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Invited Conference Paper

The Role of Charged Groups in the Adsorption of Proteins at Solid Surfaces*

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Interaction between electric charges is an important factor in protein adsorption. Similar charge signs on the protein and the sorbent surface opposes adsorption, but whether it prevents adsorption depends on other factors such as dehydration of the protein and the sorbent and structural rearrangements in the protein molecule. Ribonuclease, that does not strongly change its structure upon adsorption, adsorbs on hydrophilic surfaces only if its charge sign is opposite that of the sorbent. On increasing the hydrophobicity of the sorbent adsorption also takes place in the case of the same charge sign. Blood plasma albumin, on the other hand, adsorbs at any interface even if the sorbent is hydrophilic and has the same charge sign as the protein. In this case the driving force for adsorption stems from dehydration and/or conformational changes in the protein molecule. Although a same charge sign may not prevent adsorption to occur, it may slow down the adsorption process. This has been demonstrated for albumin at negatively charged polystyrene surfaces. The Gibbs free energy of the net electrostatic interaction is relatively insensitive for the charge on the protein and the sorbent. This is due to the role of small ions in the system: ions are eventually transferred from the aqueous solution into the contact region between the protein and the sorbent in order to prevent the development of high electrostatic potentials in this region. The chemical effect of the medium change of these ions is unfavorable and, since it is proportional to the number of trasferred ions, it increases with decreasing charge contrast between the protein and the sorbent surface.

INTRODUCTION

Proteins in aqueous media carry charged groups partly from (de)protonation of amino acid residues and partly from ions that are physically bound to the protein molecule. Under most conditions the protein contains both positive and negative charges. They occur primarily at the periphery of the molecule and where they reside in the interior of the protein they are likely to have formed ion pairs.

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Surfaces of solid materials, whether they are synthetic or biological, that are in contact with water are also usually charged. The charge is due to ionization and/or binding of ions from the medium. In most natural systems the sorbent surface charge is negative.

The charge on the protein molecule and on the sorbent surface (including the ions bound within the hydrodynamic slipping layers of these species) is balanced by counterions that are diffusely distributed. Figure 1 schematically represents the charge distribution at the protein molecule and the sorbent surface.

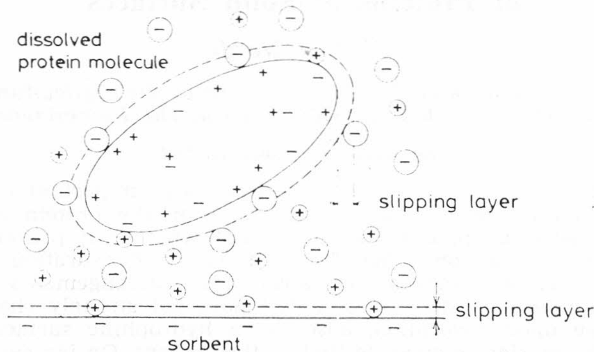

Figure 1. Schematic representation of the charge distribution in and around a dissolved protein molecule and at a sorbent surface.

When the protein molecule and the sorbent surface approach each other the electrical double layers overlap. This would result in electrostatic repulsion if the protein and the sorbent have the same charge sign and in attraction if the charges are opposite. Double layer overlap becomes effective at relatively large separations, say a few nm, between the two charged bodies. The most well-known treatment of these long-range interactions is described in the DLVO theory for colloid stability.[1,2] It states that the net interaction results from electrostatic and dispersion forces. This total interaction determines whether the protein molecule can *reach* the sorbent surface.

In calculating the Gibbs free energy G_E resulting from double layer overlap one has to assume that either the electric potential or the charge density at the interacting surfaces remains constant. Anticipating the discussion on the participation of small ions in the overall-adsorption process, the assumption of constant potential seems to be the most realistic one. Then, in the case of a flat surface (1) and a sphere (2) with radius R at a separation H across a medium (3) G_E is given by[3,4]

$$G_E = \pi \varepsilon \varepsilon_o \mathrm{R} (\psi_{13}^2 + \psi_{23}^2) \left\{ \frac{2 \psi_{13} \psi_{23}}{\psi_{13}^2 + \psi_{23}^2} \ln \left[\frac{1 + \exp(-\varkappa H)}{1 - \exp(-\varkappa H)} \right] + \ln [1 - \exp(-2 \varkappa H)] \right\} \tag{1}$$

where $\varepsilon\varepsilon_o$ is the dielectric permittivity of the medium, ψ the potential at the phase boundary indicated by the index and $\varkappa$ the reciprocal Debye length. The latter quantity depends on the ionic strength of the bulk solution, according to

$$\varkappa^2 = \frac{e^2}{\varepsilon \varepsilon_0 kT} \Sigma_i c_i z_i^2 \qquad (2)$$

where e is the elementary charge, kT one unit of thermal energy, c_i and z_i the concentration and valency of ionic species i.

For ψ the electrokinetic potential (= ζ-potential), i. e. the potential at the hydrodynamic slipping plane, is usually taken. The values of ψ and $\varkappa$ decrease with increasing ionic strength; hence, G_E is a decreasing function of the ionic strength.

The Gibbs free energy G_A from dispersion forces between a sphere and a flat surface can be approximated as follows[4]

$$G_A = -\frac{A}{6}\left[\frac{2R(H+R)}{H(H+2R)} - \ln\left(\frac{H+2R}{H}\right)\right] \qquad (3)$$

where A is the Hamaker constant for the whole system. It is composed of the Hamaker constants for the individual materials

$$A = (A_1^{1/2} - A_3^{1/2})(A_2^{1/2} - A_3^{1/2}) \qquad (4)$$

In aqueous media A usually attains a positive value so that G_A is negative, i. e. the dispersion forces are attractive.

According to the DLVO theory the total interaction free energy G_{tot} is the sum of G_A and G_E. Typical curves for G_E, G_A and G_{tot}, for the case the sphere and the flat surface have the same charge sign, are shown in Figure 2.

At low ionic strength, say $\leq 10^{-1}$ M, the maximum in $G_{tot}(H)$ is positive and therefore constitutes a barrier for deposition of the molecules at the

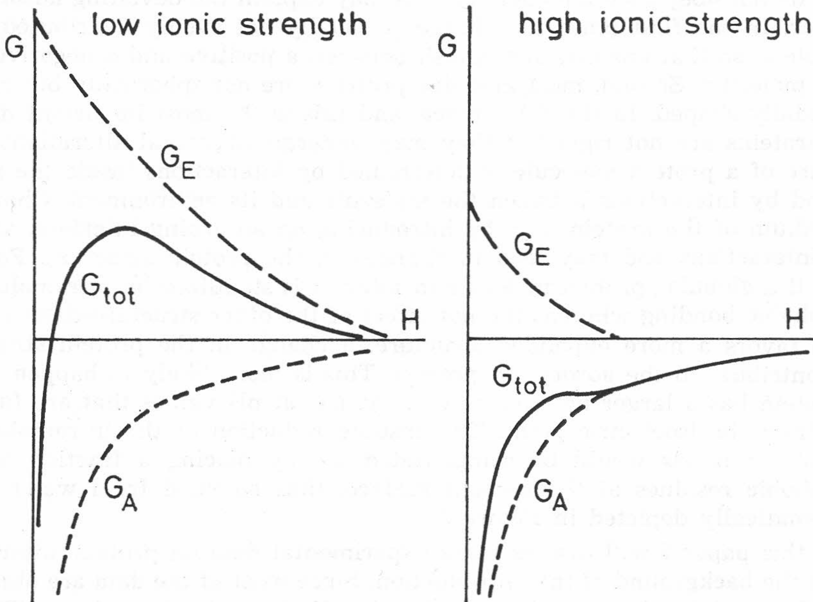

Figure 2. Interaction free energy between two bodies having the same charge sign.

surface. It is obvious that in the case of opposite charges as well as in the case of the same charge sign at high ionic strength G_{tot} is attractive at all separations.

When the protein molecule has approached the sorbent surface at very short distances, say within 0.5 nm, other interactions become effective. They are e. g. ion-ion interactions, hydrogen bonding, dipole-dipole, dipole-induced dipole and hydrophobic interactions. These short-range interactions are especially important in aqueous media. This is related to the uniqueness of the water structure being the result of hydrogen bonding and dipole interactions between the water molecules. Introducing a phase boundary in the system induces local order in the structure of the vicinal water, which at a hydrophilic surface has a lower and at a hydrophobic surface a higher free energy than bulk water. Therefore, the removal of water from a hydrophilic surface will be experienced as a repulsive force whereas dehydration of a hydrophobic surface is attractive (hydrophobic bonding). The short-range interactions determine whether the protein molecule *attaches* to the sorbent surface.

When the surface becomes covered with the adsorbate two features have to be considered

(*i*) the electrostatic potential at the adsorbing surface changes as a result of adsorbed protein molecules; in the case of opposite charges between sorbent and protein the potential may even reverse.

(*ii*) the lateral interaction between the adsorbed protein molecules may affect the degree of coverage. The coverage would increase with decreasing charge on the protein and with increasing ionic strength.

Thus far, we considered the protein molecule as a rigid sphere with the charge smeared out over its surface. In several respects globular protein molecules do not obey such a model and this may explain the deviating adsorption behavior. In the first place the charge is usually not evenly distributed over the protein, so that one can distinguish between a positive and a negative side on the molecule. Second, most globular proteins are not spherically but rather ellipsoidally shaped. In the third place, and this is the most important difference, proteins are not rigid but they may undergo structural alterations. The structure of a protein molecule is determined by interactions inside the molecule and by interactions between the molecule and its environment. Changing the medium of the protein, e. g. by introducing an adsorbing interface, affects those interactions and may lead to changes in the protein structure. For instance, if a globular protein molecule in solution is stabilized by intramolecular hydrophobic bonding whereas the net effect of the other structure-determining factors favors a more expanded structure, a change in the protein structure may contribute to the adsorption process. This is more likely to happen when the protein has a larger net change density, i. e. at pH values that are further away from the isoelectric point. The ensuing reduction of the intramolecular hydrophobic bonds would be compensated for by placing a fraction of the hydrophobic residues at the sorbent surface, thus shielded from water. This is schematically depicted in Figure 3.

In this paper I will discuss some experimental data on protein adsorption against the background of this introduction. Since most of the data are obtained using the proteins human plasma albumin (HPA) and ribonuclease (RNase)

from bovine pancreas, some characteristics of these proteins are listed in Table I and in Figure 4.

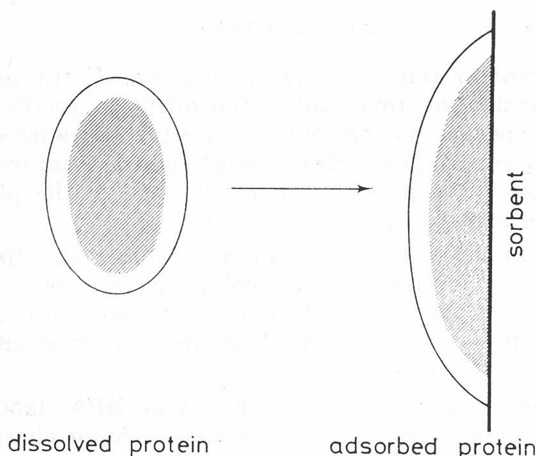

Figure 3. Possible rearrangement in the structure of an adsorbing protein molecule. Shaded areas indicate hydrophobic regions.

TABLE I

Some Properties of HPA and RNase

	HPA	RNase
molecular weight (g mole^{-1})	69,000	13,680
dimensions (nm^3)	11.6 × 2.7 × 2.7	3.8 × 2.8 × 2.2
isoelectric point (pH units)	4.2—5.0	ca. 9.2
point of zero charge (pH units)	5.5	9.4
hydrophobicity (kJ mole amino acid^{-1})[5]	4.68	3.57
diffusion coefficient (cm^2 s^{-1})[6,7]	0.70 × 10^{-6}	1.07 × 10^{-6}
structure stability towards charge density in the protein molecule	low	high

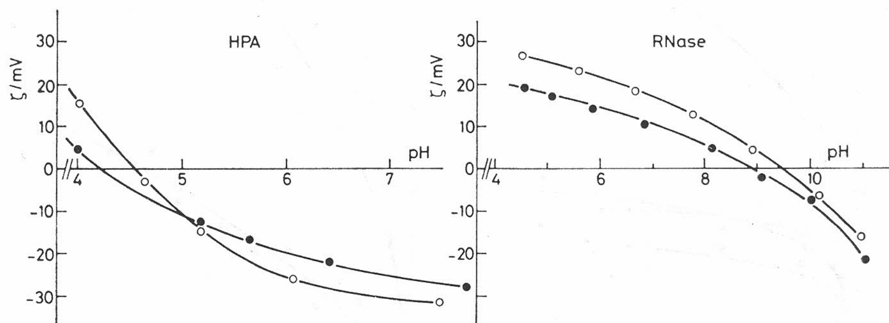

Figure 4. Electrokinetic potential. ζ, of dissolved HPA and RNase. Ionic Strengths 0.01 M (○) and 0.05 M (●) T = 25 °C.

RATE OF ADSORPTION

The sorbent surface, when first exposed to the protein solution, is void of adsorbed protein molecules. If there is no barrier, whatsoever, for the protein

molecule to be deposited at the sorbent surface, the rate of adsorption is controlled by diffusion. Initially ($t \rightarrow 0$),

$$d\,\Gamma = 2\,c\,(D/\pi)^{1/2}\,dt^{1/2} \tag{5}$$

where c is the protein concentration in solution, Γ the amount of protein adsorbed, t the incubation time and D the diffusion coefficient. Although in most adsorption experiments the solutions are (gently) stirred, there will be a stagnant layer adjacent to the sorbent surface where mass transfer occurs only by diffusion. Thus, for a given c the initial slope of the plot of Γ vs $t^{1/2}$ is determined by the diffusion constant D.

At other conditions, e. g. when both the protein and the sorbent have a net charge of the same sign, the adsorbing protein molecules may have to cross an energy barrier (see Figure 2). The adsorption rate will then be reduced by a factor of $\exp[-A/kT]$, where A is the activation energy required to overcome the energy barrier.

We have studied the adsorption kinetics of HPA, labeled with ^{125}I, on polystyrene (PS) surfaces under different conditions of charge on the protein and the PS. The protein charge is varied by controlling the pH and the charge on the PS film is due to the presence of either $—N—C(CH_3)_2—C^+(NH_3)_2$ or to $—OSO_3^-$ groups. The PS films were prepared from PS latices of which the surface charge densities are $+\,4\ \mu C\ cm^{-2}$ and $-15\ \mu C\ cm^{-2}$, respectively. During the preparation of the films reorientation of charged groups are likely to occur so that the charge densities at the films are unknown. However, in view of the differences on the latex particles, it is probable that the surface charge

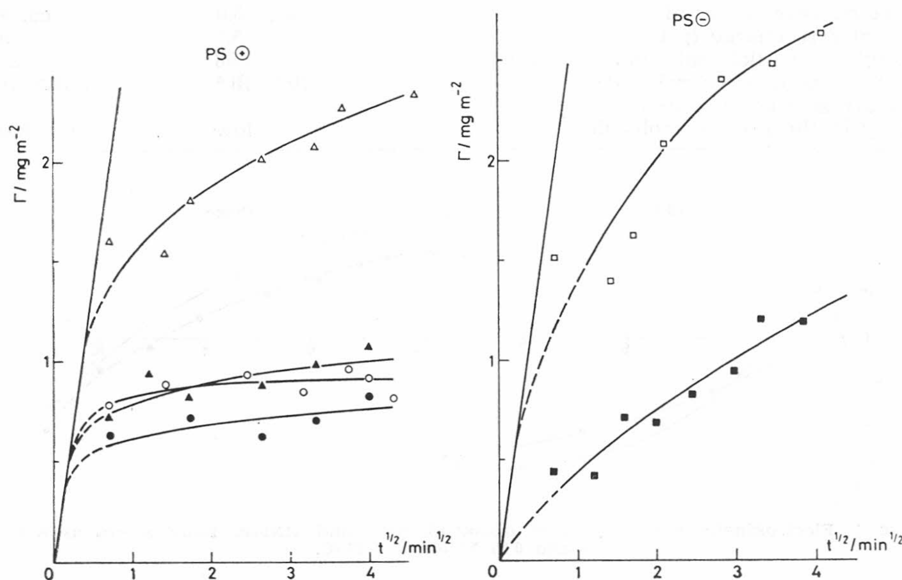

Figure 5. Rates of adsorption of ^{125}I-HPA on PS surfaces that are positively (left) and negatively (right) charged. Concentration of ^{125}I-HPA in solution 38 mg dm^{-3}. $T = 22\ ^{\circ}C$. pH of adsorption 4.0 (open symbols) and 7.4 (filled symbols). Ionic strength 0.001 M (circles); 0.01 M (squares); 0.1 M (triangles). The straight line represents the initial slope calculated for a diffusion coefficient of HPA of 0.70×10^{-6} cm^2 s^{-1}.

on the negative film is more dense than on the positive film. For further experimental details is referred to.[8]

In Figure 5, Γ is plotted vs $t^{1/2}$. In each experiment the first sample was taken after 30 s. Consequently, the initial part of the curves have to be established by interpolation between 0 and 5.5 $s^{1/2}$. The values for D derived from these slopes are therefore rather uncertain. Nevertheless, it can be concluded that for opposite charges on the protein and the sorbent the initial adsorption rates are in agreement with a diffusion-controlled rate based on the diffusion constant quoted in the literature (Table I). Adsorption of negatively charged HPA (pH 7.4) on negatively charged PS is significantly slower. The value derived for the activation energy is 1.6 RT per mole. It is noted that the adsorption of positively charged HPA (pH 4) at positive PS is not retarded. At pH 4 the electrokinetic potential of the HPA molecule is relatively low and, as mentioned above, also the potential at the positive PS surface is expected to be small. Hence, the electrostatic repulsion may be too weak to (over)compensate the dispersion attraction.

Using the equations (1), (2), (3) and (4) and adopting appropriate values for the various model parameters ($\varepsilon = 78.5$, $R = 3.82$ nm, $A_1 = 6.3 \times 10^{-20}$ J, $A_2 = 11.0 \times 10^{-20}$ J and $A_3 = 4.4 \times 10^{-20}$ J) the ζ-potential at the polystyrene film, ψ_{13}, can be evaluated. Thus, for the adsorption on negative PS at pH 7.4 and 0.01 M ionic strength ($\psi_{23} = -30$ mV and $\varkappa = 3.16 \times 10^{6}$ cm^{-1}) an activation energy of 1.6 RT corresponds to $\psi_{13} = -18$ mV. Assuming zero activation energy for the adsorption of HPA at pH 4 and ionic strength 0.001 M on positive PS yields for ψ_{13} a value of less than 10 mV. In view of the electrophoresis of the corresponding latices[9,10] the calculated values for ψ_{13} are rather low. They seem to underscore the real values for ψ_{13}. In other words, the influence of the charge on the adsorption rate can qualitatively be explained by the DLVO theory, but this theory overestimates the energy barrier that has to be passed by the protein molecule. Regarding the limited validity of the model assumptions involved, this lack of quantitative agreement between theory and experiment is not surprising.

AMOUNT OF PROTEIN ADSORBED AT DIFFERENT SURFACES

Many proteins adsorb from aqueous solution at almost any interface, even if the protein and the sorbent have the same charge sign. In that case the repulsion between equally charged groups is overcompensated by other attractive forces, such as dehydration of hydrophobic areas on the sorbent and/or the protein surface and changes in the protein structure on transferring the molecule from the dissolved to the adsorbed state.

Figure 6 shows some typical adsorption isotherms of HPA at various substrates. These are (*a*) polyoxy methylene (POM), (*b*) silveriodide (AgI), (*c*) negatively charged polystyrene (PS⊖), (*d*) positively charged polystyrene (PS⊕), (*e*) hematite (α-Fe_2O_3) and (*f*) calcium hydroxy-apatite (CaHA).[11] All these materials are supplied as colloidal suspensions. Information on the particle surface charge is given in Figure 7, where the electrophoretic mobility (u/E) is plotted vs the pH. The mobilities of the PS and the AgI particles are independent of the pH (over the range studied), whereas those of α-Fe_2O_3 and of

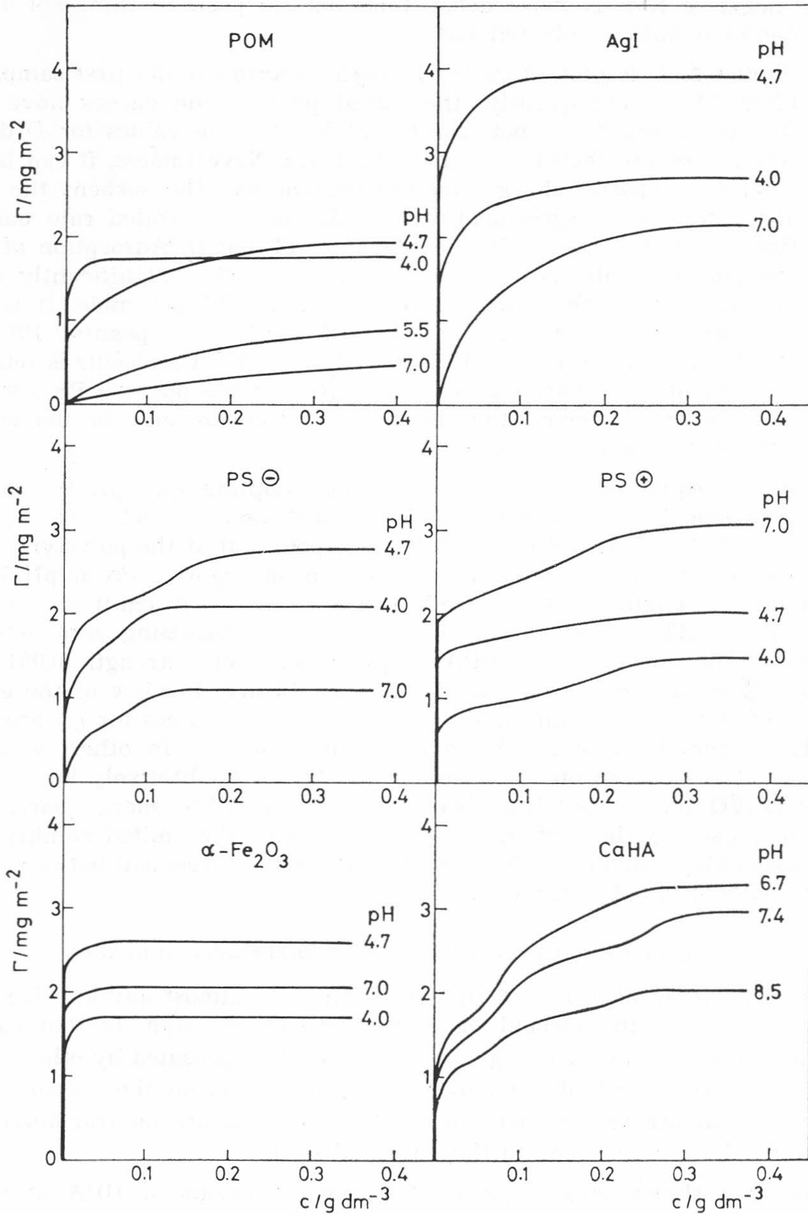

Figure 6. Adsorption isotherms of HPA at various substrates. The pH of adsorption is indicated. Ionic strength is 0.01 M, except for adsorption at POM where it is 0.05 M. $T = 22\,^{\circ}C$.

CaHA vary with pH, showing isoelectric points at pH 6.2 and 7.0, respectively. The corresponding points of zero charge are at pH 9.5 for α-Fe_2O_3 and at pH 8.5 for CaHA. Preliminary streaming potential measurements in plugs of POM crystals have shown that this material is essentially uncharged.

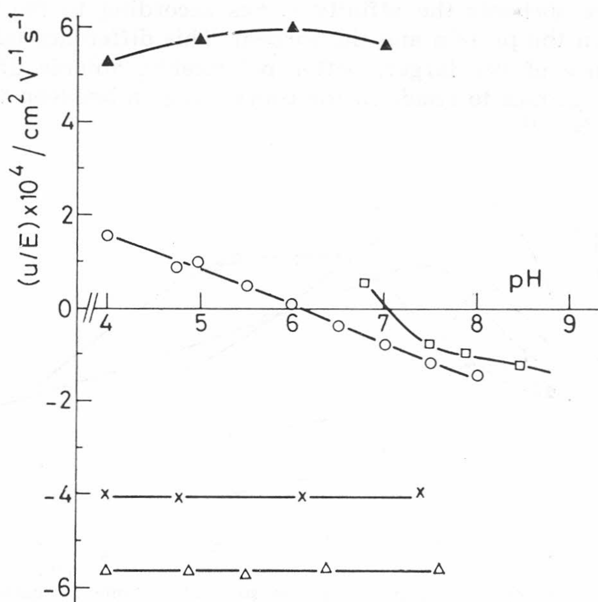

Figure 7. Electrophoretic mobilities of sorbent particles. Ionic strength 0.01 M. T = 25 °C. x AgI, △ PS⊖, ▲ PS⊕, ○ α-Fe_2O_3, □ CaHA

The adsorption on the POM surface is probably the most simple one. This substrate is chemically inert and the surface is homogeneous without ionizable groups. The surface is rather polar, i. e. it is readily wetted by water. Since the substrate is uncharged, any influence of charge on the adsorption behaviour must be attributed to the HPA molecules. Figure 6 shows a prominent effect of pH, i. e. of protein charge, on the adsorption isotherms at a POM surface. The initial slopes evidently indicate that the affinity between the HPA molecule and the POM surface decreases with increasing (negative) charge on the protein molecule. In Figure 4 it can be seen that the electrokinetic potential at pH 4.0 and 4.7 does not exceed $\pm$ 15 mV. At pH 5.5 and 7.0 this potential amounts to — 18 and — 25 mV. It can, therefore, not be concluded whether the lower affinity is a general charge effect or that it is due to an increased (decreased) number of anionic (cationic) charged groups. It could as well be that the affinity reflects the influence of the pH on the solvent quality. At all other surfaces, whether they are positively or negatively charged, the initial slopes for pH > i. e. p. of HPA are much steeper than at the POM surface. Since this is the case both for hydrophobic (AgI and PS) and hydrophilic (α-Fe_2O_3 and CaHA) surfaces, the higher affinity must be due to favorable ionic interactions. With positive surfaces this would be expected, but the relative high affinity for negative surfaces suggests strong interaction between the negative sorbent charge and positively charged groups of the protein, although the net charge of the protein is negative. Indeed, with PS⊖ the formation of ions pairs between cationic groups of the protein and —OSO_3^- groups at the PS surface have been demonstrated.[12]

Another interesting feature is that for the positively charged surfaces all isotherms, at each pH value studied, show a high affinity character, whereas

for the negative sorbents the affinity varies according to the overall charge contrast between the protein and the sorbent. This difference might be related to the preference of the larger, better polarizable, anionic groups over the smaller cationic groups to reside in the contact region between the protein and the sorbent surface.[12]

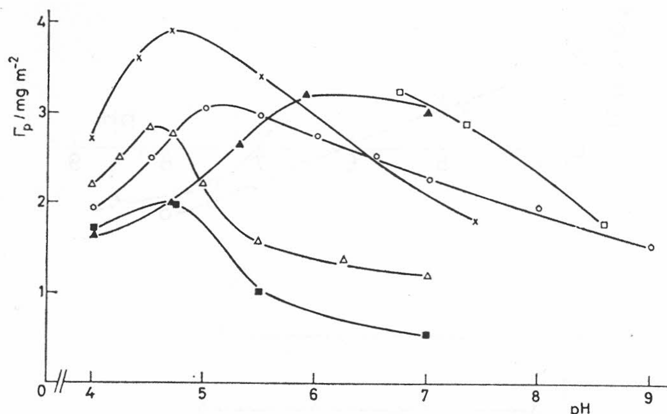

Figure 8. Plateau adsorption of HPA at various substrates. Ionic strengths as in Figure 6. T = 22 °C. ■ POM, x AgI, △ PS⊖, ▲ PS⊕, ○ α-Fe_2O_3, □ CaHA

Figure 8 gives the plateau-values Γ_p of the adsorption isotherms as a function of pH. For all substrates, except PS⊕, Γ_p is at a maximum in the isoelectric region of the protein and in most cases the maximum ranges between 2 and 3 mg m^{-2}. This corresponds to a close-packed monolayer of side-on adsorbed HPA molecules in their native shape (see the dimensions given in Table 1), allowing for a hydration layer of ca. 0.5 nm around the protein molecules. Higher surface concentrations could be obtained by tilting the adsorbed molecules, reaching ca. 8.3 mg m^{-2} in the case of end-on positions. The reduction of the amount adsorbed with increasing protein charge may be the result of two different mechanisms: (*a*) increased electrostatic repulsion between the adsorbed molecules and (*b*) progressive structural rearrangement of the adsorbing molecule. At ionic strengths 0.05 M and 0.01 M the Debeye lengths are 1.4 nm and 3.2 nm, respectively. Hence, lateral repulsion could lower the plateau adsorption by a factor of about 2 at POM and about 3.5 at other substrates. With POM the decrease in Γ_p clearly exceeds a factor of 2, which suggests that repulsion between the adsorbed molecules is not the only factor responsible for the variation of Γ_p with pH. Since the other surfaces show a higher affinity towards the HPA molecule, it is expected that Γ_p is less sensitive to lateral interaction. In the case of HPA adsorption on PS⊖ we have collected ample experimental evidence to conclude that the reduced adsorption away from the isoelectric point is caused by structural rearrangements rather than by lateral repulsion.[12,13] The similar trends in Γ_p (pH) observed with the other surfaces may as well be due to changes in the protein structure. The high values for Γ_p on AgI surfaces suggest a tilted position of the HPA molecules at this material. The curve for PS⊕ is quite different. Here, it seems that the contrast between the sorbent charge and the overall--charge of the protein plays a dominant role. At high pH Γ_p raises beyond

side-on monolayer coverage. Why does this not happen at the other positively charged surfaces? At pH 7 the positive charge on the α-Fe_2O_3 and the CaHA surfaces have almost diminished. Still, it is seen that also at both these surfaces the adsorption is relatively large. Furthermore, in the case of α-Fe_2O_3 the adsorption at pH 7 is larger than at pH 4, whereas the reverse is true for the adsorption at the negatively charged surfaces.

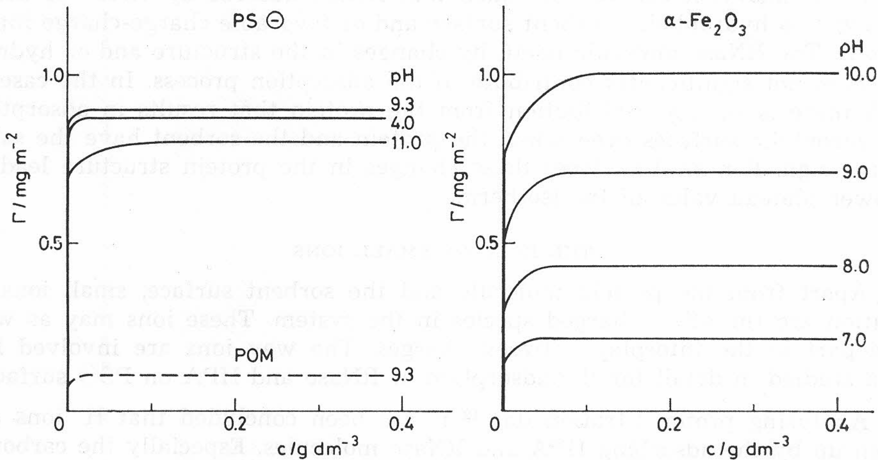

Figure 9. Adsorption isotherms of RNase on different sorbents. The pH of adsorption is indicated. Ionic strength 0.01 M; with POM 0.05 M. $T = 22$ °C.

In Figure 9 isotherms for RNase adsorption are presented. Although they have been obtained for only three sorbents, POM, PS⊖ and α-Fe_2O_3, the data are very informative, especially by comparing them with the HPA adsorption data. At POM surfaces RNase adsorbs in a very slight amount from its isoelectric solution (pH 9.3). At other pH values adsorption is undetectable. The fact that HPA does and RNase does not adsorb implies that the contribution from the protein molecule, including its hydration water, to the Gibbs free energy of adsorption is for RNase apparently smaller than for HPA. Like HPA, RNase readily adsorbs at the hydrophobic PS⊖ surface. The surface concentration is almost independent of the pH, reaching ca. 1.0 mg m^{-2}. This value corresponds well with a close-packed monolayer of hydrated RNase molecules oriented with its longest axes parallel to the surface. It suggests that RNase does not change its structure significantly upon adsorption, even not at pH values where the protein molecule has reached a high charge density. Furthermore, the constant value of Γ_p demonstrates that lateral repulsion does not lead to lower adsorption. Since the net charge/mass ratio for RNase at pH 4 is 6.2×10^{-4} eq. g^{-1}, whereas that of HPA at pH 7 is 3.2×10^{-4} eq. g^{-1}, the same would hold for HPA adsorption. This is another indication that the lower Γ_p values for HPA at pH $\neq$ i. e. p. is caused by flattening of the adsorbing protein molecule. Such structural rearrangements would support the adsorption process. It could very well be responsible for obtaining a negative value for $\Delta_{ads} G$ at POM surfaces, whereas $\Delta_{ads} G$ of RNase at this surface is under most conditions positive.

Finally, the adsorption of RNase at the hydrophilic, charged α-Fe_2O_3 surface occurs only at pH > 6.5, the isoelectric point of the sorbent. Thus, RNase does not adsorb when both the protein and the sorbent have a net positive charge. This, again, is in contrast with the adsorption behavior of HPA. Beyond pH 6.5 the RNase adsorption increases even up to pH 10 where the overall charge in the RNase molecule is slightly negative.

In summary, it can be concluded that RNase adsorbs by virtue of dehydration of a hydrophobic sorbent surface and/or favorable charge-charge interactions. The RNase molecule itself, by changes in the structure and/or hydration, does not significantly contribute to the adsorption process. In the case of HPA there is such a contribution from the protein that results in adsorption at hydrophilic surfaces even when the protein and the sorbent have the same charge sign. For most surfaces these changes in the protein structure lead to a lower plateau value of the isotherm.

THE ROLE OF SMALL IONS

Apart from the protein molecule and the sorbent surface, small ions in solution are the other charged species in the system. These ions may as well take part in the interplay between charges. The way ions are involved has been studied in detail for the adsorption of RNase and HPA on PS⊖ surfaces.

Analyzing proton titration data[12] it has been concluded that H^+ ions are taken up by the adsorbing HPA and RNase molecules. Especially the carboxyl groups undergo a considerable shift to higher pK values, from which it was inferred that a relatively large fraction of the carboxyl groups resides close to the negatively charged PS⊖ surface. Electrophoresis experiments[9] show that ions, other than H^+, are also transferred between the solution and the adsorbed layer. Based on a model for the adsorbed protein layer[14] we published a few years ago, the amount of cations incorporated was estimated. According to the model the co-adsorption of ions is of electrostatic nature, i. e. it occurs to prevent charge accumulation in the contact region between the protein and the sorbent. It, therefore, predicts an uptake of charge that is idependent of the type of cation. However, the transfer of ions from an aqueous into a non-aqueous proteinaceous medium also involves a chemical, non-electrostatic contribution. This contribution generally differs between different ions. In fact, the ion uptake is determined by the molar free energy of transfer, i. e. by the difference between the partial molar free energy g_i of the ion i in the protein layer p and in the bulk solution s, respectively

$$g_i^p - g_i^s = (\mu_i^p - \mu_i^s) + z_i F (\psi^p - \psi^s) \qquad (6)$$

where μ_i is the chemical potential of ion i and ψ the electrostatic potential in the indicated phase. F is the Faraday constant and z_i the valency of the ion, sign included. Obviously the second term in the r. h. s. of equation (6) represents the electrostatic contribution and the first term the chemical contribution to the free energy of ion transfer.

The hypothesis of ion incorporation was tested experimentally using the radioactive isotopes $^{22}Na^+$ and $^{133}Ba^{2+}$ and the paramagnetic Mn^{2+} ion.[15] The radionuclides were determined by γ-spectrometry and Mn^{2+} by ESR spectroscopy. In Figure 10 the predicted ion uptake and the experimental data are

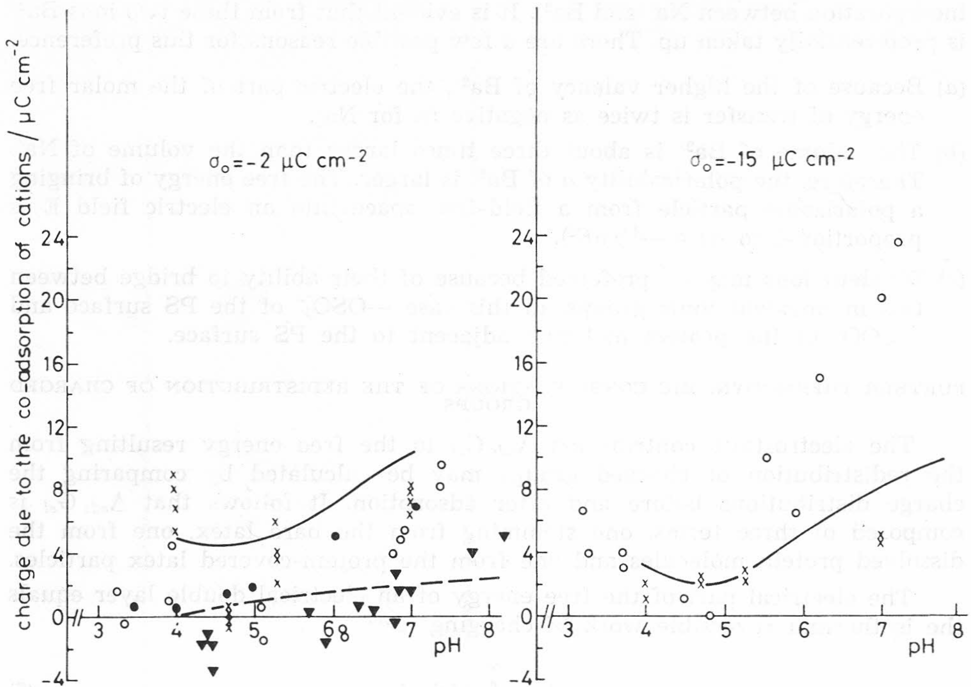

Figure 10. Incorporation of cations in adsorbed layers of HPA (x Na^+; ○ Ba^{2+}; ● Mn^{2+}) and RNase (▼ Na^+) on polystyrene latices having different surface charge densities (σ_0). The curves show the ion uptake predicted from the model: solid curves for HPA and dashed curve for RNase.

compared. The measured values do not coincide with the predicted ones, but they are at least comparable and the trends with pH are in agreement with the prediction. The observation that with the various cations the amounts of charge taken up are rather similar underlines that ion co-adsorption is dominated by electrostatic forces.

This conclusion does not imply that the uptake of one kind of ion is not preferred over the other. Table II summarizes results for the competitive

TABLE II

Competition Between Na^+ and Ba^{+2} for Incorporation in Layers of HPA Adsorbed at Polystyrene Latices Having Different Surface Charge Densities. The Incorporated Amounts are Expressed in μC per cm^2 of Polystyrene Surface. pH 7, $T = 22\,^0C$

electrolyte	$\sigma_o = -2\ \mu C\ cm^{-2}$		$\sigma_o = -15\ \mu C\ cm^{-2}$	
	Ba^{2+}	Na^+	Ba^{2+}	Na^+
0.0050 M $BaCl_2$ +				
0.0050 M *NaCl		0.4		0.4
0.0150 M *NaCl		5.0		7.2
0.0050 M *$BaCl_2$ +				
0.0050 M NaCl	4.4		10.8	
0.0075 M *$BaCl_2$	5.9		9.5	

incorporation between Na^+ and Ba^{2+}. It is evident that from these two ions Ba^{2+} is preferentially taken up. There are a few possible reasons for this preference.

(*a*) Because of the higher valency of Ba^{2+}, the electric part of the molar free energy of transfer is twice as negative as for Na^+.

(*b*) The volume of Ba^{2+} is about three times larger than the volume of Na^+. Therefore, the polarizability α of Ba^{2+} is larger. The free energy of bringing a polarizable particle from a field-free space into an electric field E is proportional to α ($= -{}^1/_2\, \alpha E^2$).

(c) Divalent ions may be preferred because of their ability to bridge between two monovalent ionic groups, in this case $-OSO_3^-$ of the PS surface and $-COO^-$ of the protein molecule adjacent to the PS surface.

FURTHER THERMODYNAMIC CONSIDERATIONS OF THE REDISTRIBUTION OF CHARGED GROUPS

The electrostatic contribution $\Delta_{ads} G_{el}$ to the free energy resulting from the redistribution of charged groups may be calculated by comparing the charge distributions before and after adsorption. It follows that $\Delta_{ads} G_{el}$ is composed of three terms, one stemming from the bare latex, one from the dissolved protein molecules and one from the protein-covered latex particles.

The electrical part of the free energy of an electrical double layer equals the isothermal reversible work of charging it[2,15]

$$G_{el} = \int_0^{\infty} \psi_o' \, d\, \sigma_o' \tag{7}$$

where ψ_o' and σ_o' are the variable surface potential and surface charge density during the charging process.

The model assumptions for the distribution of charge in layers of HPA and RNase on PS$\ominus$ surfaces,[14] make it possible do derive ψ_o' as a function of σ_o'. It is obtained from the integration of the Poisson-equation, $d\,\psi(x) = (\sigma(x)/\varepsilon\varepsilon_o)dx$, taking into account the proper boundary conditions. Similarly, ψ_o' (σ_o') can be obtained for the bare sorbent surface and the dissolved protein molecules. Analyzing microcalorimetric data[17] we concluded that both the enthalpy effect, $\Delta_{ads} H_{el}$, and $\Delta_{ads} G_{el}$ are only little dependent on the pH of adsorption, i. e. little dependent on the charge contrast between the HPA or the RNase molecule on the one hand and the PS$\ominus$ surface on the other. The reason for this insensitivity is the co-adsorption of cations, which do adsorb in a larger number the smaller the charge contrast between the protein and the sorbent is (see Figure 10). Figure 11 shows $\Delta_{ads} H_{el}$ (pH) and $\Delta_{ads} G_{el}$ (pH) as obtained from the thermodynamic analysis referred to above.

It is noticed that in each case $\Delta_{ads} G_{el} < 0$ and that $\Delta_{ads} H_{el} > \Delta_{ads} G_{el}$, which implies that the entropy change $\Delta_{ads} S_{el}$ must be positive. Apparently, the rearrangement of the charged groups leads to a lower degree of order.

As a result of the medium change of the ionic groups at the sorbent surface and of those at the protein surface that, after adsorption, are oriented towards the sorbent and also because of the medium change of the ions transferred from solution into the protein layer, the chemical contribution, $\Delta_{ads} G_{medium}$, to the free energy of the redistribution of charged groups will in

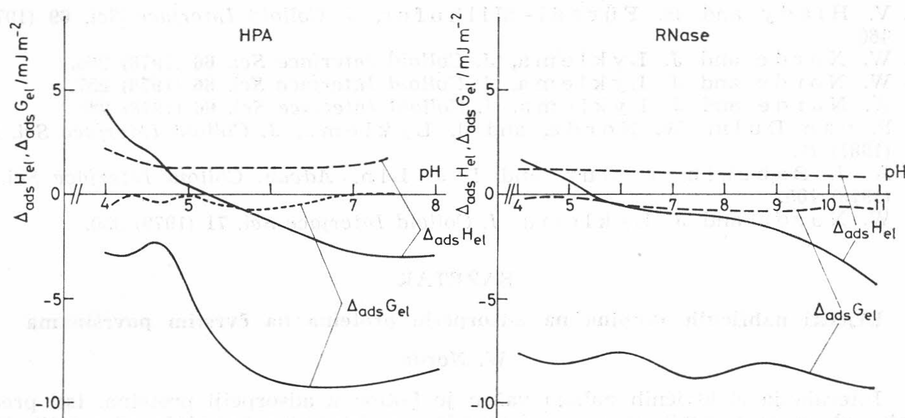

Figure 11. Adsorption of HPA and RNase on polystyrene latices (——— $\sigma_o = -15$ μC cm^{-2}; — — — $\sigma_o = -2$μC cm^{-2}). Electrostatic contributions to the enthalpy and the free energy of the adsorption process. Ionic strength 0.01 M. $T = 25$ °C.

general be positive. Thus, the medium change of the transferred ions opposes the overall adsorption process. If no ions were incorporated, however, either

(*i*) the electric potential inside the protein layer, having a low dielectric permittivity, would reach high values, which gives rise to high positive values for $\Delta_{ads}\, G_{el}$

or

(*ii*) the protein molecule would unfold into a loose structure that is freely penetrable by the solvent and the electrolyte. The general experience that globular proteins seldom form such loose layers indicates that exposure of hydrophobic amino acid residues to the aqueous medium is more unfavorable than the medium change of transferred ions.

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SAŽETAK

Utjecaj nabijenih skupina na adsorpciju proteina na čvrstim površinama

W. Norde

Interakcija električnih naboja važan je faktor u adsorpciji proteina. Isti predznak naboja proteinskih molekula i površine sorbenta ne pogoduje adsorpciji. No, hoće li adsorpcija biti spriječena ovisi još o nizu drugih faktora, kao što su dehidratacija proteina i sorbenta te strukturne promjene proteinskih molekula. Ribonukleaza koja adsorpcijom bitno ne mijenja svoju strukturu, adsorbirat će na hidrofilnim površinama samo u slučaju kad je predznak naboja proteina suprotan predznaku naboja supstrata. Albumin iz krvne plazme, pak, adsorbira na bilo kojoj površini, čak i kad je sorbent hidrofilan i ima isti predznak naboja kao i protein. U tom slučaju pogonska snaga adsorpcije potječe od dehidratacije i/ili konformacijskih promjena u proteinskoj molekuli. Iako isti predznak naboja neće spriječiti adsorpciju, smanjit će, ipak, brzinu procesa. To je pokazano za adsorpciju albumina na negativnim površinama polistirenskih lateksa. Gibbsova slobodna energija elektrostatskih interakcija razmjerno je neosjetljiva na naboj proteina i sorbenta. Taj se efekt pripisuje ulozi malih iona u sistemu: da bi se spriječilo nastajanje visokih elektrostatskih potencijala u graničnom sloju između proteina i sorbenta, mali ioni iz vodene otopine postupno ulaze u taj sloj. Kemijski je efekt toga prijelaza nepovoljan, a kako je proporcionalan broju transferiranih iona, on raste kako pada razlika u naboju između proteina i površine sorbenta.