On the Complexation of Proteins and Polyelectrolytes

Fernando Luís B. da Silva,*,† Mikael Lund,‡ Bo Jönsson,‡ and Torbjörn Åkesson‡

Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Av. do café, s/no., Universidade de São Paulo, 14040-903 Ribeirão Preto, SP, Brazil, and Department of Theoretical Chemistry, Lund University, POB 124, S-221 00 Lund, Sweden

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Both natural and synthetic polyelectrolytes form strong complexes with a variety of proteins. One peculiar phenomenon is that association can take place even when the protein and the polyelectrolyte carry the same charge. This has been interpreted as if the ion−dipole interaction can overcome the repulsive ion−ion interaction. On the basis of Monte Carlo simulations and perturbation theory, we propose a different explanation for the association, namely, charge regulation. We have investigated three different protein−polymer complexes and found that the induced ionization of amino acid residues due to the polyelectrolyte leads to a surprisingly strong attractive interaction between the protein and the polymer. The extra attraction from this charge-induced charge interaction can be several *kT* and is for the three cases studied here, lysozyme, α-lactalbumin, and *β*-lactoglobulin, of the same magnitude or stronger than the ion−dipole interaction. The magnitude of the induced charge is governed by a response function, the protein charge capacitance $\langle Z^2\rangle - \langle Z\rangle^2$. This fluctuation term can easily be calculated in a simulation or measured in a titration experiment.

I. Introduction

The complexation of polyelectrolytes and proteins is extensively used in pharmaceutics, foods, and cosmetics.[1−9] The subject has been addressed by a number of authors exploring it from experimental measurements[8−13] to theoretical modeling.[10,14,15] The strength of interaction is to a large extent regulated by electrostatic interactions, governed by key parameters such as pH and salt concentration.[8−10]

A particularly interesting issue is the apparently paradoxical formation of soluble complexes at conditions where the net charges of the protein and the polyelectrolyte have the same sign. Experimental studies of Dubin, Kruif, and co-workers[9−12,16] have demonstrated this special feature of the polymer/protein complexation. The term complexation "on the wrong side" has been used, meaning that a polyanion forms a complex with a protein at a pH above the isoelectric point of the protein.[4,9,14,16] The molecular interpretation of such studies has focused on the assumption of "charged patches" on the protein surface.[9,11,12,14,17] This mechanism has also been used in order to explain protein chromatography data.[18]

Following this reasoning, a polyanion monomer should bind in a positive protein region, and vice versa. The same kind of argument has been used when discussing the interaction between two protein molecules at the isoelectric point, pH ≈ pI. A more formal way to describe the interaction between oppositely charged patches on two protein molecules is in terms of a multipole expansion. That is, for two neutral protein molecules the leading terms would then be dipole−dipole, dipole−quadrupole, etc. Other electrostatic properties of the protein, however, may be more important, and Kirkwood and Shumaker[19] demonstrated theoretically in 1952 that fluctuations of residue charges in two proteins can result in an attractive force. Recently, Lund and Jönsson[20] have taken up this idea and used Monte Carlo simulations and a charge regulation theory in order to explain protein−protein association in a purely electrostatic model.

In a system were electrostatic interactions are known to be relevant, protein charge fluctuations could be an important component of models aimed to describe such systems. This potentially relevant contribution was neglected in previous simulations.[9,14−16] Conversely, the authors invoked an additional $1/R^6$ attractive potential in order to better describe the complexation "on the wrong side".[9,14,15]

The purpose of this work is 2-fold: The first is to demonstrate that a polyelectrolyte and a protein molecule at its isoelectric point do form complexes in a *purely* electrostatic model. We will also show that the driving force for the complexation can be due to charge fluctuations in the protein. The second issue is the relative importance of "charged patches", i.e. charge−dipole interactions, etc., versus the charge regulation term. Second-order perturbation theory offers an easy way to get a qualitative picture of the significance of these terms. We have chosen a set of globular proteins extensively investigated in the literature in order to demonstrate these interactions, namely, lysozyme (lys), α-lactalbumin (α-lac), *β*-lactoglobulin (*β*-lac), bovine serum albumin (bsa), insulin (ins), and calmodulin (CaM).

Lysozyme, α-lactalbumin, and *β*-lactoglobulin were chosen to be extensively studied due to both their biological relevance and particular physicochemical features. These milk proteins exhibit some interesting properties and are often used as models of protein folding, stability, complex formation, and other biophysical and biochemical studies.[21−28] Lysozyme is a small enzyme that hydrolyzes the glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine. In the human body it acts as a barrier preventing infections.[21,22] α-Lactalbumin is a calcium metalloprotein that is responsible for the formation

* To whom correspondence may be addressed. Phone: +55 (16) 3602 42 19. Fax: +55 (16) 3633 29 60. E-mail: fernando@fcfrp.usp.br.
† Universidade de São Paulo.
‡ Lund University.

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of lactose in the mammary gland.[23] Different other functional properties such as apoptosis and induction of cell growth inhibition have also been attributed to this whey protein.[23−25] Even though β-lactoglobulin, the primary component of whey, might be related to milk allergy, shows the ability to bind small hydrophobic molecules such as retinol and fatty acids, and has significant industrial interest,[26−30] its biological function remains unclear.[27,28,30]

II. Model and Simulation

The proteins were modeled as rigid bodies in full atomistic detail according to the X-ray structures provided by the Protein Data Bank[31,32] (PDB identities are 2LZT, 1HFY, 1BEB, 1AO6, 1APH, and 1CLL for lys, α-lac, β-lac, bsa, ins, and CaM, respectively). To account for the acid−base equilibrium, the initial atomic charges were allowed to change their charge state according to the solution pH.[33]

All protein atoms present in the X-ray structure are described by hard spheres of radius $R_a = 2$ Å. This is a reasonable size, and the results are not sensitive to this particular detail. The protein was kept fixed at the center of an electroneutral spherical cell, whose radius R_{cell} was determined by the protein concentration. This so-called *cell model*[34,35] has been used successfully in the past.[36,37] The electrolyte solution surrounding the macromolecule, including neutralizing counterions, is described by the restricted primitive model.[38] Each mobile ion k with charge q_k is treated explicitly as a hard sphere of radius $R_a = 2$ Å, while the solvent is treated as a structureless dielectric medium characterized by a relative dielectric permittivitty ϵ_s.

The interaction between any two particles is given by

$$u(r_{ij}) = \begin{cases} \infty & r_{ij} \le 2R_a \\ \dfrac{q_i\, q_j}{4\pi\epsilon_0\epsilon_s r_{ij}} & \text{otherwise} \end{cases} \tag{1}$$

where ϵ_0 is the vacuum permittivity, q_i and q_j denote the charges on particles i and j, respectively, and r_{ij} represents their separation.

A single flexible polyelectrolyte is modeled as a chain of $N_{mon} = 21$ charged hard spheres of radii $R_{mon} = 2$ Å and charges of $q_{mon} = -e$ (e is the elementary charge) connected by harmonic springs. The bond interaction potential between neighboring monomers is calculated as

$$\beta u^{bond} = \frac{l_B}{2r_{min}^3} \sum_{i=1}^{N_{mon}-1} (r_{i,i+1})^2 \tag{2}$$

where $r_{i,i+1}$ is the distance between monomer i and $i+1$, r_{min} is the separation corresponding to the energy minimum for a dimer and $l_B = e^2/4\pi\epsilon_0\epsilon_s kT$ is the Bjerrum length. We have used a value of 4 Å for r_{min}, which results in an average monomer−monomer separation of approximately 7.4 Å. The polymer is not allowed to titrate. The total energy of the system for a given configuration is then

$$U = \sum_{i=1}^{N_{mob}} \upsilon^{ex}(r_i) + \frac{1}{2}\sum_{i=1}^{N}\sum_{j=1}^{N} u(r_{ij}) + u^{bond} \tag{3}$$

where $N_{mob} = N_c + N_s + N_{mon}$ is the total number of mobile particles comprising N_c counterions, N_s added salt ions, and N_{mon} number of polyanion beads and $N = N_{mob} + N_p$ is the total number of particles including the N_p protein atoms. The term $\upsilon^{ex}(r_i)$ is the imposed hard wall that defines the cell

$$\upsilon^{ex}(r_i) = \begin{cases} 0 & r_i \le R_C \\ \infty & \text{otherwise} \end{cases} \tag{4}$$

The dielectric constant, ϵ_s, was set to 77.8 at room temperature of 300 K.

Simulation Details. Single protein properties, average residue charges on lysozyme, α-lactalbumin, and β-lactoglobulin together with their dipole moments, and capacitances at different pH values were obtained initially in the simulations. The simulation cell, $R_C = 189$ Å, contained a single titrating protein fixed at the center plus 20 ion pairs as well as neutralizing counterions. The polyelectrolyte was absent in these simulations, and the corresponding pI values for the studied proteins were acquired from the average protein charge as a function of pH. Partial residual charges of the protein at pI were saved and used in simulations B and C.

The complexation between the protein and the polyelectrolyte was studied in the second set of simulations. The protein was kept fixed at the center of the cell, while the polyelectrolyte was free to move within the cell. The probability distribution, $P(R)$, for the separation between the center of mass of the two was sampled, and during a first production run an approximate potential of mean force, $w(R)$, was generated

$$\beta w(R) = -\ln P(R) + \text{constant} \tag{5}$$

The probability distribution, $P(R)$, was updated during a second production run, and a final well-converged potential of mean force was obtained. The cell boundary introduces an artificial repulsion in the potential of mean force at separations close to R_C. It is easy to correct for this depletion effect by subtracting off the potential of mean force for a polyelectrolyte in a cell without a protein. All simulations were done with a protein concentration of 0.06 M and salt concentration of approximately 1 mM. An appropriate number of counterions was always present in order to obtain an electroneutral system.

The simulations were performed in a semi-grand-canonical ensemble using the standard Metropolis Monte Carlo algorithm[39] with random displacements of mobile species (salt, counterions, and polyanion beads) within the cell. In addition, the simulation cell was coupled to a proton bath in order to establish a constant pH in the system. After every tenth attempted move of the mobile charges, an attempt was made to delete/insert protons on the titrating groups. In reality, protonation of an acidic group means that acid has been added to the solution. Hence in the simulation, a protonation was balanced by the insertion of a negative mobile charge in order to maintain electroneutrality. The acceptance/rejection of an attempt to change the ionization state of a residue was based on the trial energy

$$\Delta U_{titra} = \Delta U_c \pm kT \ln 10(\text{pH} - \text{p}K_0) \tag{6}$$

where ΔU_c is the corresponding change in Coulomb energy, and pK_0 is the dissociation constant of the model compound. These values were taken from ref 40 and are given in Table 1. This method is accurate for weakly charged systems, whereas for really highly charged titrating objects, it has to be corrected for the excess chemical potential of the proton.

Perturbation Theory. In this section we present a perturbation approach for the interaction between a protein molecule at pH = pI and a charged molecule, i.e., in our case a polyelectrolyte. If the charges of a neutral protein are fixed, that is, the amino acid residues are not allowed to titrate, then the leading term in the perturbation expansion is the ion−dipole interaction; the polyelectrolyte net charge interacts with the dipole moment

TABLE 1: Titrating Residues in the Investigated Proteins[a]

protein	residues	Asp	Glu	His	Tyr	Lys	Cys[b]	Arg
pK_0[40]		4.0	4.4	6.3	9.6	10.4	10.8	12.0
lysozyme (2LZT)	129	7	2	1	3	6	0	11
α-lactalbumin (1HFY)	123	14	4	3	4	13	0	1
β-lactoglobulin (1BEB)	320	20	32	2	8	30	2	6
bovine serum albumin (1A06)	585	35	62	15	18	58	35	24
insulin (1APH)	41	0	4	2	4	1	6	1
calmodulin (1CLL)	142	16	19	1	2	5	0	6

[a] The PDB identities are reported together with the corresponding protein name. The dissociation constants for the isolated amino acids are given in the second line and the corresponding pK_0 for C- and N-termini are 3.8 and 7.5, respectively.[40] [b] Only cysteins not engaged in sulfide bridges can titrate.

of the protein. If the amino acid residues can titrate and change their charges as a function of protein−polyelectrolyte separation, then an additional induced charge−charge interaction will appear (this term is sometimes referred to as the "regulation term"). Both terms are identically zero in first order, but contribute attractively to the free energy in second order. The thermally averaged ion−dipole term varies as R^{-4}, while the ion-induced charge interaction is more long ranged and decays like R^{-2}. The relative importance of these two terms is the focus of the present work. Below follows a formal derivation of the terms.

Consider a protein described by a charge distribution $[\mathbf{r}_i,q_i]$, assuming its center of mass is placed at the origin, and the polyelectrolyte simply modeled by a point charge Q_α at $[\mathbf{R}]$. The electrostatic energy can then be written as

$$\beta U(R) = \sum_i \frac{l_B z_i Z_\alpha}{|\mathbf{R} - \mathbf{r}_i|}$$

$$R = |\mathbf{R}|$$

where we have used $q_i = ez_i$. From statistical mechanical perturbation theory[41,42] we can, provided that $\beta U(R)$ is small, write the interaction free energy as

$$\beta A(R) = -\ln\langle e^{-\beta U(R)}\rangle_0 \approx -\ln\left[1 - \langle \beta U(R)\rangle_0 + \frac{1}{2}\langle(\beta U(R))^2\rangle_0\right]$$

where $\langle ...\rangle_0$ denotes an average over all configurations in the unperturbed system. Assuming that $R \gg r_i$, we now perform a multipole expansion and obtain the final expression for the ion−ion, ion-induced charge, and ion−dipole interactions

$$\beta A(R) \approx l_B Z_\alpha \left(\frac{\langle Z\rangle_0}{R}\right) - l_B^{\,2} Z_\alpha^{\,2}\left(\frac{C}{2R^2} + \frac{\langle \mu\rangle_0^{\,2}}{6R^4}\right) \qquad (7)$$

where $\langle Z\rangle_0$ is the protein average charge number, $\langle \mu\rangle_0 = |\sum_i z_i \mathbf{r}_i|$ the average dipole moment number,and finally C is the charge fluctuations or the *protein charge capacitance*[43]

$$C \equiv \langle Z^2\rangle_0 - \langle Z\rangle_0^{\,2} = -\frac{1}{\ln 10}\frac{\partial \langle Z\rangle_0}{\partial \mathrm{pH}}$$

The capacitance is an intrinsic property of a protein defining its ability for charge regulation. It is easily obtained as the derivative of the titration curve and is thus strongly pH-dependent. Since charge fluctuations are largest when pH = pK_a of a certain residue, the capacitance of a protein rich on, say, glutamic acid will peak at pH 4−5 (p$K_a^{glu} \approx 4.4$).

The leading term in eq 7 is the ion−ion interaction, but this vanishes at the isoelectric point where $\langle Z\rangle_0 = 0$. Left is the regulation and the ion−dipole terms—which one is more important depends of course on C and $\langle \mu\rangle_0$ but it is interesting to note that the charge regulation term is more long ranged than the ion−dipole interaction.

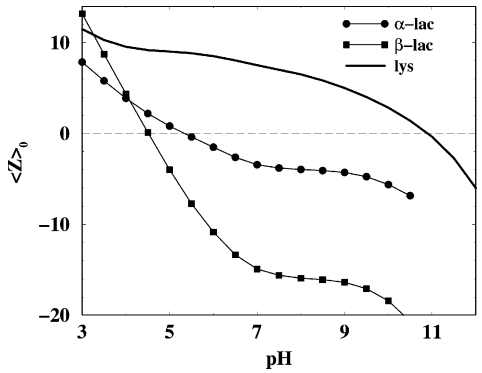

Figure 1. The simulated charge number of α-lactalbumin (spheres), β-lactoglobulin (squares), and lysozyme (no symbols). The salt concentration is 1 mM, and the protein concentration is 0.1 mM.

Equation 7 is valid in the limit of no salt. To approximately account for the effect of salt, the above expansion can be performed using a screened potential, where the ionic strength is expressed through the Debye length, $1/\kappa$. In the case of the ion−ion term, this brings about an additional factor of exp(−κR), while the ion-induced charge interaction is screened by exp(−2κR).

III. Results

The Isoelectric Point. As a starting point, we have simulated a single protein in salt solution varying the pH in order to determine the isoelectric point. Figure 1 shows the net charge of the three proteins as a function of pH. The corresponding pI values for α-lactalbumin, β-lactoglobulin, and lysozyme are 5.4, 4.5, and 10.9, respectively. These values have been obtained at low salt concentration. Addition of salt changes pI, and dimer or oligomer formation can also affect the isoelectric point. For example, the addition of 1 M salt to an α-lactalbumin solution decreases pI by approximately 0.4 units. Due to these facts, the experimental values for α-lactalbumin varies between 4.1 and 4.6, while for β-lactoglobulin the experimental data are slightly more scattered, between 4 and 5.5.

Figure 2 describes how the capacitance and the protein dipole moment vary with pH. The dipole moment is strictly well defined only at pI. For a nonneutral molecule the value of μ will depend on the coordinate origin—here we have used the center of mass. Both α-lactalbumin and β-lactoglobulin have large dipole moments over an extensive pH interval, while lysozyme has a comparatively small dipole moment. The capacitance for the three proteins varies significantly with pH, but at pI it is considerable for all three proteins. The smallest capacitance value is found for α-lactalbumin [$C_{\alpha-lac}$(pH = pI = 5.4) = 0.99], and it is related to the number of amino acid residues that titrate around pI. The relevant properties for the three proteins are collected in Table 2. Note, however, that the data for β-lactoglobulin is obtained for the dimer.[26,28]

Perturbation Calculations. Table 2 contains the basic physical data for the proteins used in this study. We now use this information to analytically calculate the ion-induced charge and ion−dipole contributions to the interaction free energy according to eq 7. The magnitude of the regulation and ion−dipole terms at contact are also given in Table 2. The results indicate that the regulation term is by far the most important term for lysozyme, while for α-lactalbumin and β-lactoglobulin

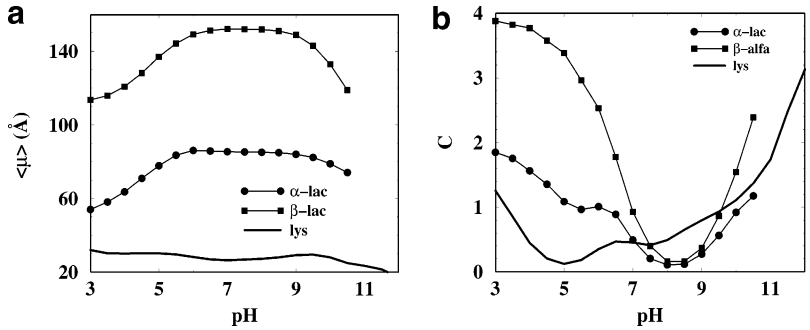

Figure 2. (a) The simulated dipole moment number, $\mu = \langle|\sum z_i \mathbf{r}_i|\rangle$, of α-lactalbumin (no symbols), β-lactoglobulin (spheres), and lysozyme (squares). The salt concentration is 1 mM, and the protein concentration is 0.1 mM. (b) Same as (a) but the protein capacitance, C.

TABLE 2: Charge Capacitance and Dipole Moment Number for the Investigated Proteins at Their Isoelectric Points[a]

	pI	C	μ	$R_p + R_{pe}$	$-\beta A_{reg}$	$-\beta A_{dip}$
lysozyme	10.9	1.7	24	58	5.7	0.2
α-lactalbumin	5.4	0.99	82	58	3.3	2.2
β-lactoglobulin	4.5	3.5	128	73	7.4	2.2
bovine serum albumin	5.5	3.2	297	81	5.5	7.7
insulin	5.4	0.36	49	51	1.6	1.3
calmodulin	4.0	3.7	51	58	12	0.9

[a] R_p is an estimate of the protein radius. The two last columns give the interaction between the protein and the polyelectrolyte at contact, that is $\beta A_{reg} = -l_B^2 Z_\alpha^2 C/2(R_p + R_{pe})^2$ and $\beta A_{dip} = -l_B^2 Z_\alpha^2 \mu^2/6(R_p + R_{pe})$,[4] where R_{pe} has been chosen as half the end-to-end separation of the corresponding neutral polymer (30 Å) and $Z_\alpha = -21$.

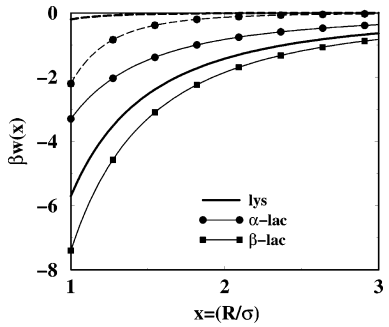

Figure 3. The contribution to the free energy of interaction from the charge−induced charge term (solid lines) and the ion−dipole term (dashed lines). Lines without symbols describe lysozyme, filled circles refer to α-lactalbumin, and filled squares refer to β-lactoglobulin, respectively. The free energies are calculated from eq 7 using simulated capacitances and dipole moments from Table *2* and $\sigma = R_p + R_{pe}$. Note that the ion−dipole terms for α-lactalbumin and β-lactoglobulin coincide.

the two terms are of comparable magnitude. The actual numbers in Table 2 should of course be regarded as qualitative and not quantitative. However, they still give, as will be seen below, a correct picture of the behavior of the three proteins. The contact separation has been defined as the protein radius plus the polyelectrolyte radius, $R_p + R_{pe}$. The latter has been chosen as half the end-to-end separation of the corresponding neutral ideal polymer. Both the protein and polyelectrolyte radii are approximate, but even with a rather generous variation of these values, the general picture of Table 2 will remain the same.

The regulation term decays slower than the ion−dipole term, which means that it will gain in relative importance at larger separation; see Figure 3. This means that even if the two terms are comparable at contact, the regulation term can still dominate the contribution to, for example, the second virial coefficient.

Monte Carlo Simulations. We have performed four different simulations for each protein: A, the "neutral" protein, that is all charges have been set to zero; B, the protein with fixed charges at each amino acid residue; C, the protein with an ideal dipole at its center of mass; D, the protein with titrating amino acid residues.

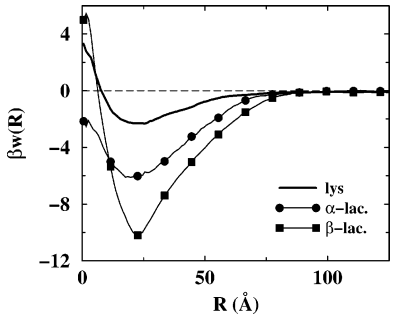

Figure 4. The potential of mean force between the centers of mass of the protein and the polyelectrolyte obtained from MC simulations with model D. The curves have been calculated at the respective isoelectric points for lysozyme (no symbols), α-lactalbumin (filled circles), and β-lactoglobulin (filled squares).

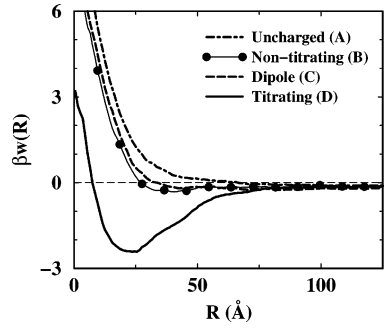

Figure 5. The potential of mean force between the centers of mass of lysozyme and the polyanion. The curves have been calculated at pI, and the four curves correspond to the different cases mentioned in the text.

The first set of simulations (A) describes only the shape of the protein, and the free energy of interaction is of course everywhere repulsive. These energy curves also give an indication of how difficult it is to deform the polyelectrolyte. The second set of simulations (B) uses fixed fractional charges on all residues, which has been determined in a separate simulation of the isolated protein at the appropriate pH. In the next set (C), the charge distribution of the protein is replaced by an ideal dipole; see Table 2. In the fourth and final set (D) the amino acids are allowed to titrate and this simulation contains all electrostatic contributions including the ion-induced charge term. The difference between set B and C describes the importance of higher order electrostatic moments, quadrupole, octupole, etc., in the protein, while a comparison of sets B and D will reveal the effect of the regulation mechanism.

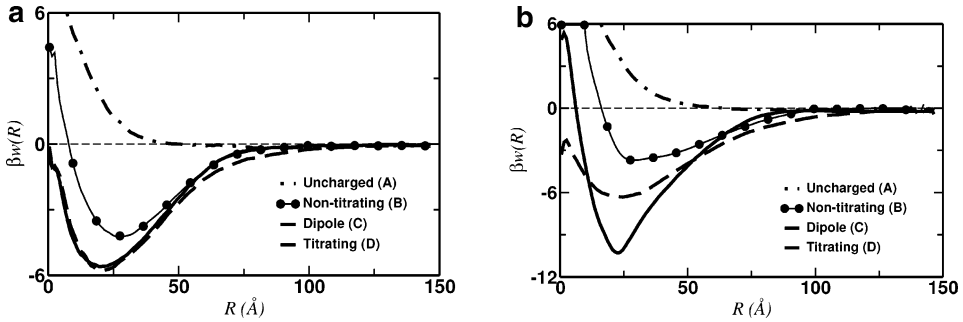

Figure 6. The potential of mean force between the centers of mass of (a) α-lactalbumin and (b) *β*-lactoglobulin and a polyanion, calculated at the respective pI. The four curves correspond to the different cases mentioned in the text.

The calculated potential of mean force, $w(R)$, for the three proteins at their respective pI all show a clear minimum; see Figure 4. The relative strength of the minima are in qualitative agreement with perturbation calculations, cf. Figure 3, while the actual numbers are approximately half the values predicted by second-order perturbation theory. A more quantitative comparison is difficult since the size of the polyelectrolyte is not unambiguously defined. The minima appear at roughly the same separation despite the fact that *β*-lactoglobulin is more than twice as big as the two others. This can be explained by the elongated form of the former, which also results in a more long ranged attraction. The separation R can approach zero, which corresponds to a situation where the polyelectrolyte wraps around the protein. Note, however, that $w(0)$ is repulsive indicating that the "wrapping" of the chain around the proteins is an entropically unfavorable structure.

β-Lactoglobulin forms a stronger complex with the polyanion than α-lactalbumin. Experimental results with whey proteins and gum arabicum by Weinbreck et al.[44] indicate a significant complex formation between the polyelectrolyte and *β*-lactoglobulin, which was the main component in the whey protein in their experiment. An experimental comparison of the relative strength of complex formation between α-lactalbumin and *β*-lactoglobulin at their respective pI is difficult, since the charge of gum arabicum varies in this pH interval. The attractive minimum in the protein-polyelectrolyte complex is reduced upon addition of salt,[14] and we can use the minima of $w(R)$ in Figure 4 in order to estimate the critical ionic strength. Assuming that the salt screening can be described by simple Debye−Hückel theory and that the complex can be defined as dissolved when the interaction is less than kT, we get the following relation

$$\exp(-2\kappa R_{\min})|\beta w(R_{\min})| \leq 1 \qquad (8)$$

The factor of 2 in the exponent comes from the fact that the second-order terms dominate the interaction. Following this recipe we find that approximately 10 and 20 mM salt is sufficient to dissociate the α-lactalbumin and *β*-lactoglobulin polymer complexes, respectively. This is in fair agreement with experiments,[9,14] but the actual numbers are of course dependent on the criterion in eq 8.

Thus, we have shown that a polyanion can form a complex with a neutral protein molecule. Next, we will make a numerically more rigorous partitioning of contributions to the potential of mean force shown in Figure 4. The minimum for lysozyme is solely due to charge regulation, Figure 5. If the charge distribution on lysozyme is considered fixed, then the polyanion−lysozyme interaction is essentially everywhere repulsive. Replacing the detailed charge distribution with an ideal dipole at the mass center has a small effect on the free energy. This means that the ion−dipole interaction gives a very small attractive contribution, while the effect from higher order moments is negligible.

As shown in Figure 6, the polyanion interacts more strongly with α-lactalbumin and *β*-lactoglobulin than with lysozyme. For α-lactalbumin the regulation term increases the depth of the minimum from approximately 4 to 6kT. An interesting effect is that the dipolar protein shows a stronger interaction than the protein with a detailed but fixed charge distribution. This means that the ion−quadrupole interactions etc. add repulsive contributions to the interaction. The potential of mean force for *β*-lactoglobulin is even more attractive (≈10kT) in good agreement with the predictions based on the perturbation calculations.

IV. Conclusions

Strong protein−polyelectrolyte complexes can form due to purely electrostatic interactions even when the protein has a zero net charge at its isoelectric point. Two mechanisms contribute to the attractive interaction. One is the ion−dipole term and the other is a charge−induced charge interaction. The latter is often the dominating term and its strength is related to charge regulation of titrating amino acid residues in the protein. This ability can be quantified by the protein charge capacitance $C = \langle Z^2 \rangle - \langle Z \rangle^2$. The capacitance can be calculated from a simulation or measured in a titration experiment. Three different protein−polymer complexes have been investigated here by Monte Carlo simulations: lysozyme, α-lactalbumin, and *β*-lactoglobulin. The contribution from the charge−induced charge interaction to the free energy of interaction can be several kT s and is for the three cases studied here of the same magnitude or stronger than the ion−dipole interaction. We believe that this type of interaction is of importance also for the interaction between two proteins.

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Note Added after ASAP Publication. The following Note Added in Proof was submitted by the authors after this paper was published on the Web on 2/9/2006.

After publication of our paper on the Web, we were informed of the paper by P. M. Biesheuvel and M. A. Cohen Stuart, *Langmuir* **2004**, *20*, 2785, which, using mean-field theory, also suggests charge regulation as a relevant mechanism to describe polyelectrolyte−protein complexation at the wrong side of the isoelectric point.

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