed for the adsorption of BLG onto SPB is endothermic throughout the entire range. This finding immediately indicates that the driving force for protein adsorption cannot be of enthalpic origin (see also the discussion in ref 30). Moreover, it is obvious from Figure 2b that two adsorption steps are needed (TSIS model, solid line) in order to fit the integrated heats Q (circles). The maximum amount that can be adsorbed per gram of brush particles is 1300 mg of β-lactoglobulin. Since the abscissa of the panel in Figure 2b is the molar ratio of the protein to the brush particles, the number of protein molecules per particles can be directly read off the diagram. The subsequent analysis of the data by the TSIS model is shown by the solid line in Figure 2b. The change of entropy derived from this analysis is positive for both steps. Obviously, the adsorption process must be driven by entropy as expected for a process driven by counterion release (see Introduction). Table 1 summarizes the parameter of the fit with the TSIS model.

It must be kept in mind that the BLG dimers may associate in the adsorbed state partly to form larger aggregates due to the lower pH value inside the brush layer.[38,39] This association of BLG in the adsorbed state should be followed by a negative entropic effect. This indicates that the entropic effect by the PMPA may be even larger than that originating from the association of BLG within the brush layer.

The above findings are in accordance with studies by small-angle X-ray scattering.[40,41] These investigations demonstrated that the proteins are adsorbed inside the polyelectrolyte layer of the SPB and are closely connected to the polyelectrolyte chains.[40,41] Moreover, the results obtained from these studies suggest the presence of at least two different binding sites. This finding could be explained as follows: The inner part of the polyelectrolyte layer has a higher chain density, resulting from the spherical geometry. In this inner region the proteins can interact with more than one chain, leading to a high binding strength. In the outer part, on the other hand, the bound proteins will interact only with one chain, which will be followed by a concomitantly lower binding strength. The present thermodynamic analysis is the first direct evidence for the existence of binding sites that differ considerably with regard to the binding strength.

Additional measurements were performed at higher ionic strength to prove the entropic origin of the protein adsorption of BLG on the SPB. These measurements were performed at 30 °C due to the better signal-to-noise ratio that can be achieved at higher temperature. Figure 3 presents the result for the adsorption of BLG at an ionic strength of 30 mM. In Figure 3a the raw data for the adsorption of BLG onto SPB particles (black) and the dilution of BLG by buffer (green) are shown. For these experiments the heat of protein dilution cannot be neglected because the fraction of free protein is much greater than previously found for low ionic strength. Therefore, this contribution has to be subtracted to yield the corrected adsorption enthalpy. Compared with data collected at lower ionic strength shown in Figure 2, the enthalpic effect is still positive but much smaller (cf. Figure 3). Hence, the driving force for the adsorption process is still of entropic nature but much weaker. This indicates that the effect of the counterion release is diminished. Evidently, the hydrophobic interaction contributes as well to the overall measuring signal. The hydrophobic interaction is known from the literature to be endothermic in many systems comparable with the present one.[30] The overall

Table 1. Thermodynamic Data for the Adsorption of BLG on the Spherical Polyelectrolyte Brushes at Low Ionic Strength

	N	K_A (L/mol)	ΔH_A (kJ/mol)	ΔS_A [kJ/(mol K)]
1. adsorption step	1800 ± 100	$(3 \times 10^7) \pm (2 \times 10^7)$	155 ± 4	0.68 ± 0.02
2. adsorption step	10300 ± 100	$(1.0 \times 10^6) \pm (0.1 \times 10^6)$	113 ± 3	0.494 ± 0.008

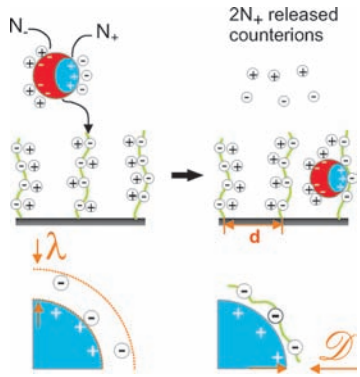

Figure 4. Schematic illustration of the electrostatic model used for the description of the protein interaction with polyelectrolyte brushes. The protein surface carries negatively charged groups. The number N_- of these groups is slightly greater than the number of positive charges N_+ (if pH > p*I*). During the adsorption process the positive patch on the protein surface becomes a N_+-fold counterion of the polyelectrolyte chains in the brush layer. This releases N_+ negative counterions of this positive patch together with N_+ positive counterions of the brush layer. The Gouy−Chapman length of the dissolved protein is λ. This is depicted in the bottom of the left panel. The thickness of the adsorbed polyelectrolyte layer on the protein surface, $\mathscr{D}$, is illustrated in the bottom of the right panel.

adsorbed amount of protein is significantly lowered, as was already observed in earlier studies.[20] However, the measured heat is too small for a meaningful evaluation in terms of the TSIS model. If the ionic strength would still be further increased, the signal-to-noise ratio would become even worse because of the reduced interaction between the protein and the polyelectrolyte. Therefore, the data shown in Figure 3 presents the experimental limit for these measurements.

Concluding the experimental part of this investigation, it can be stated that the entropic origin of the protein adsorption at low ionic strength has been clearly demonstrated by a direct caloric experiment. In the following section, we present a simple electrostatic model that provides a semiquantitative explanation of these findings.

Theoretical Considerations

We consider a simplified model of a protein depicted in Figure 4. The protein is modeled as a sphere of radius R_P and its surface has one negative patch with N_- charges and one positive patch carrying N_+ charges. Since the present modeling refers to a pH greater than the isoelectric point p*I*, the overall charge is negative and $N_- > N_+$. The charge of both patches is balanced by an equal number of counterions with opposite charge.

The decisive assumption for the present model is that the size of these patches, and their charge is high enough to localize a significant part of the counterions. In other words, the charge of a given patch must be sufficiently large in order to localize its counterions within a Gouy−Chapman length λ (counterion condensation). The electrostatic interaction in a system with a dielectric constant ε can be characterized in terms of the Bjerrum length $l_B = e^2/4\pi\varepsilon_0\varepsilon kT$, where e is the elementary charge and ε_0, k, and T have their usual meanings. Since we present a semiquantitative approach only, we omit numerical factors on the order of unity in the following. If σ denotes the number of elementary charges per unit area, the Gouy−Chapman length λ can be estimated as[42,43]

$$\lambda \approx \frac{1}{l_B\sigma} \tag{1}$$

With these definitions, we now can distinguish two different cases: (1) $\lambda \gg R_P$: This condition refers to a weakly charged protein surface; thus, there is no localization of the counterions in this case. (2) $\lambda \ll R_P$, which follows if $R_P l_B \sigma_+ \approx R_P l_B \sigma_- > 1$, where σ_+ and σ_- refer to the number of charges per unit area of the positive and the negative patch, respectively. In this case, there is a localization of the counterions at a given patch.

Evidently, counterion release can only take place for case 2, that is, if a certain fraction of the counterions of the protein are localized near a patch. Hence, we shall consider this case in the following, that is, $R_P > \lambda$. Moreover, we shall consider an "osmotic" brush[33] in which practically all counterions are localized within the surface layer of polyelectrolyte chains. As discussed above, the case refers to a low (monovalent) salt concentration, c_{salt}, in the protein solution in which a strong adsorption of proteins onto the brush particles is observed.

We now consider the transfer of one protein molecule from the solution to the brush layer and estimate the change of free energy, ΔF, for this process. Given the above premises, we assume that the N_- positive counterions of the negative patch are fully localized and thus carried into the brush layer. Moreover, the positive patch of the protein becomes the counterion of the polyelectrolyte chains within the brush layer, thereby releasing the N_+ negative counterions that previously balanced the charge of the positive patch. In addition, N_+ positive counterions that previously balanced the charge of the polyelectrolyte chains are released from the brush as well (see Figure 4). Then the change of free energy ΔF effected through introducing one protein into the brush layer is given by three contributions, $\Delta F = \Delta F_1 + \Delta F_2 + \Delta F_3$. The first term, ΔF_1, is given in units of RT by

$$\Delta F_1 = N_+ \ln\left(\frac{c_{salt}}{c_{brush}}\right) \tag{2}$$

where c_{brush} is the salt concentration within the brush layer. The term ΔF_2 is the gain in free energy by the adsorption of the polyelectrolyte chain on the positive patch of the protein. Finally, ΔF_3 is related to the release of the N_+ negative counterions from the positive patch to the solution. Given the various approximations used so far, it is reasonable to assume that

$$\Delta F_2 + \Delta F_3 \approx N_+ \ln\left(\frac{c_{salt}}{c_{si}}\right) \tag{3}$$

where c_{si} denotes the concentration of the counterions near the surface of the positive patch. The Coulombic contribution of the protein double layer is

$$F_{\text{Coulomb double layer}} = l_B\sigma^2\lambda \tag{4}$$

(38) Verheul, M.; Pedersen, J. S.; Roefs, S. P. F. M.; De Kruif, K. G. *Biopolymers* **1999**, *49*, 11.
(39) Majhi, P. R.; Ganta, R. R.; Vanam, R. P.; Seyrek, E.; Giger, K.; Dubin, P. L. *Langmuir* **2006**, *22*, 9150.
(40) Henzler, K.; Wittemann, A.; Breininger, E.; Ballauff, M.; Rosenfeldt, S. *Biomacromolecules* **2007**, *8*, 3674.
(41) Henzler, K.; Rosenfeldt, S.; Wittemann, A.; Harnau, L.; Finet, S.; Narayanan, T.; Ballauff, M. *Phys. Rev. Lett.* **2008**, *100*, 158301.
(42) PDB code 3BLG.
(43) *The Colloidal Domain*, 2nd ed.; Evans, D. F., Wennerström, H., Eds.; Wiley-VCH: New York, 1999.

Furthermore, the Coulombic contribution of the adsorbed polyelectrolyte layer is

$$F_{\text{Coulomb adsorbed layer}} = l_B \sigma^2 \mathscr{D} \tag{5}$$

where $\mathscr{D}$ is the thickness of the adsorbed polyelectrolyte layer on the protein surface (cf. Figure 4). Equation 3 thus amounts to the assumption that all energetic contributions are approximately canceling each other. This is justified if λ is equal to $\mathscr{D}$. Moreover, it is assumed that the N_- ions attached to the negative protein patch have no contribution to ΔF. This assumption is valid if λ is smaller than the screening length in the brush layer or c_{brush} is smaller than c_{si}. Moreover, the distance d between two polyelectrolyte chains is much larger than λ, unless the protein is adsorbed near the grafting points of the chains.

Since we assume that the ions of a patch are confined within the length λ, $c_{\text{si}} \approx \sigma^2 l_B$. Thus, in units of RT

$$\Delta F = N_+ \ln\left(\frac{c_{\text{salt}}}{c_{\text{brush}}}\right) + N_+ \ln\left(\frac{c_{\text{salt}}}{c_{\text{si}}}\right) \tag{6}$$

The concentration c_{brush} can be calculated from the Donnan equilibrium through (see refs 20, 34 and further references given there)

$$\frac{c_{\text{brush}}}{c_{\text{salt}}} = \left[1 + \left(\frac{c_p}{2c_{\text{salt}}}\right)^2\right]^{1/2} + \frac{c_p}{2c_{\text{salt}}} \tag{7}$$

where c_p is the concentration of charged monomers within the brush layer.

Equation 6 demonstrates that the change of free energy ΔF calculated under the above assumption is mainly of entropic origin inasmuch as all energetic contribution caused by Coulombic interaction are canceling each other within the limits of accuracy of the present model. Also, possible contributions of the hydrophobic interaction are neglected in this simple electrostatic model, too. Thus, the free energy can directly be converted to the entropy:

$$\Delta S = -RN_+\left[\ln\left(\frac{c_{\text{salt}}}{c_{\text{brush}}}\right) + \ln\left(\frac{c_{\text{salt}}}{c_{\text{si}}}\right)\right] \tag{8}$$

where R is the gas constant. Thus, Equation 8 predicts that the entropy of protein adsorption is related to the difference of the salt concentration c_{salt} in the solution by two different terms: The first term in Equation 8 is determined by the ratio of c_{salt} to c_{brush}, that is, to the salt concentration within the brush layer adjusted through the Donnan equilibrium (Equation 7). It provides a quantitative correlation for the successful correlation of the amount of adsorbed protein to the Donnan pressure within the brush layer suggested in ref 20 recently. It should be noted that this term gives always a positive contribution to ΔS, that is, even when $\lambda > R_P$, the only condition being that multivalent patches are localized on the surface of the protein. The second term is related to the concentration c_{si} of ions condensed directly on the positive patches of the protein. Depending on the size of the patches, it may be even more important than the first term. Furthermore, Equation 8 shows that $\Delta S = 0$ for higher salt concentrations c_{salt}, since in this case $c_{\text{brush}} \approx c_{\text{salt}} \approx c_{\text{si}}$.

A comparison of Equation 8 with experiments can be done in different ways: For a given protein with known patch size, one may estimate the change of ΔS and compare this to experimental data. Here we estimate the number N_+ by Equation 8 from the measured ΔS with the following parameters referring to the present system: $c_{\text{salt}} = 0.005$ mol/L; $c_p = 0.08$ mol/L; $c_{\text{brush}} = 0.08$ mol/L. The parameter $c_{\text{si}} \approx \sigma^2 l_B$ can be estimated to 0.3 mol/L if a reasonable value of σ, namely, 5×10^{15} dm^{-2}, is assumed. This charge density follows from a positive patch on the surface of the BLG dimer with a size of 10 nm^2 carrying five positive charges. Such a value of σ can been estimated from the crystal structure of BLG.[44] l_B is 0.714 nm in water at 298.15 K. Given these numbers, N_+ is determined to be 12 using the experimental $\Delta S = 0.68$ kJ/(mol K) for the first adsorption step. In the second adsorption step, N_+ is 9, as calculated from the experimental $\Delta S = 0.494$ kJ/(mol K). The present result thus indicates that several positive patches interact with the brush layer. The protein BLG contains a total of 36 positive charges on its surface. Thus, N_+ is on the order of 10, which appears to be a reasonable figure. Since the total number of counterions released is twice this figure, ca. 20 counterions are released in total for the uptake of one protein molecule. This underscores the enormous gain of entropy reached during the uptake of protein if the ionic strength in the system is low.

Conclusion

We have presented an investigation of the adsorption of β-lactoglobulin onto spherical polyelectrolyte brushes (SPB). Two limits of the polyelectrolyte brush have been considered: (i) the osmotic brush resulting for low ionic strength in the system and (ii) the salted brush resulting for a high ionic strength in solution. Isothermal titration calorimetry demonstrated that the adsorption of β-lactoglobulin onto the SPB in the osmotic limit is accompanied by a positive enthalpy. The subsequent analysis of the adsorption isotherm demonstrates in a quantitative fashion that the protein adsorption is driven by an increase of entropy. If the salt concentration is raised (salted brush limit), the amount of adsorbed protein is greatly diminished. A simple theoretical model shows that the protein adsorption in the osmotic limit is indeed of entropic origin inasmuch as possible Coulombic contributions cancel each other. The entire analysis demonstrates the importance of the counterion release force for protein adsorption. Hence, the counterion release should be considered for all systems characterized by a lower ionic strength. In general, the present results demonstrate clearly that effects due to the surface charges of the proteins must be taken into account in addition to hydrophobic forces when considering protein adsorption. Hence, possible contributions due to counterion release may play an important role in various fields, e.g., nanomedicine or biotechnology.

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Supporting Information Available: Experimental Section, including the properties of the SPB system and the BLG, the procedure of the ITC experiments, and the analysis of the ITC data. This material is availble free of charge via the Internet at http://pubs.acs.org.

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