Interaction of Dissolved Proteins with Spherical Polyelectrolyte Brushes

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Summary: We consider the adsorption of bovine serum albumin (BSA) on spherical polyelectrolyte brushes (SPB). The SPB consist of a solid polystyrene core of 100nm diameter onto which linear polyelectrolyte chains (poly(acrylic acid), (PAA)) are grafted. The adsorption of BSA is studied at a pH of 6.1 at different concentrations of added salt and buffer (MES). We observe strong adsorption of BSA onto the SPB despite the effect that the particles as well as the dissolved BSA are charged negatively. The adsorption of BSA is strongest at low salt concentration and decreases drastically with increasing amounts of added salt. The adsorbed protein can be washed out again by raising the ionic strength. The various driving forces for the adsorption are discussed. It is demonstrated that the main driving force is located in the electrostatic interaction of the protein with the brush layer of the particles. All data show that the SPB present a new class of carrier particles whose interaction with proteins can be tuned in a well-defined manner.

Keywords: polyelectrolyte brush, proteins, BSA, adsorption

Introduction

The adsorption of proteins from solution to solid substrates is an important phenomenon.[1] Often protein adsorption is to be avoided as e.g. in the field of biocompatible materials. In this case the surface must be modified by suitable groups to resist protein adsorption.[2] On the other hand, proteins may be immobilized on macroscopic surfaces or on the surface of colloidal particles in order to obtain functional systems.[3] In general, aqueous suspensions of colloidal particles are well-suited to study the adsorption of proteins because these particles create a large and well-defined surface in the system. Therefore there is a large number of studies on the adsorption of proteins on e.g. polymer latex particles[3-17] or on silica particles.[18,19] The driving forces for protein adsorption are not fully understood yet since the interaction of biomolecules with solid

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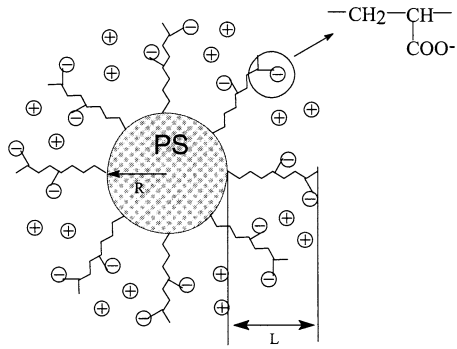

Fig. 1 Schematic presentation of a spherical polyelectrolyte brush (SPB). Long linear poly(acrylic acid) chains are grafted to a colloidal poly(styrene) sphere. The diameter of the core is ca. 100nm whereas the contourlength is 36nm. The thickness *L* of the brush layer depends on the pH well as on the salt concentration in the solution.[24-26]

surfaces is in general a complex process.[20] Investigations of protein adsorption onto colloidal particles with a well-defined surface are very helpful in this regard because the surface properties can directly be related to the adsorbed amount of the respective biomolecules.

Here we wish to present the first steps of an investigation devoted to the adsorption of proteins to spherical polyelectrolyte brushes (SPB). Fig. 1 depicts schematically the structure of these particle: Linear poly(acrylic acid) chains are grafted onto poly(styrene) cores that have typical diameters of 100nm. The number of chains per unit surface is quite high so that the distance between the chains directly at the surface is much smaller than their overall dimensions. Hence, a polyelectrolyte brush[21-23] has been generated on these strongly curved surfaces since the average lateral distances between the polyelectrolyte chains are much smaller than their contour length.

These particles are generated by photo-emulsionpolymerization[24] and have recently been studied comprehensively by dynamic light scattering (DLS).[25,26] They are characterized in terms of the

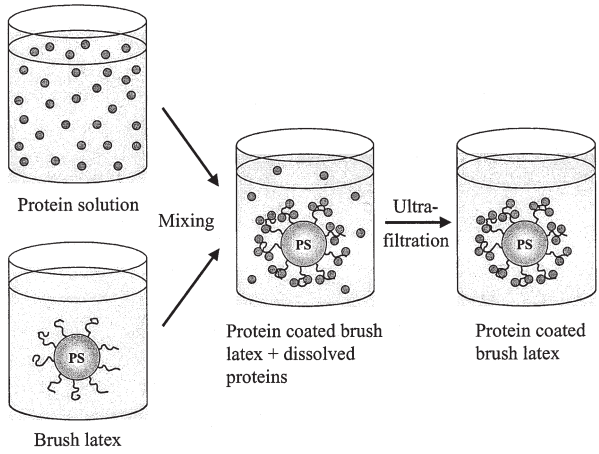

Fig. 2 Schematic representation of the experiment: Solutions of BSA were prepared in buffer solutions with defined concentrations of added salt. These solutions were added to the SPB dissolved in the same buffer having the same ionic strength. After equilibration for 24 hours the non-adsorbed protein was removed by careful serum replacement.

core radius R_C, the contourlength L_c of the grafted chains and the grafting density σ. Osmometry[27] demonstrated that the counterions are mostly confined within the brush as predicted by theory.[21] For an annealed SPB the overall thickness L (see fig. 1) of the brush-layer on the surface of the particles depends on the *pH* as well as on the ionic strength in the solution.[25,26] Hence, these particles present a well-defined model system that allows a comprehensive investigation of the interaction of dissolved proteins with polyelectrolyte brushes.

Experimental

The SPB have been prepared by photo-emulsionpolymerization. Detail of the method of preparation and characterisation have been described elsewhere.[24] The synthesis of these particles includes a careful ultrafiltration to remove all traces of free polymer. The SPB were generated by affixing PAA-chains onto the surface. Latex KpS13 used here is characterised by the method of reference[24] as follows: core radius R_C = 51nm, contourlength L_c = 36nm, the grafting density $\sigma = 0.13\text{nm}^{-2}$.

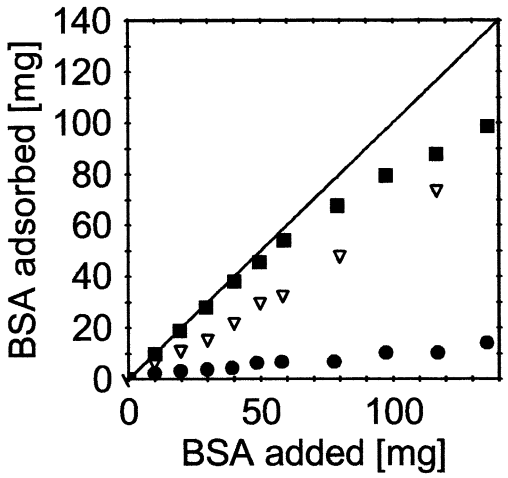

Fig. 3 Adsorption of BSA on the SPB KpS13 at pH = 6.1 and a concentration of the MES buffer of 10mM. The amount of adsorbed protein is plotted against the total amount of BSA. The amount of particles was 100mg in each run. Parameter of the different curves is the concentration of added salt. Filled squares: no added salt; triangles: 50mM NaCl; filled circles: 150mM NaCl.

All aqueous solutions of the SPB were carefully adjusted to a given ionic strength and pH. Here we used N-morpholinoethane-sulfonic acid (MES) to adjust the pH of the solutions to 6.1. In all investigations reported here a MES-concentration of 10mM has been used. Higher ionic strengths were adjusted through addition of NaCl to these solutions. 2mM NaN_3 were added to all solutions to avoid possible microbial growth. Bovine serum albumin (BSA, fatty acids free, Sigma A-6003) was used without further purification.

Results and Discussion

Fig. 2 shows the method employed here: Solutions of BSA in buffer solutions with defined concentrations of added salt were added to the SPB in buffer solution. These solution were equilibrated for 24 hours. Thereafter the solutions were filled into a serum-replacement cell and flushed eight times with buffer solution of same pH and salinity. The amount of protein washed

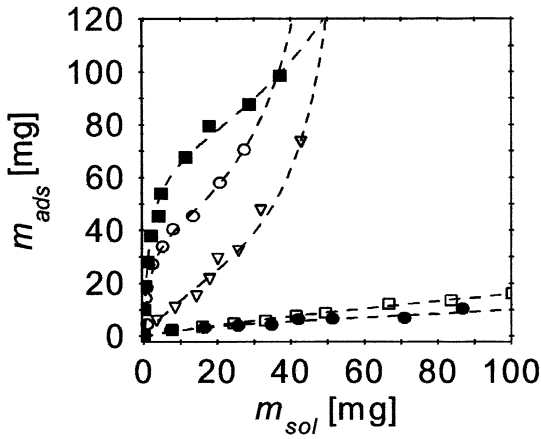

Fig. 4 Adsorption isotherm of BSA on the SPB KpS13 at pH = 6.1 and at a concentration of the MES buffer of 10mM. The amount of adsorbed BSA is plotted against the amount of protein left unadsorbed in solution. The data has been taken from the data shown in fig. 3. Parameter of the different curves is the concentration of added salt. Filled squares: no added salt; hollow circles: 25mM NaCl; triangles: 50mM NaCl; hollow squares: 100mM NaCl; filled circles: 150mM NaCl. The dashed lines are drawn as a guide for the eye.

off by this procedure was determined spectroscopically from the known absorption coefficient. This data and the total amount was used to calculate the amount of adsorbed BSA. In all cases the same time of equilibration as well as the same procedure for serum replacement was used to ensure a meaningful comparison of the data. Parameter of the different experiments is the ionic strength as adjusted through adding NaCl.

Fig. 3 displays the principal result of this study. In fig. 4 the amount of adsorbed BSA is plotted against the total amount of protein. The total amount of SPB was 100mg in all runs. Fig. 4 gives the same data in a plot of the adsorbed amount versus the amount of BSA not adsorbed to the particles. The total concentration of SPB particles has been kept constant to 1 wt. % throughout all experiments reported here.

Fig. 3 and 4 immediately demonstrate that BSA is strongly adsorbed if the ionic strength in the system is low while virtually no adsorption takes place for high ionic strength. This is in opposite

to the usual finding that a high ionic strength furthers the adsorption of proteins to polymeric surfaces.[1] In case of flat substrates the high ionic strength lowers the electrostatic repulsion between the surface and the dissolved protein. The same repulsion should operate in the present mixtures, too, and an increased adsorption is to be expected at high ionic strength. Conversely, a low ionic strength is expected to prevent adsorption of dissolved proteins because both the protein and the particle are both negatively charged. Moreover, the layer of densely grafted polymer chains, i.e., the brush layer on the surface of the particles should exert a strong steric repulsion onto the protein molecules that can be envisioned as small colloidal particles. The steric repulsion between solute objects and planar or curved brushes is a well-known phenomenon and no adsorption is expected for the present mixtures at all.

Additional experiments[28] demonstrate that this unexpected effect is not only occurring for BSA but for other proteins as well. It is clear that the adsorption of dissolved proteins onto spherical polyelectrolyte brushes is a general phenomenon. All data available by now show that the decisive parameter is the ionic strength. This finds further support by the observation that the bound protein can be washed off the particles by a salt solution of much higher ionic strength. Thus, flushing the particles carrying BSA by a NaCl solution of 0.5M concentration removes the protein quantitatively. This is a very important observation inasmuch it shows that the adsorption is not irreversible. It may be reversed upon increasing the ionic strength to values where the electrostatic interaction is fully screened. In this way the SPB present a new class of carrier particles whose interaction with proteins can be adjusted by the ionic strength.

Summarizing these observations one can state that there is a strong adsorption of BSA at low ionic strength. Raising the ionic strength afterwards removes the protein but it cannot be washed off by a buffer solution of the same ionic strength. The amount of bound protein, however, is directly related to the amount added to the solution as clearly demonstrated by fig. 3 and 4. The data shown in fig. 4, however, do not present a true adsorption isotherm because there can be no true equilibrium anymore (see fig. 2).

These findings therefore suggest that the process of adsorption must contain two steps: The first step must be connected to an adsorption/desorption equilibrium which in turn leads to the well defined isotherm-like adsorption curve shown in fig. 4. This first step must hence define the number of available places on a brush particles at a given ionic strength. In a second step the

binding of the BSA becomes much stronger so that the washing process shown in fig. 2 does not remove the bound protein.

It must be noted that the second step cannot be related to an irreversible change of conformation of the adsorbed protein as e.g., the unfolding of the secondary structure or at least a partial denaturation. Such a process would lead to an irreversible adsorption of the protein and a washing off by salt solution would no more be possible. Small changes of the structure of the adsorbed protein, however, may well exist and may held responsible for the strong binding once the protein is confined within the brush.

The findings presented here can be explained by the following model: The *pH* adjusted by the buffer is higher than the isoelectric point of BSA. The *pH* within the brush, however, may be lower because of the Donnan equilibrium between the brush layer and the solution. Hence, depending on the *pH* there will be a finite number of cationic groups on the surface of the protein that may interact with the linear polyelectrolyte chains. Thus, the positive patch of the protein becomes a multivalent counterion which neutralizes several negative charges of the linear polyelectrolyte chain. The activity of the counterions within a brush, however, is quite low and even monovalent counterions are mainly confined within the brush.[27] For each neutralized patch the respective negative counterions together with the positive counterions of the PAA chains are released. This is connected to a concomitant gain of entropy of the entire system. If the proteins were to be released from the brush again, the concomitant number of counterions must be brought back to the brush. The loss of entropy related to such a process renders it much less probably and the proteins are bound tightly to the brush ("counterion release force"; see the discussion in ref.[29]). This would also explain the strong adsorption of BSA that persists if the ionic strength in the solution is kept constant: The activity of the protein molecules serving as counterions is dropped to very low values and the equilibrium concentration of the proteins outside is in consequence negligibly small. If salt is added, however, the effect vanishes and the protein molecules are replaced by the ions of the added salt.

Conclusion

A study of the adsorption of BSA onto spherical polyelectrolyte brushes has been presented. Strong adsorption of BSA onto the SPB is observed at low ionic strength despite the effect that

the particles as well as the dissolved BSA are charged negatively. The adsorption of BSA decreases drastically with increasing amounts of added salt. The possible reasons for the strong adsorption can directly related to the electrostatic interaction of the proteins with the brush layer. All results presented here therefore demonstrate that the SPB present a new class of carrier particles whose interaction with proteins may be tuned by the ionic strength.

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