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Can a Protein Adsorb on its Own? The Thermodynamics of Ion Participation*

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We present a general thermodynamic method to obtain information about co-adsorption and co-partitioning of low molecular weight ions upon adsorption or partitioning of proteins between two phases. Esin-Markov coefficients are central quantities. By this procedure useful additional information is obtained that, in turn, can be used in mechanistic interpretations. Applications are presented for (1) ion binding to free BSA, (2) charge adjustments in the protein and on the surface upon adsorption of BSA on silver iodide and (3) ion co-participation upon solubilization of cytochrome-C into micro-emulsion droplets.

Two answers can be given to the title question. The first is obvious and it is »no«, the reason being that for any adsorption from solution solvent has to be desorbed, hence it co-participates.

The second answer is less trivial and refers to co-sorption of low molecular weight electrolytes, including acids and bases. In the present paper this issue will be addressed.

Studying ion co-adsorption is relevant for a better understanding of the mechanism(s) of protein adsorption. In particular, its occurrence can point to the adjustment of the electrostatic contribution to the interaction when non-electrostatic forces dominate. For instance, a negatively charged protein may well adsorb spontaneously onto a negative adsorbent by, say, entropic forces provided co-adsorption of cations takes place between protein and surface, to screen the opposing electrostatic repulsion.

In literature, the occurrence of ion co-adsorption is usually disregarded but in those cases where it was specifically studied more often than not ion uptake or expulsion was found. One approach to measure it is to take the algebraic difference between the electrokinetic charge(s) before and after adsorption. For the adsorption of human plasma albumin (HPA) on negative latices

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this was reported by Norde and Lyklema¹ and for immunoglobulins by Elgersma et al.² Van Dulm et al.³ measured the co-uptake of Na⁺ and Ba⁺² ions radio-metrically upon the same adsorption. As expected, substantial uptake took place for pH > i.e.p., but it was non-zero at the i.e.p. and tended to increase again with decreasing pH below the i.e.p. There is no purely electrostatical reason for this observation. One of the interpretations is that in this system HPA tends to enrich the carboxyl group concentration in the interfacial region⁴, a process that takes place irrespective of the charge on the protein, so that it must have a chemical origin. Unexpected ion incorporations are, so to say, detectors for such electrostatic phenomena.

In the present contribution the problem will be approached thermodynamically. We shall show that the sought uptake or expulsion can be obtained in a general way from acid-base titration data at various electrolyte concentrations provided these data are accurate. Such an analysis is most conveniently carried out in terms of so-called *Esin-Markov coefficients*. By our procedure, pertinent additional information on the interaction is obtained without directly measuring the co-partitioning of ions.

The Thermodynamic Approach. General Considerations

When oxides or proteins are potentiometrically titrated with acid and base it is observed that surface charge density σ° -pH curves depend on c_{salt} . This is due to screening. The more counterions accumulate near a charged surface, the higher the absolute value of σ° becomes at given pH. Distances between different curves are therefore measures of the extent of counterion (and, hence co-ion) adsorption.

Quantitatively this is conveniently expressed in terms of Esin-Markov coefficients. The name dates back to an old paper on the shift of the electrocapillary maximum of mercury due to surfactant adsorption.⁵ For oxides we redefined this coefficient as.⁶

$$\beta \equiv \left(\frac{\partial \text{pH}}{\partial \ln a_{\pm}} \right)_{\sigma} \quad (1)$$

In words, β measures the change in pH required to keep σ° constant if the mean salt activity is changed by $d \ln a_{\pm}$. Temperature and pressure are taken constant. For one (z_+ , z_-) electrolyte we derived

$$\beta = - \frac{1}{z^+} - \frac{z_+ + z_-}{z_+ z_-} \left(\frac{\partial \sigma_-}{\partial \sigma^{\circ}} \right)_{a_{\pm}} = \frac{1}{z_-} + \frac{z_+ + z_-}{z_+ z_-} \left(\frac{\partial \sigma_+}{\partial \sigma^{\circ}} \right)_{a_{\pm}} \quad (2)$$

Here, σ_- and σ_+ are the ionic components of charge, i.e. the contribution of the anion and cation of the electrolyte respectively to the compensation of σ° . It is seen that σ_- and σ_+ are obtainable from experiment, except for a constant.

For a protein in solution we redefine β slightly differently

$$\beta \equiv \left(\frac{\partial \text{pH}}{\partial \ln a_{\pm}} \right)_{r^{\sigma}, c_p} \quad (3)$$

Here, c_p is the protein concentration and r^σ the number of H^+ ions minus the number of OH^- ions bound on one protein molecule. In the next section we shall define r^σ thermodynamically; for the moment it suffices to note that r^σ is a measure of the surface charge on the protein. For a protein in a KCl solution, titrated by KOH and HCl, r^σ is *operationally* defined as

$$r^\sigma \equiv r_{HCl} - r_{KOH} \quad (4)$$

It is stressed that a protein, together with its surrounding double layer as a whole is electroneutral. Therefore, only electroneutral combinations of electrolytes occur in the equation. Identification of r^σ as the surface charge is an (appropriate) model interpretation.

By the same token, binding coefficients can be introduced for the other ions in the system. First it is recalled that the countercharge consists of two parts: an excess of counterions and a deficit of co-ions, the latter also known as *negative adsorption*. The expulsion of co-ions leads to an increase of the concentration of these ions in the equilibrium solution, as compared with the situation in which the colloid was uncharged. Again because of electroneutrality, this increase is experimentally observed as an increase in the concentration of *electroneutral* electrolyte. This phenomenon is nothing else than the *Donnan effect*.

More specifically, in the above system expulsion of KCl takes place. The coefficient r_{KCl} is the number of KCl molecules, bound per protein molecule; it is negative.

With the above in mind, *ionic binding* can be introduced as follows

$$r_{K^+} = r_{KOH} + r_{KCl} \quad (5a)$$

$$r_{Cl^-} = r_{HCl} + r_{KCl} \quad (5b)$$

Equation [5a] tells us that the amount of K^+ in a double layer is determined by the amount of K^+ co-adsorbing with OH^- minus the amount of K^+ lost by the Donnan effect.

It follows from [5a,b] that individual co-adsorption coefficients are measurable (except for a constant, see below). In fact, this is the reason that in [2] individual ionic components of charge (σ_- and σ_+) occur.

The Relation Between Ionic Binding Ratios and the Esin-Markov Coefficient for an Isolated Protein

Here we give the essential steps in the derivation, accepting some simplifying assumptions. A more rigorous derivation will be given elsewhere⁷.

In the Donnan membrane equilibrium of Fig. 1 the binding ratio of each component i is given as

$$r_i \equiv \frac{c_i^L - c_i^R}{c_p} \quad (6)$$

where c_i is the molar concentration of i .

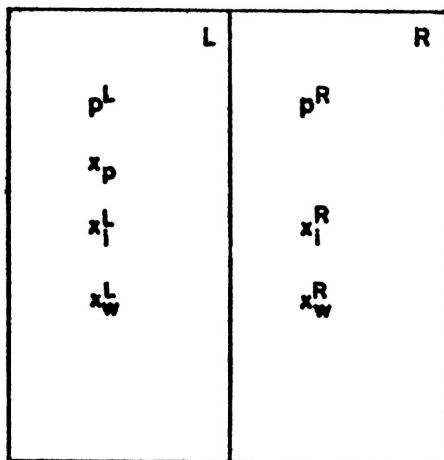


Figure 1. Membrane equilibrium between a protein-containing solution L and its dialysate R. The outer pressure and temperature are taken constant; *i* refers to electroneutral components and the osmotic pressure *p* is equal to $p^L - p^R$. The solutions are assumed to be dilute ($x_w^L \sim x_w^R \sim 1$).

Consider one protein (*p*), titrated with HCl or/and KOH in a KCl solution, with $c_{KCl} \gg c_{HCl}, c_{KOH}$. The solutions are so dilute that $d\mu_w \sim 0$ (except for the osmotic term). Writing the Gibbs-Duhem equation in the sol (L) and dialysate (R), then subtraction gives

$$d\mu_p = -\Gamma_{HCl} d\mu_{HCl} - \Gamma_{KOH} d\mu_{KOH} - r_{KCl} d\mu_{KCl} + \frac{d\pi}{c_p} \quad (7)$$

If $\Lambda = \mu_p - \pi/c_p$, then

$$d\Lambda = -\Gamma_{HCl} d\mu_{HCl} - r_{KOH} d\mu_{KOH} - r_{KCl} d\mu_{KCl} + \pi d \ln c_p / c_p \quad (8)$$

Because of the chemical equilibrium condition $d\mu_{HCl} + d\mu_{KOH} = d\mu_{KCl}$ ($+ d\mu_w \sim 0$), either μ_{KOH} or μ_{HCl} can be eliminated, leading to this pair of equivalent expressions

$$d\Lambda = -(r_{HCl} - r_{KOH}) d\mu_{HCl} - (r_{KOH} + r_{KCl}) d\mu_{KCl} \quad (9a)$$

$$d\Lambda = (r_{HCl} - r_{KOH}) d\mu_{KOH} - (r_{HCl} + r_{KCl}) d\mu_{KCl} \quad (9b)$$

which, using [4,5a and 5b] can be rewritten as

$$d\Lambda = -r^\sigma d\mu_{HCl} - r_{K^+} d\mu_{KCl} \quad (10a)$$

$$d\Lambda = r^\sigma d\mu_{KOH} - r_{Cl^-} d\mu_{KCl} \quad (10b)$$

Starting from [10a] the differential $d(\Lambda + r^\sigma \mu_{HCl}) = \mu_{HCl} dr^\sigma - r_{K^+} d\mu_{KCl}$ is cross-differentiated, leading to

$$\begin{aligned} \frac{\partial r_{K^+}}{\partial r^\sigma} &= -\frac{\partial \mu_{HCl}}{\partial \mu_{KCl}} = -\frac{\partial \mu_{H^+} + \partial \mu_{Cl^-}}{\partial \mu_{K^+} + \partial \mu_{Cl^-}} = -\frac{RT d \ln a_{H^+} + RT d \ln a_{Cl^-}}{RT d \ln a_{K^+} + RT d \ln a_{Cl^-}} = \\ &= -\frac{d \ln a_{H^+}}{2 d \ln a_{\pm}} - \frac{1}{2} = \frac{1}{2} (\beta - 1) \end{aligned} \quad (11a)$$

where we have used [3] to introduce the Esin-Markov coefficient. If we had started from [10b], we would have obtained

$$\frac{\partial r_{Cl^-}}{\partial r^\sigma} = \frac{1}{2} (\beta + 1) \tag{11b}$$

Integration of [11a,b] gives eventually

$$r_{K^+}(r^\sigma) = r_{K^+}(r^{\sigma^0}) + \frac{1}{2} \frac{r^{\sigma^0}}{r^\sigma} \int (j' - 1) dr^\sigma \tag{12a}$$

$$r_{Cl^-}(r^\sigma) = r_{Cl^-}(r^{\sigma^0}) + \frac{1}{2} \int_{r^{\sigma^0}}^{r^\sigma} (\beta + 1) dr^\sigma \tag{12b}$$

Equations [12] show that binding ratios of individual ionic species can be obtained from experiment except for a constant. The constant can only be determined by non-thermodynamic considerations, such as the presumption that certain species do not adsorb specifically or by computing negative adsorption of the co-ion from diffuse double layer theory (because this ion is expelled it does not accumulate close to the surface, so that for this purpose diffuse theory is relatively good).

Application to Tanford's Titration Data

Already many years ago, Tanford et al.⁸ have published titration data as a function of c_{KCl} for bovine serum albumin (BSA). Using the theory developed above, we have used their data to obtain r_{Cl^-} and r_{K^+} as functions of pH. The reference point for the integrations was the assumption that no specific adsorption of K^+ takes place at the i.e.p. Further details have been given by one of us⁹. In Fig. 2 the result is represented.

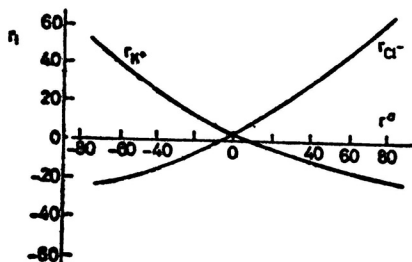


Figure 2. Binding fractions of K^+ and Cl^- on BSA, derived from Tanford et al's data; $c_{KCl} = 0.07$ M.

At any value of r^σ electroneutrality prevails, i.e. $r_{K^+} - r_{Cl^-} = -r^\sigma$.

At negative r^σ , chloride ions are negatively adsorbed, at positive r^σ it is the other way around. Apart from minor quantitative differences, the curves for K^+ and Cl^- are each other's mirror image; the minor deviations from exact mirroring are due to the specific adsorption of chloride ions; at positive r^σ , r_{Cl^-} increases more rapidly than r_{K^+} increases with increasing negative r^σ . At the isoelectric point ($r^\sigma = 0$) there is a slight binding of chloride ions, compensated by an equal amount of potassium in the diffuse part of the double layer.

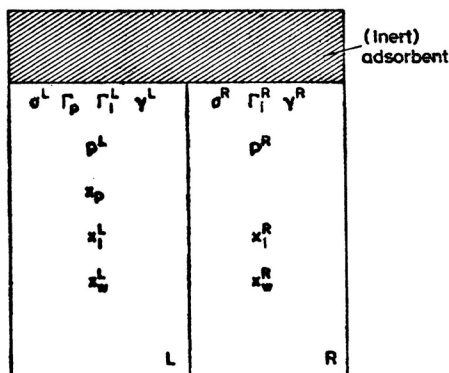


Figure 3. Membrane equilibrium between sol and dialysate, both in equilibrium with the same adsorbent. Symbols and conditions as in Fig. 1; σ is the surface charge, Γ_i the surface concentration of i and γ the interfacial tension ($\gamma^R - \gamma^L$ equals the surface pressure π^σ).

Co-adsorption of Ions

Having the method developed for ion binding to proteins it is only one step to extend it to ion co-adsorption with proteins. To that end we must compare r_i in the adsorbed state with the same for the free, unadsorbed protein. The difference is called the co-adsorption ratio Δr_i . Its operational definition is introduced in Figure 3. In line with the foregoing

$$\Delta r_i \equiv r_i \text{ (ads. state)} - r_i \text{ (free)} \equiv \frac{\Gamma_i^L - \Gamma_i^R}{\Gamma_p} - \frac{c_i^L - c_i^R}{c_p} \quad (13)$$

Similar definitions can be given for the extent of ion co-uptake in other protein partitioning processes, such as the distribution between reverse micelles and protein solutions, of which an illustration will be given below. In those cases it is more appropriate to call Δr_i a *co-partitioning ratio*.

As an example of co-adsorption ratios we refer to Fig. 4, dealing with the adsorption of BSA on AgI. Measurements of the surface charge

$$r^\sigma = r_{\text{AgNO}_3} - r_{\text{Kl}} \quad (14)$$

as a function of pH at various BSA adsorptions⁹ enabled us to find both Δr^σ (the change in r^σ due to the adsorption of BSA) and Δr_{H^+} (the co-adsorption of protons on the protein upon adsorption). Details of the experiment and analysis will be presented elsewhere¹⁰.

The figure illustrates the point made in the introduction: *grosso modo* the curves behave according to expectation on the basis of simple electrostatic assumptions, but there are definite deviations, pointing to idiosyncracies that deserve further study.

In figure (a), at low ψ^0 the trend is as expected: below the i.e.p. (4.7) the protein is positive, when it adsorbs, the surface picks up Kl or expels AgNO_3 ;

above the i.e.p. this is the other way around. However, negative values for Δr^σ at ψ° more negative than -160 mV cannot be explained this way.

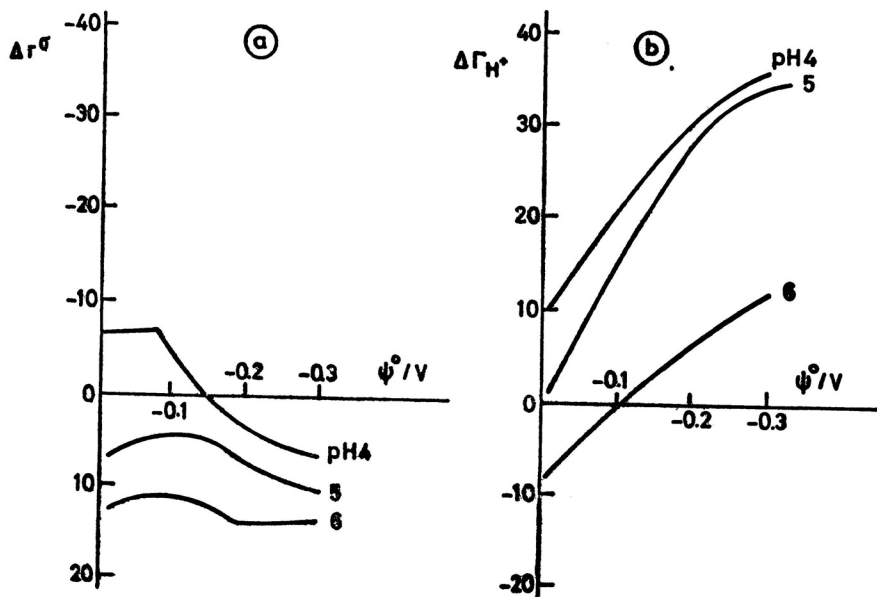


Figure 4. Charge adjustments upon BSA-adsorption on AgI. Fig. (a) represents the adjustment of the surface charge $\Delta r^\sigma = r_{AgNO_3} - r_{KI}$. Fig. (b) the co-uptake of protons. Adsorbed amount Γ_p , 1 mg m^{-2} ; electrolyte 0.1 M KNO_3 . The surface potential ψ° and the pH are indicated.

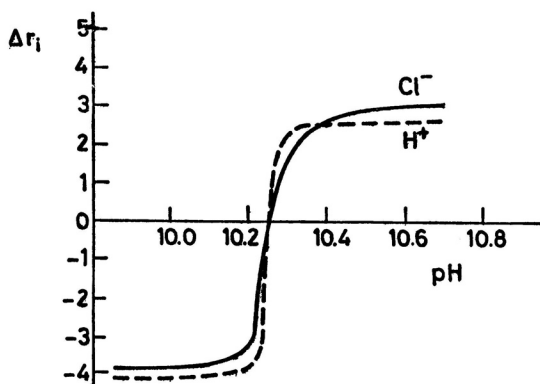


Figure 5. Co-partition ratios for the solubilization of ferri cytochrome-C in W/O micro-emulsions in isooctane, stabilized by TOMAC and octanol. $V_{water} = V_{octane} = 2 \text{ ml}$; C_{TOMAC} in isooctane 10 mM ; $C_{NaCl}(\text{aq.}) = 0.0075 \text{ M}$; octanol 0.1% in isooctane. Buffer: ethylene diamine.

By the same token, in Fig. (b) it is expected that the BSA picks up more protons when ψ° becomes more negative, but a release of protons for pH 6 below -100 mV and a non-zero value at pH 4 and $\psi^{\circ} = 0$ cannot be explained this way.

We intend to return to these unexpected observations in a forthcoming paper¹⁰. Among other things, the co-adsorption of K^{+} and NO_3^{-} must be also considered.

The last illustration concerns ion-copartitioning when proteins are extracted from an aqueous solution into reverse micelles (or micro-emulsion droplets). Figure 5. gives results, obtained by Hilhorst et al.¹¹ using a procedure analogous to the one developed by us.

The protein is ferri cytochrome-C, which is solubilized in the heart of micro-emulsion droplets of the W/O type, stabilized by the cationic surfactant triocetylammmoniumchloride (TOMAC) and the co-surfactant octanol; isooctane constituting the oil phase. For this system co-partition of Na^{+} and buffer (ethylene diamine) is small, maximally one charge per molecule of cytochrome¹¹ so that H^{+} and Cl^{-} are the essential participating ions. In line with this, $\Delta r_{H^{+}} \sim \Delta r_{Cl^{-}}$, meaning that essentially electroneutral HCl is co-adsorbed or expelled.

For this system, an interpretation in terms of the Donnan effect appears satisfactory. Below pH 10.25 protein and micelle are both positively charged, their negative adsorptions amplify each other, so that the total Donnan exclusion is more than that of the components ($\Delta r_i < 0$). On the other hand, at $pH > 10.25$ the negative adsorption of the one is compensated by positive adsorption of the other ($\Delta r > 0$).

Contrary to the previous example, ion co-partition seems to obey simple electrostatic laws. Moreover, the zero point of 10.25 is very close to the i.e.p. of bulk cytochrome-C. It may be concluded that cytochrome-C, solubilized in micro-emulsion droplets is very similar to free protein, a fact that is of relevance in the interpretation of micellar kinetics. On the other hand, BSA molecules may undergo structural changes upon adsorption.

This last conclusion is in line with our general division of proteins into two classes: »soft« and »rigid« with respect to their resilience against conformational changes upon adsorption or solubilization¹²⁻¹⁴. There is substantial evidence that BSA, together with the immunoglobulins and various other big protein molecules belongs to the former category, whereas RNase, lysozyme, and cytochrome-C belong to the latter.

The overall conclusion is that the presented method is general and powerful and that it is helpful to obtain structural information of free and interacting proteins. The range of application is not exhausted; for example Füredi-Milhofer¹⁵ suggested that it may be used to study the role of calcium uptake in biomineralisation. As a caveat it may be repeated that in order to apply our technique the availability of good experimental data is mandatory. In particular, the titrations should be hysteresis-free.

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SAŽETAK

Može li se protein spontano adsorbirati? Termodinamika sudjelovanja iona

J. G. E. M. Fraaije i J. Lyklema

Predstavljena je opća termodinamička metoda kojom se mogu dobiti informacije o ko-adsorpciji i ko-particiji iona niske molekulske težine u procesu adsorpcije ili particije proteina između dvije faze. Esin — Markovljevi koeficijenti u tom su postupku centralne veličine. U radu je pokazano da se predloženim postupkom dobivaju dodatne informacije, koje dozvoljavaju i mehanistička tumačenja tih procesa. To je pokazano trima primjerima: (1) u vezivanju iona na slobodni goveđi serumski albumin, (2) u raspodjeli naboja u proteinu i na površini srebrnog jodida kod adsorpcije tog proteina, i (3) u ionskom sudjelovanju u solubilizaciji citokroma-C u mikro-emulzijske kapljice.