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Review

Factors affecting protein interaction at sorbent interfaces

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Abstract

Interactions between surfaces and macromolecules are the fundamentals in separation and detection of diverse solutes. In this very brief review the central aspects of protein–surface interactions are discussed with the intention of identifying the important factors influencing such processes and placing them in relation to the established knowledge in this field. Some perspectives of new techniques related to scanning probe microscopy for studying interactions at the nanometer level are also discussed. © 1997 Elsevier Science B.V.

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Contents

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1. Introduction

If the amounts of the various components in a system vary, the chemical potential is the driving force within the system. There will be a flow of molecules between all of the different states of the system until Eq. (1) is satisfied which means that equilibrium is reached when the value of the chemical potential is uniform throughout.

$$\mu^{i}n + kT\log X_n = \text{const. for all states } n = \mu \qquad (1)$$

where μ is known as the chemical potential and $kT\log X_n$ as the ideal solution entropy.

What parameters and factors influence these driving forces apart from those included in Eq. (1)?

Before a protein molecule can be adsorbed it must reach the interface. Since surfaces are covered by layers of water molecules which are not broken by convection, the transport in the water layer close to the surface must be based on diffusion. In this case, simple diffusion theory may be applied to predict the rate of adsorption. The rate of adsorption will be simply equal to the rate of this diffusion step given by classical diffusion theory as:

$$dn/dt = C_0(D/\pi)^{1/2}t^{-1/2} \qquad (2)$$

where C_0 is the bulk concentration, D is the diffusion coefficient, t the time.

The effect of co-solvents as a driving force on protein and hydrophobic surface interactions have been extensively studied [1–3]. Basically, two different theories have been proposed to explain the effects of co-solvents on the binding of proteins to these type of surfaces: the preferential protein–solvent interaction effect which originates from Arakawa [1] and the surface tension effect proposed by Melander and Horváth [4]. Upon addition of co-solvents the chemical potential of the protein in solution will increase, which enhances the binding of the protein to the surface simply by the law of mass action. The relation between surface tension and the degree of protein adsorption onto non-polar surfaces originates from the work of Frank and Evans [5] and Sinanoglu and Abdulnur [6].

The effect of conformational changes of proteins upon adsorption to surfaces as a driving force for adsorption of proteins has been studied by Norde et al. [7] for hydrophilic and hydrophobic surfaces. They showed that $D_{ads}H>0$, which means that the adsorption process must be entropically driven. They conclude that properties of the protein molecule itself govern the adsorption process by conformational changes and increase in rotational mobility. These conformational effects can be so large that they overcome the unfavorable contributions from dehydration of a hydrophilic surface and/or overall electrostatic repulsion between the protein and the sorbent.

Another thermodynamic aspect of conformational changes is the effect on hydration of non-polar groups of proteins. As a consequence of release of more flexible segments, new areas of the protein will became exposed to water molecules. According to Sinanoglu [3] the water molecules covering surfaces have a lower entropy than the bulk water. This means that hydration of those newly exposed surfaces is an unfavorable process. According to Privalov [8] the balance between the entropy of hydration of internal groups of proteins that become exposed upon protein unfolding almost completely balances the entropy due to the conformational transition of the polypeptide chain into a random coil conformation.

When the protein molecules are sufficiently close to the surface at the interface different types of intermolecular interactions will take place. One traditional way to classify forces and interactions is: electrostatic interactions, Van der Waal interactions, hydrogen bonding and hydrophobic interactions. Israelachvili [9] prefered to classify the forces as: purely electrostatic forces, ion–dipole forces, dipole–dipole forces, quantum-mechanically based forces (covalent bonds, charge–transfer interactions). The Coulomb force between two charges is expressed by:

$$F = Q_1Q_2/4\pi\cdot\varepsilon_0\varepsilon r^2 \qquad (3)$$

where F is the force between the two charges Q_1 and Q_2 and r the distance between them. ε is the dielectric constant of the medium and ε_0 the dielectric constant in vacuum.

Since the protein molecule and the sorbent surface are usually electrically charged, Coulomb interaction forces expressed by Eq. (3) play an important role in the adsorption process of proteins onto surfaces.

The surface, together with the adjacent part of the solution, is always electrically neutral under equilib-

rium conditions. An electrical double layer will be established consisting of a surface charge and an equal but opposite charge in the solution, the counter charge, and an ensuing electric potential. Different double layer models have been developed, of which the Gouy–Stern double layer is a consensus of other models [10–12]. According to Jönsson and Wennerström most of the counter-ions are located within a few Å from the surface [13]. Co-ions are also distributed in this area, together with the counter-ions, in such a way that an excess of counter-ions exists together with a deficit of co-ions in relation to the bulk solution resulting in an electrically neutral surrounding at a certain distance from the surface.

London showed [14] that molecules without permanent dipoles attract each other because of the mutual perturbation of their electron orbits. He named this type of interaction the dispersion force attraction and proposed that the perturbation of orbiting electrons could be treated as a sum of fluctuating dipoles and quadropoles.

In addition to the dispersion force, two other forces contribute to the long-range interaction between polar molecules which we today collectively know as the Van der Waals force, namely the induction force and the orientation force.

$$W_{VDW}(r) = -C_{VDW}/r^6 = -(C_{ind.} + C_{orient.} + C_{disp.})/r^6 \quad (4)$$

The net Van der Waals energies between two polar molecules at a certain distance r are given by C_{VDW}, $C_{ind.}$, $C_{orient.}$ and $C_{disp.}$ which are interaction free energy coefficients for inductive forces, orientational forces and dispersion forces, respectively [9].

Since London's theory cannot handle the interactions of molecules in a solvent a new theory was developed by McLachlan [15]. This theory treats Van der Waals forces between molecules, whereas the Lifshitz theory deals with Van der Waals forces between surfaces [16]. The Coulomb interaction forces and the Van der Waals forces act together and this is the basis for the DLVO theory. (Derjaguin and Landau [17], Verwey and Overbeek [18]).

When we consider long range interactions between molecules and surfaces in liquids the two most important forces are the Van der Waals and electrostatic forces. At a shorter distance, 1–3 nm, the DLVO theory fails and additional short range forces are observed, the so called solvation forces which often dominate over both the electrostatic and Van der Waal forces [9]. Solvation forces in water are short-range exponentially repulsive forces and are commonly referred to as hydration or hydrophilic forces [19]. Hydration forces arise whenever water molecules bind to surfaces containing hydrophilic groups and their strength depends on the energy needed to disrupt the ordered water structure.

When interactions between a molecule and a hydrophobic surface are taken into consideration in the presence of water, Van der Waals forces are strongly reduced. However for low-molecular-mass alkanes interacting in water, the major contribution comes from the hydrophobic interaction [20]. This type of interaction is classified as a strong attractive force. At any kind of hydrophobic surface there is no binding of water molecules but, a cooperative hydrogen bonding interaction gives rise to solvation effects that are quite different from those occurring at hydrophilic surfaces.

Concerning stabilisation of proteins by the hydrophobic effect, Kauzmann [21] assumed that non-polar side chains along the polypeptide backbone have the same character as non-polar molecules.

According to Ben-Naim [22] the latter hypothesis has two weak aspects: it ignores solvent-induced effects other than those from hydrophobic groups and it treats the side groups of the polypeptide backbones as though they had the same characteristic as low-molecular-mass alkanes.

According to Ben-Naim [22], the effect of hydrophilic groups in forming hydrogen bonds between functional groups, the so called "hydrophilic effect" can also contribute. Since functional groups are solvated by water molecules, hydrogen bonds have to be broken and new ones formed [23]. Such a process argues against the hydrophilic effect. According to Ben-Naim [22], available data supports neither the hydrophobic effect nor the hydrophilic effect hypothesis. He concludes that the required data is quite different from that currently used to estimate protein stability and driving forces in different molecular surface interactions.

The electron donor acceptor concept is based on Mullikens theory of charge transfer complexes [24,25]. In this theory, the wave function of the ground state of a 1:1 complex, n, is described by the expression:

$$\psi_n(DA) = a\psi_0(DA) + b\psi_1(D^+A^-) \quad (5)$$

Two electronic states are assumed here: non bonding (DA) and dative (D^+A^-). The ψ_0 term represents a no bond wave function of A and D in close proximity with no charge transfer between them. The ψ_1 term is a dative wave function corresponding to the total transfer of an electron from D to A.

The main factor influencing the stability of AD complexes is the electronic structure of A and D. Mulliken [24,25] stresses the orientation character of charge transfer interactions. Specific interactions taking place in the liquid or on solid surfaces can be explained on the basis of a modern concept of acid–base interactions.

2. Effect of the type of surface

Proteins become adsorbed onto all types of surfaces. The amount adsorbed varies considerably for different types of surfaces and depends on factors presented in the introduction, where mainly four different type of forces are involved: attractive Van der Waals forces, repulsive double layer forces, repulsive hydration forces and attractive hydrophobic interactions. The contribution from the different forces will be different depending on the type of surface as well as the influence of the type of molecule. A widely accepted generalization is that the more hydrophobic the surface, the greater is the extent of adsorption. The strongest support for this comes from numerous articles published in the field of chromatography which have been confirmed many years later by work performed by Elwing et al. using gradient surfaces [26]. The results of this study show that the adsorption of proteins from protein solutions containing only one type of protein is greater on the hydrophobic end of the gradient for a variety of proteins.

Conclusions about protein adsorption onto electrically charged surfaces are easy to draw if we only have to handle purely electrostatic phenomena, since it seems obvious that attractive interactions should dominate between opposite charges and repulsive interactions between like charges. The effect of the electrical double layer in combination with Van der Waals and solvation forces makes the situation more complicated to evaluate. The contribution of hydration changes, Coulombic interaction and structural rearrangements of the protein molecule have been studied by Arai and Norde [27]. They investigated the adsorption behavior of the proteins lysozyme, ribonuclease, myoglobin and α-lactalbumin onto surfaces of different hydrophobicity and electrokinetic potential. Hydrophobic polystyrene adsorbs all proteins. At the hydrophilic hematite surface lysozyme and ribonuclease adsorb only if electrostatically attracted. Myoglobin and α-lactalbumin adsorb even to electrostatically repulsive surfaces. The explanation for this behavior of myoglobin and α-lactalbumin is that proteins of low structural stability contain an additional driving force for adsorption which is related to structural alterations.

Sometimes low adsorptive surfaces are needed for different purposes. One important characteristic of gel filtration media is a low adsorptive solid-phase, which was the reason for development of chromatographic media such as cross-linked dextrans [28] and beaded agarose [29]. Later on, polyethylene oxide (PEO) grafts showed greatly reduced protein adsorption [30]. The mechanism of resistance to protein adsorption is not well understood but appears to be related to the high energy needed to disrupt the ordered water structure around the hydrophilic surfaces and/or the high flexibility of the PEO chains [31].

3. Adsorption of proteins to different type of surfaces in the presence of co-solvents

3.1. Effect of the type of surface

A vast number of articles have been published in the area of co-solvent based chromatography performed with the intention to separate molecules of different kinds from each other. At the same time a lot of information concerning the factors influencing adsorption of proteins onto different types of adsorbents has been obtained. It is impossible to make a complete review of this area. Instead, I would like to look into this field from the perspectives I have presented in the Introduction. Of the different forces involved in protein–surface interactions, at least three different co-solvent mediated chromatographic principles can, in principle, exist: chromatography

based on hydrophobic interactions explained by Sinanoglu [3] and Melander and Horváth [4], chromatography based on the electron donor acceptor concept explained by Mulliken [24,25] and, finally, chromatography based on hydration or hydrophilic forces. As early as 1948, Tiselius noticed that proteins could be adsorbed onto surfaces that in the absence of salt exhibited no tendency to bind these proteins [32]. Later on, chromatographic methods based on the first two principles discussed above were described by Porath et al. [33,34] and Melander and Horvath [35]. Hydrophobic interaction chromatography originates from observations made during affinity chromatography experiments [36]. Concerning classification of co-solvent promoted interactions, Porath [37] classifies immobilized metal ion affinity chromatography (IMAC) as the third type of chromatographic principle.

Do we have other types of co-solvent based chromatographic principles? As pointed out by Israelachvili [9] the interplay of the repulsive hydration forces and attractive hydrophobic solvation forces occurring between surfaces containing both hydrophilic and hydrophobic groups is not understood. The question is what the balance between hydrophobic and hydrophilic forces will be with molecules exhibiting a gradient of polarity between water and hexane.

Among the above mentioned principles, surfaces have been developed which favor hydrophobic interactions whereas others are more purely electron donor acceptor based interactions. In between these two extremes mixed effects can be obtained where the influence of hydration forces might also be involved.

One interesting type of interaction is the so called "thiophilic interaction", first described by Porath and coworkers, whereby a surface containing a combination of a sulphur and a sulphone group [38] or sulphur and a mercaptopyridine group [39] preferentially adsorbs immunoglobulin under certain conditions. Several groups have been elaborated by combining different amounts of sulphur groups and different combinations of pyridines with the intention to reveal the role of sulphur [40,41].

3.2. Effect of co-solvents

In the Introduction I presented the different theories developed to explain the effect of co-solvents on protein adsorption. According to Arakawa and Narhi [42] co-solvents will be preferentially excluded from the protein surface. The preferential interaction is directly correlated to the chemical potential of the protein. This alteration in the chemical potential of the protein can be translated into the free energy of binding by:

$$L(L_{m2}) = L^{l}_{m2} - L^{f}_{m2} = LG_s - LG_w \quad (6)$$

where L^{l}_{m2} and L^{f}_{m2} are the transfer free energy of the protein when bound to the ligand and in free solution, respectively and LG_s and LG_w are the free energies of binding in water and in solution containing the co-solvent. According to Arakawa, the co-solvents will be less excluded from the protein bound to the ligand on the adsorbent due to the smaller surface of the protein presented to the solvent, and as a consequence, $L(L_{m2})<0$. This leads to $LG_s<LG_w$ and hence the binding becomes more favorable in the presence of additive.

If, according to Arakawa [1], the effect of co-solvents is to change the chemical potential of proteins, all type of protein–surface interactions will be affected by addition of co-solvents. This is also supported by our own studies of covalent binding of human serum albumin (HSA) via thiol–disulphide interactions [43].

According to Tanford [44] the dissolution of any solute in water proceeds by some initial disruption of the ordering of water molecules. This first event is followed by a reorganisation which frequently results in a negative entropy indicative of the ordering of water molecules around the ions by ion–dipole forces.

During the study of the salt-dependent covalent immobilization of HSA [45] it became obvious that the increased chemical potential of the protein is not the only factor influencing the improved covalent immobilization. Since the thiol group in HSA is not exposed on the surface of the protein in the native conformation it must in some way became exposed to the reactive groups on the surface, otherwise, no reaction can occur. Conformational changes as a consequence of adsorption to the surface are strongly supported by complementary experiments with thiolated HSA, where the thiol group is exposed on the surface. In this case no difference in the reactivity of

HSA was observed [46]. Despite the stabilizing effect of salts on the protein structure [47] conformational changes are one of the main problems in co-solvent dependent adsorption [48]. The occurrence of changes in the native structure of proteins, even in solution, in the presence of different salts at high concentration has been experimentally reported by us [49] and by other groups [50,51].

The effect of different salts on protein interaction with hydrophobic surfaces has been investigated by Arakawa [52] and Melander and Horváth [35] from a thermodynamic point of view. The effect of the type of salt on the type of proteins which will be adsorbed to hydrophobic surfaces has been investigated by Fausnaugh et al. [53,54]. They conclude that protein hydration as well as specific interactions between the protein and the salt ions influence the protein retention. Strop [55] studied the effects of the concentration of cations and anions on the retention of proteins. Nishikawa and Bailon [56] tested a number of compounds for their effect on the elution of β-lactalbumin. Their results are in qualitative agreement with the preferential interaction and surface tension data. The studies of Påhlman et al. [57] on the effects of high concentration of neutral salts on the retention of human serum albumin show good agreement with the existing theories.

We showed in a study that sorbitol can promote the adsorption of proteins onto mercaptomethylenepyridine derivatized agarose whereas glycerol cannot do so [58]. Therefore, we have recently extended our study to the investigation of polyol-promoted adsorption of serum proteins to amphiphilic agarose-based adsorbents. The results show that polyols containing three and five carbon atoms were inefficient in promoting protein adsorption whereas polyols containing six carbon atoms could promote serum protein adsorption onto mercaptomethylene-pyridine derivatized Sepharose as well as onto octyl and phenyl-Sepharose [59].

4. Competitive and sequential adsorption. Protein–protein interaction

When a mixture of proteins is present in the solution, competitive adsorption will occur when only a limited amount of interaction sites are available on the adsorbent. As pointed out by Brash [60] the factors influencing the relationship between the surface and solution composition in competitive protein adsorption to a surface can be divided into kinetic factors and affinity factors. The kinetic factors will be dependent on the relative concentration of the proteins, the sizes of proteins and the activation energy of adsorption.

Important affinity factors are the electrical charge of the surface, the hydrophobicity of the protein surface, the chemical functional groups on the proteins, the conformational stabilities and the sizes of the proteins.

An experimental manifestation of a sequential competitive adsorption is the so-called "Vroman effect". Using immunochemical methods of detection, Vroman and Adams [61] were the first to observe that fibrinogen becomes rapidly adsorbed following blood–surface contact, but is subsequently displaced from the surface. Several other groups have recently confirmed this behavior [62,63]. Competitive adsorption of ^{125}I-labeled fibronectin, fibrinogen and immunoglobulin to polystyrene latex by unlabeled fibrinogen, fibronectin, immunoglobulin and albumin was studied by Bale et al. [64] to determine the relative affinities of these proteins for the surface. The results indicate that the order is fibrinogen > fibronectin > immunoglobulin > albumin. The competitive adsorption of HSA, immunoglobulin G (IgG) and fibrinogen at radio frequency plasma polymer surfaces was studied by in situ ellipsometry and total internal reflection fluorescence [65] whereas both IgG and fibronectin were adsorbed preferentially over HSA at hydrophobic surfaces, preadsorption of the latter protein dramatically reduced the adsorption of the two former. Furthermore, the preadsorbed HSA does not exchange with HSA added after preadsorption, presumably as a result of conformational changes in the adsorbed HSA. Different plasma polymer surfaces behave differently regarding competitive protein adsorption. Elgersma et al. [66] studied the competitive adsorption between albumin and monoclonal IgGs onto polystyrene latices and found that preadsorbed IgG was more easily displaced by bovine serum albumin (BSA) than vice versa. The first stages in the adsorption process determine the final composition of the adsorbed layer. Structural rearrangements take

place more rapidly in BSA than in adsorbed IgG, which probably explains why BSA withstands competition better than later-arriving IgG.

Differential surface binding of albumin, IgG and fibrinogen was studied by Warkentin et al. [67] by use of a wettability gradient on silicon wafers. By use of radiolabeled BSA and IgG they investigated the preferential adsorption of these proteins to the hydrophobic gradient. The behavior of ^{14}C-labeled albumin incubated for 10 min as a single protein indicates that adsorption occurs to a greater extent onto surfaces with an intermediate contact angle than onto those which are purely hydrophobic or hydrophilic. Moreover subsequent incubation with fibrinogen indicated that more radiolabel was present on the hydrophobic surface than when it was incubated for the same period in phosphate buffered saline (PBS). Hydrophilic surfaces showed less albumin after fibrinogen treatment. When radiolabeled albumin was co-incubated with unlabeled IgG, the total amount adsorbed at a 45° water contact angle remained the same, but substantially more albumin bound to the hydrophobic surfaces. Radiolabeled IgG incubated alone bound more to the hydrophobic surfaces than to the intermediate or the hydrophilic ones. Fibrinogen treatment after IgG had no significant effect. Co-incubating the surfaces with equal amounts of radiolabeled IgG and unlabeled albumin showed that the binding of IgG onto hydrophobic surfaces was reduced by half but was essentially unchanged on the hydrophilic surfaces. When transferred to a fibrinogen solution, the IgG surface content remained unchanged. The results of ellipsometric studies showed that when IgG and HSA were combined in the same experiment, a greater thickness was obtained at the hydrophilic than at the hydrophobic end. The influence of the sequence of proteins in contact with the surface shows that when HSA preceded IgG, IgG adsorption was enhanced in the hydrophilic region.

In order to investigate the influence of the underlying surface on the first adsorbed biomolecule and how this first layer will adsorb additional biomolecules, porous silica beads were modified with aminoalkyl and thioalkyl groups of different chain lengths [68]. Chromatography in combination with frontal analyses were used for evaluation of the surfaces. A higher tendency to adsorb IgG to HSA- covered underivatized surfaces was observed. Decylamine and decylmercaptan-derivatized silica covered by HSA had the opposite effect from underivatized silica. In the case of decylmercaptan the capacity diminished up to nine-fold compared to the native surface. The diminishing adsorption capacities with increasing hydrophobicities of the derivatized surfaces are explained in terms of orientational effects and conformational effects which result in exposure of new surface features. The same effect was observed when IgG-covered surfaces were investigated with respect to HSA adsorption; i.e., the more hydrophobic surfaces had a diminished capacity to adsorb HSA following coverage with IgG. Displacement studies showed that HSA was displaced by IgG from all of the surfaces except thioalkyl-derivatized silica. On the other hand, IgG was displaced only from the decanthiol-derivatized silica and butylamine silica by HSA.

5. Conformational changes as a consequence of protein adsorption

Conformational changes in proteins as a consequence of adsorption to different types of surfaces have been reported for many proteins and especially for serum albumin [69–71]. Further reports supporting this fact come from several other groups. Hoffman [72] reported that the type of surface is important for the extent of conformational changes. Kochwa et al. [73] found that the density of the protein molecules is important for the extent of the conformational changes in gamma globulins. This effect was more pronounced on hydrophobic surfaces. This more pronounced effect of conformational changes on hydrophobic surfaces has been confirmed by Cuypers et al. [74]. Walton and Maenpa [75] studied the adsorption of albumin onto benzyl-esterified polypeptide particles and reported a decrease in the α-helix content of the desorbed molecules. Sandwick and Scray [76] observed inactivation of enzymes upon contact with hydrophobic surfaces but not with hydrophilic ones. The same effect has been reported by Elwing et al. [77] for complement factors.

An important new experimental development in the study of the structure of adsorbed proteins was

reported by Kondo et al. [78]. They used circular dichroism in combination with nanoparticles of silica (15 nm) as adsorbent to examine changes in the α-helix content. They showed that albumin and hemoglobulin undergo extensive loss of α-helix content upon adsorption, whereas smaller molecules like ribonuclease do not.

6. Effect of pH

A general observation is that proteins adsorb maximally at their isoelectric points [12,79]. This can been explained in terms of a minimum of lateral repulsions between protein molecules at isoelectric point (i.e.p.) but according to Norde and Lyklema [80] the reduction can be explained by structural rearrangements in the adsorbed molecules which they verified experimentally for albumin. The more pronounced conformational change at both sides of i.e.p. is an effect of a decrease in the protein stability at increased net charge of the molecule surface.

7. Effect of the protein structure

The variations in structure between different type of proteins are too great to allow interpretations, but the use of protein mutants affords new opportunities to elucidate even finer details of the adsorption process. Pioneering work has been done by McGuire et al. [81]. Minimal changes in amino acid composition can change adsorption behavior significantly [82].

8. Organisation of the adsorbed protein layer

The influence of the protein concentration on the organization of molecules has been reported by Morrissey [83]. He found that the thickness of the adsorbed layer was 140 Å when a low protein concentration was used compared to 320 Å when a more concentrated protein solution was brought in contact with a silica plate. Morrisey suggested that at a low protein concentration the protein can "expand" compared to the situation when the surface is totally covered by molecules with a resulting orientation of the molecules in an "upright position".

Söderquist and Walton have proposed a model for protein adsorption onto surfaces [84]. According to this model, the surface becomes covered with protein molecules gradually. When the surface coverage exceeds 50% a reorganisation of the molecules occurs which leads to a more efficient packing followed by conformational changes and desorption.

Lee and Belfort [85] showed that during the adsorption process of RNase molecules onto surfaces, the molecules slowly become reoriented until they lie with the longest axis perpendicular to the surface and the active site partially exposed to the free solution.

9. The degree of reversibility in the protein–surface interaction

Another important factor which indicates the degree of conformational changes of proteins in contact with surfaces is the reversibility. Our own studies of desorption efficiencies from amphiphilic surfaces show that the adsorption process is more or less irreversible with gels used for hydrophobic interaction whereas less hydrophobic gels like mercaptopyridine-derivatized agarose allow more-or-less complete desorption of proteins [86]. The pronounced conformational effects on traditional hydrophobic adsorbents were also the reason for the development of mild hydrophobic chromatographic adsorbents by Ling and Mattiasson [87]. Further results from the studies by Norde et al. [7] on hydrophilic as well as hydrophobic surfaces show that the degree of desorption from hydrophobic surfaces is minimal which can be explained as a thermodynamically unfavorable hydration of the hydrophobic surface which involves a loss of entropy. With the hydrophilic substrates hematit and silica, the adsorption was found to be almost completely reversible.

10. Effect of temperature

The effect of temperature on protein–surface interactions is often studied when hydrophobic inter-

actions are evaluated. The observed increase in protein retention with temperature has been ascribed to enhancement of hydrophobic interactions with increasing temperature due to temperature-induced conformational changes in proteins and concomitant increase in the hydrophobic contact area upon binding to the surface [88]. Basic studies of the temperature effect in hydrophobic interaction have also been made by Jennissen and Botzet [89]. Temperature effects on hydrophobic interactions between dansyl derivatives of amino acids were studied by Haidacher et al. [90] using three different stationary phases: polyethylene glycol with methoxy end groups, propyl ligates bound to cross-linked polyethyleneimine and butyl ligates attached to bonded hydrophilic layers. Plots of the logarithm of the retention factor against the reciprocal temperature were non-linear, indicating a large negative heat capacity change associated with retention in the experimental temperature interval. The heat capacity change was found to increase with temperature. The effect of surface adsorption on the thermal stability of seven different types of proteins was studied by Steadman et al. [91] who found that surface adsorption decreased the thermal stability of the bound protein as evaluated by use of differential scanning calorimetry and front surface fluorescence spectroscopy.

11. Effect of ionic interactions

Elgersma et al. [92] have investigated the influence of electrostatic interactions on displacement reactions between IgG and BSA.

If displacement of albumin or monoclonal IgG occurs, its extent depends to some degree on the electrostatic interactions between the respective proteins and the surface. The amounts of additionally adsorbed proteins are also influenced by electrostatics. If no displacement of BSA occurs by IgG, the amounts of different IgGs adsorbed follow more or less the rules of electrostatic interaction, but BSA adsorption onto IgG-coated latices does not show such a trend. Simultaneous adsorption of IgG and BSA was studied using two latices of different charge sign. In the case of electrostatic attraction, there is a preferential adsorption of BSA, whereas in the case of repulsion IgG adsorption seems initially to be favored. In the above case both proteins have the same charge sign.

Buijs et al. [93] have studied interactions between different type of surfaces and IgG and $F(ab')_2$ fragments. When these two types of molecules were adsorbed onto negatively or positively charged hydrophobic polystyrene surfaces, hydrophobic interaction dominated with electrostatically attractive surfaces. When a situation of electrostatic repulsion existed between the hydrophobic surface and the protein, a secondary influence of the charge was observed at the saturation level. This secondary influence was exemplified by studies of adsorption of two different monoclonal IgG and $F(ab')_2$ onto negatively as well as positively charged hydrophobic polystyrene as a function of pH. The maximum of protein adsorption was observed when the proteins were marginally charged with a sign opposite to that of the surface. When the net charge on the proteins rose, the adsorbed amount decreased. Such a reduction in the adsorbed amount is considerably stronger when the protein charge and surface charge repel each other. By determination of the electrophoretic mobilities of the different protein–latex complexes of the originally negatively charged polystyrene particles, they found similarities in the pH dependence of the zeta potential despite very large variations in the adsorbed amounts and charges of the proteins adsorbed. Apparently, the net amount of charges remains low to suppress adverse electrostatic interaction. From this study they also concluded that the zeta potential is positive at low pH, which implies non-electrostatic influence on the interaction for the different protein–latex complexes and that the surface charge of the bare polystyrene latex is not yet fully compensated which means that the maximal amount of protein is adsorbed neither at the i.e.p. of the protein nor at the i.e.p. of the protein–latex complex, but somewhere in between.

The effect of ionic strength gave expected results such as higher amount of proteins adsorbed when the electrostatic interactions between the protein and sorbent were repulsive, whereas the opposite applied for conditions of attractive electrostatic interactions. Lateral repulsion between proteins is always reduced at higher ionic strength. Some exceptions are pointed out. Lateral repulsive screening is not complete.

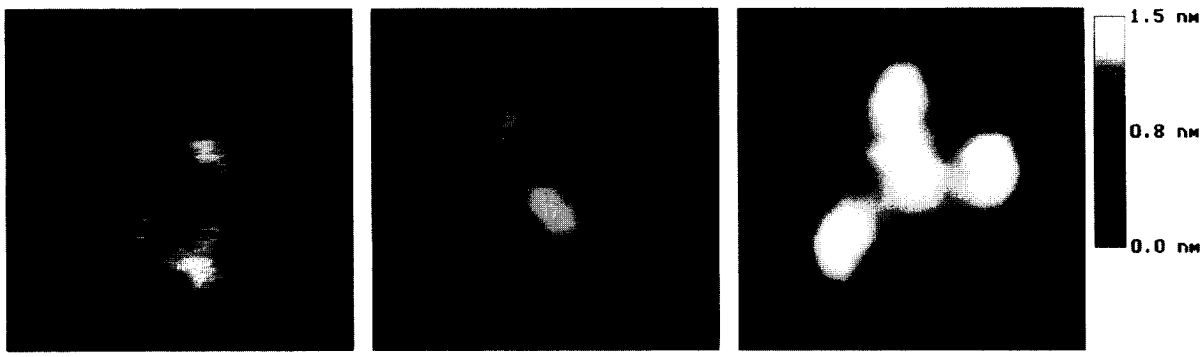

Fig. 1. TM-SFM images of single molecules adsorbed on mica surfaces. (A, left) HSA molecule, compact formation; (B, middle) HSA molecule, extended conformation; (C, right) human IgG, "Y"-shaped conformation.

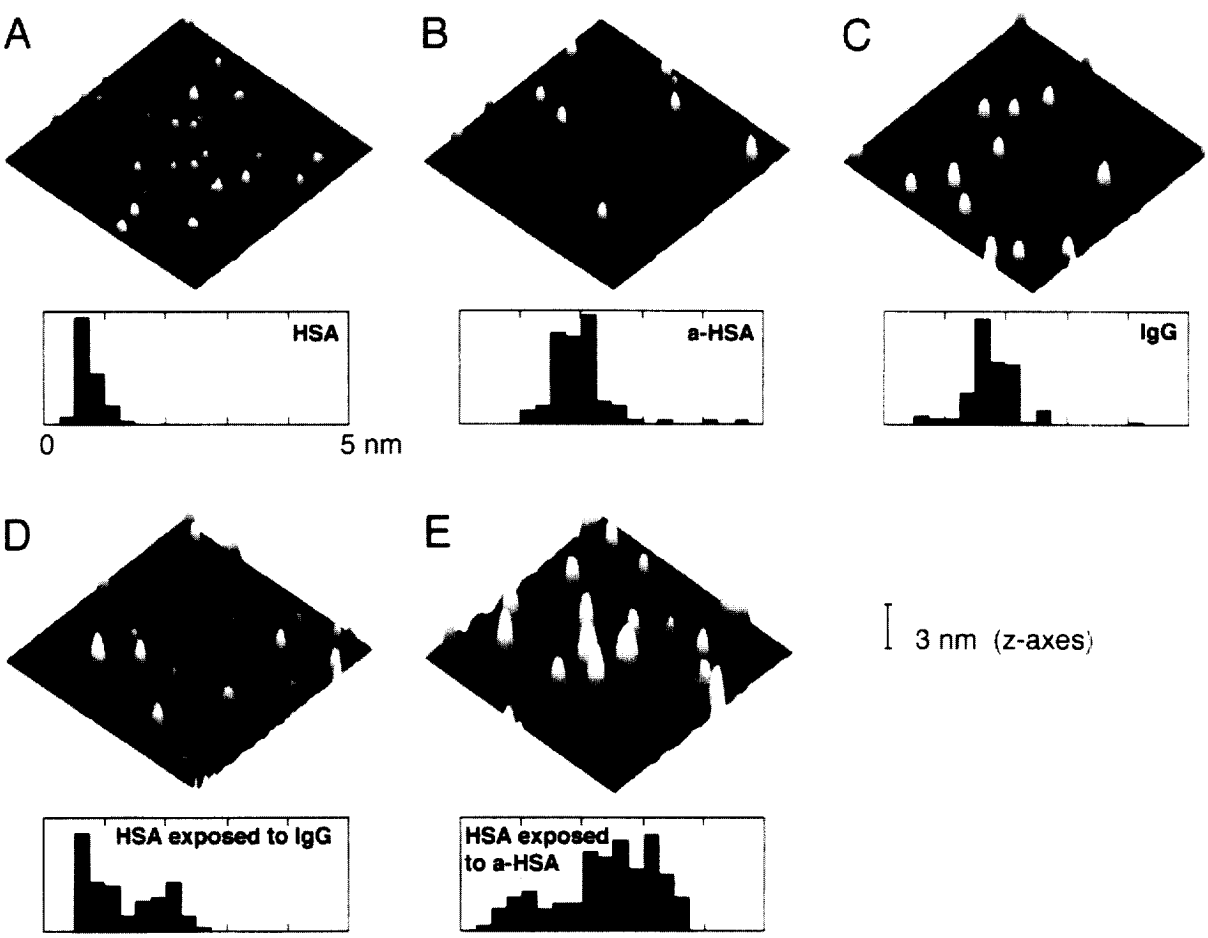

Fig. 2. TM-SFM images of mica surfaces exposed to: (A) HSA only; (B) anti-HSA only; (C) IgG only; (D) HSA only followed by exposure to IgG; (E) HSA followed by a separate exposure to anti-HSA. Each image covers an area of 0.5·0.5 μm. The histograms show the heights of observed features on the mica surfaces measured with TM-SPM. The horizontal axes below each micrograph range from 0–5 nm. Each histogram reflects measurements of at least 100 features from several comparable surfaces.

According to van Oss et al. [94] the influence of discrete heterogenous sites in hydrophilic surfaces such as glass or silica particles might be another explanation for the unexpected adsorption of HSA to electrostatically repulsive silica particles and glass slides. These discrete sites are positively charged according to van Oss et al. attract negatively charged moieties on the HSA surface.

12. Models for protein adsorption

Several models have been proposed for protein–surface interactions [95–97]. All of these models have one thing in common, they do not cover the whole interaction process and some of them even assume a reversible process for the protein–surface interaction. Instead, a more-or-less pronounced irreversible adsorption occurs [7]. One alternative adsorption model which includes the major requirement that the protein adsorption should be irreversible has been presented by Schaaf and Talbot [98]. This model on the other hand, does not fulfil the requirement that surface diffusion should occur, which has been demonstrated by Rabe and Tilton [99].

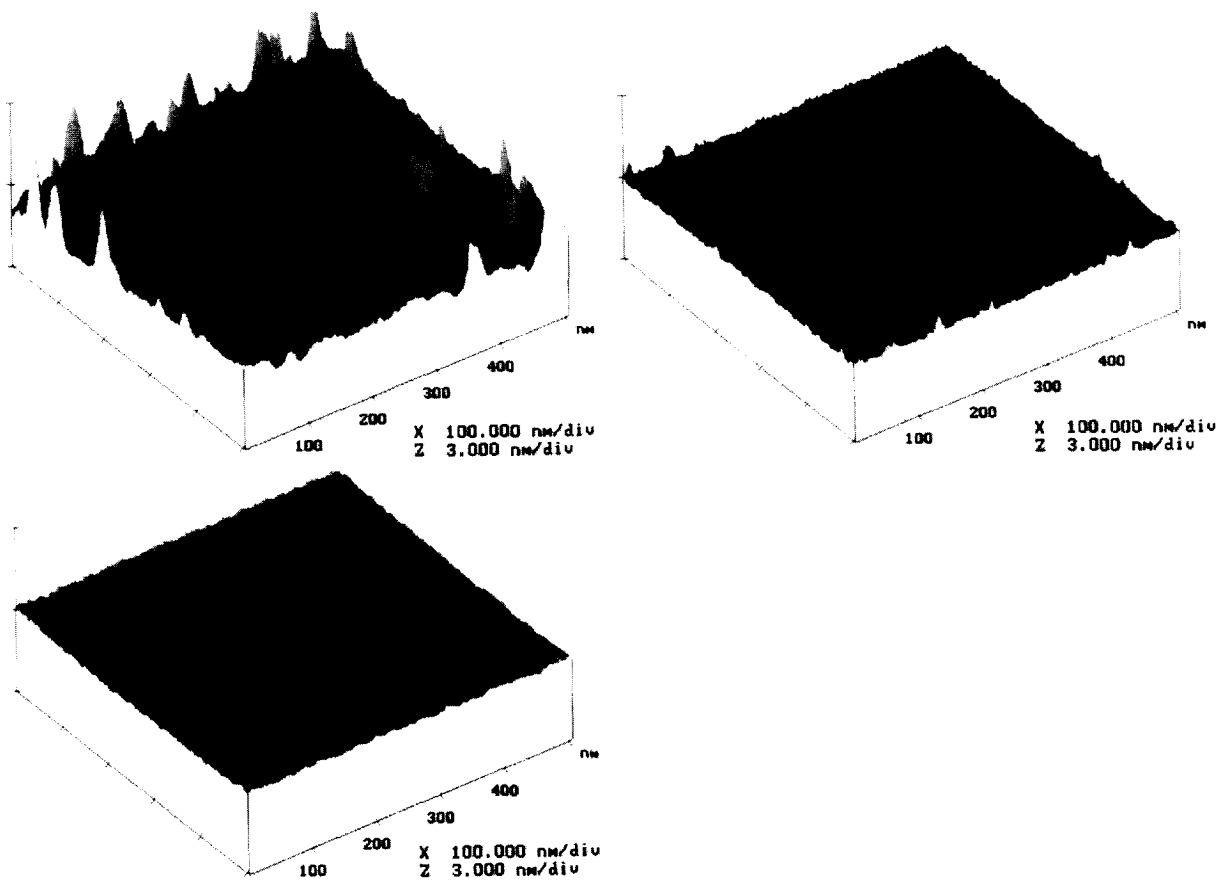

Fig. 3. Chemical modification of mica surfaces. (A, top left) and (B, top right) Derivatization of mica with γ-glycidoxypropyltrimetoxysilane in toluene. Reaction times were 10 min in all experiments and the concentration of γ-glycidoxypropyltrimetoxysilane 100 mg/ml in the experiment which is illustrated in (A) and 0.033 mg/ml of γ-glycidoxypropyltrimetoxysilane in (B). (C, bottom) The effect of evaporation of γ-glycidoxypropyltrimetoxysilane on a mica surface when placed in a dessicator under reduced pressure. The total reaction time was 1 1/2 h.

13. Future aspects

The key to further exploration of protein–surface interactions and the development of new separation techniques is new scientific tools. One of the most promising techniques is the so-called scanning probe microscopy (SPM) technique. SPM is one of several acronyms for this technique: another is atomic force microscopy (AFM). One of the most up-to-date and comprehensive reviews is that presented by Bottomley et al. [100]. To introduce this technique I would like to present some of our own results in the area of SPM. This technique permits visualization of single molecules and even sub-domains of molecules. Fig. 1A shows an image of a single HSA molecule adsorbed onto mica. The white parts of this triangularly shaped molecule are the three sub-domains. In Fig. 1B the HSA molecule has adopted its elongated structure and it is still possible to see two of the sub-domains. The elongated structure is a proof of conformational changes, since the triangularly shaped molecule is the native form [101]. 35% of the molecules displayed this type of structure [102]. During our studies of antigen–antibody docking we observed that IgG was oriented on mica in an end-on manner. This orientation was so strongly favored that actually less than 1% of IgG with a side-on orientation was observed [103]. This strongly unfavored orientation of one single IgG molecule can be seen in Fig. 1C.

In Fig. 2, the result of antigen–antibody docking experiments show that one immunocomplex between HSA and anti-HSA can be distinguished from the single antibody and albumin molecule.

Fig. 3A,B, display results from chemical derivatization with one of all the silanol reagents, γ-glycidoxypropyltriethoxysilane in toluene, according to a standard procedure often described in the literature to obtain chemically modified surfaces for investigation of protein–surface interactions. It shows that no monolayer is obtained by these techniques. Instead, a patchwise distribution results giving rise to an unevenly modified surface which greatly complicates interpretation of adsorption behavior. In the worst case, most of the published results concerning gradients of chemically derivatized surfaces are the result of imperfect derivatization. Fig. 3C shows a mica surface which has been derivatized by vaporization of the silanol reagent onto the surface. It is evenly modified according to

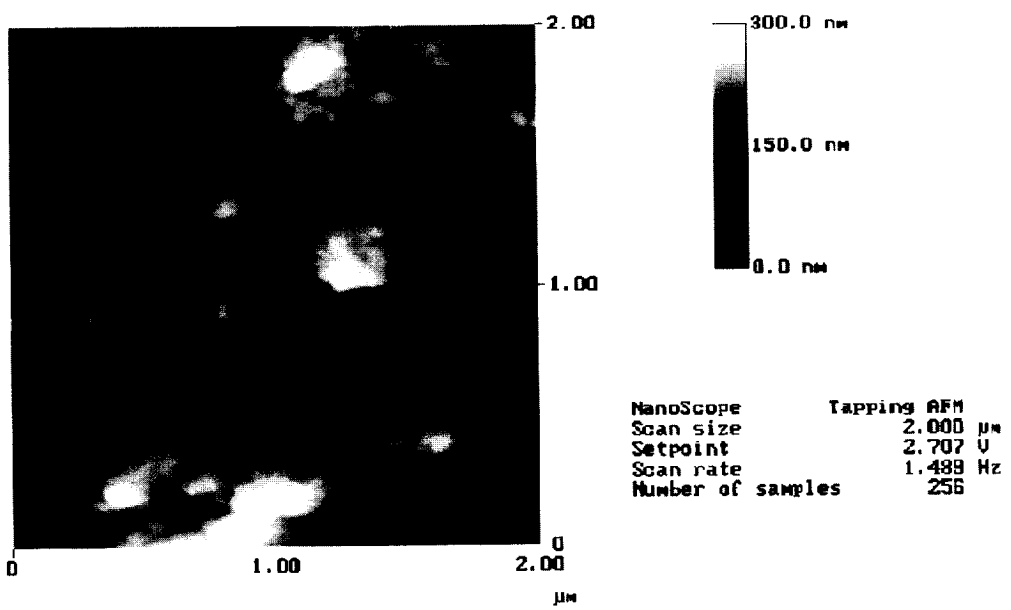

Fig. 4. TM-SFM image of the surface of an agarose bead (Sepharose 6B). The height scale is presented to the right part of the picture with a range of 300 nm. The scan area is 2 μm.

AFM, but the thickness of the layer is still under investigation.

Finally, one image of a surface which is often used for chromatographic purposes, a Sepharose 6B surface. Fig. 4 shows a result from a 2 μm scan over one single agarose bead with an approximate diameter of 40 μm. It shows the topography of the surface, where the light parts are higher areas and the dark parts are lower areas and probably pores into the inner part of the agarose bead.

14. List of symbols

μ	Chemical potential
$kT\log X_n$	Ideal solution entropy
C_0	Bulk concentration
D	Diffusion coefficient
t	Time
$D_{ads}H$	Adsorption entalpy
Q_1 and Q_2	Charges
ε	Dielectric constant
ε_0	Dielectric constant in vacuum
r	Distance between charges or molecules
W_{VDW}	Net Van der Waals energies between two polar molecules
C_{VDW}	Interaction free energy coefficient
$C_{ind.}$	$2u^2\alpha_0/(4\pi\varepsilon_0)^2$ where $\alpha_0/4\pi\varepsilon_0=$ electronic polarizability and $u=$ permanent dipole moment
$C_{orient.}$	$u^4/3kT\cdot(4\pi\varepsilon_0)^2$
$C_{disp.}$	$3\alpha_0^2 h\nu_1/4(4\pi\varepsilon_0)^2$ where $h\nu_1=I$, the ionization potential
ψ_n(DA)	Wave function of the ground state of a 1:1 charge transfer complex, n
ψ_0	No bond wave function of A and D in close proximity with no charge transfer
ψ_1	A dative wave function corresponding to the total transfer of an electron from A to D
L^l_{m2}	The transfer free energy of the protein when bound to the ligand
L^f_{m2}	The transfer free energy of the protein when in free solution
LG_s	The free energy of binding in solution containing the co-solvent
LG_w	The free energy of binding in water without the co-solvent

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