SUPPORTING INFORMATION

Interaction Between $\beta$-lactoglobuline and Weak Polyelectrolyte Chains: a Study Using Monte Carlo Simulation

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SII. Monte Carlo titration scheme

The general equilibrium properties of weak acids can be described at ideal conditions using the dissociation of monoprotic acid:

\[ HA \rightleftharpoons A^- + H^+ \]  

(1)

the corresponding thermodynamic equilibrium constant:

\[ K_a = \frac{a_{H^+}a_{A^-}}{a_{HA}} = \frac{\gamma_{H^+}\gamma_{A^-}}{\gamma_{HA}} \times \frac{c_{H^+}c_{A^-}}{c_{HA}} \]  

(2)

where \( a, \gamma \) and \( c \) are activity, activity coefficients and concentrations of each species, respectively. The free energy difference between the protonated and deprotonated form is:

\[ \beta \Delta A_{HA \rightarrow A^-} = -\ln \left( \frac{c_{A^-}}{c_{HA}} \right) = -\ln \left( \frac{\gamma_{HA}}{\gamma_{A^-} \gamma_{H^+}c_{H^+}} \right) \]  

(3)

\[ \beta \Delta A_{HA \rightarrow A^-} = -\ln \left( \frac{\gamma_{HA}}{\gamma_{A^-}} \right) - (pH - pK_a) \ln 10 \]  

(4)

where \( pH = -\log(\gamma_{H^+}c_{H^+}) \). We shall treat the charged (deprotonated) sites as the fluctuating species. The (bulk) chemical potential of these “particles” is related to the pH via\(^1\):\(^3\):

\[ \mu = k_B T (pH - pK_a) \ln 10 \]  

(5)

where \( pK_a = -\log(K_a) \).

In the simulation, the protein and polyelectrolyte molecules are represented as coarse-grained models described in the manuscript. The small cation and anion are modelled explicitly as rigid spheres and represent the added salt to the system. The protons and hydroxides are modelled implicitly. The solvent is treated as a dielectric continuum with a permittivity equal to that of water at room temperature.
The simulations were performed in the canonical ensemble with respect to protein, polyelectrolyte and using the traditional Metropolis Monte Carlo procedure. In addition, the simulation box was coupled to a bath in order to establish a constant pH and chemical potential of small ions in the system. It also acts as a proton reservoir via a chemical potential. In each attempt to delete/insert protons into a titratable group, a proton is only moved from the chain to the bath or Vice Versa. Then, the proton deleted from a titratable group is not left in the bulk solution of the system, but is taken out of the system and is conducted to the reservoir. Actually, a deletion of a proton from a group means that alkali (NaOH) has been added to the solution. Hence, in the simulation, a proton deletion from a titratable group was always accompanied by the addition of a positive mobile charge to the simulation box in order to keep the system electroneutral. The added small cation interacts with the remaining charges in the system with coulombic potential:

\[
\frac{U_{el}}{k_BT} = l_B \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \frac{\varepsilon_i \varepsilon_j}{r_{ij}} \tag{6}
\]

where \(l_B = \frac{e^2}{4\pi \varepsilon_r \varepsilon_0 k_B T}\) is the Bjerrum length. These movements of protonation or deprotonation were accepted according to the probability:

\[
\rho \sim \exp(-\beta \Delta U_{el} \pm (pH - pK_a) \ln 10) \tag{7}
\]

where the sign - or + corresponds to protonation and deprotonation and \(\Delta U_{el}\) symbolizes the change of electrostatic energy between initial and final states.

Since the protons are implicitly modelled in the solution of system, they do not modify the chemical potential of small ions. This is a good approximation as long as the concentration of protons and hydroxides is much lower than the concentration of small ions. In addition, the explicit presence of small ions is fundamental to study non-lineal phenomena as the counterion condensation on the PE chain (Manning condensation), since the protein competes with the small counterions by the adsorption on the PE chain. A screened Coulomb potential, so called Debye-Hückel potential with a characteristic decay Debye length is unable to capture non-linear effects.
All models have approximations with a limited detail of system representation. The elements contained in our model are consistent and, to our knowledge, reflect the relevant physicochemistry of the process of complex formation between proteins and polyelectrolyte.

SI2. The ionic pair formation criterion:

The binding between polyelectrolyte and protein was quantified based on a structural criterion defined by a cut radius $r_{cut} = 0.5 \text{ nm}$. But this structural criterion has an energy criterion enclosed, which is related to the Bjerrum length, $l_B$. It is defined as the distance that the electrostatic energy between two elementary charges is comparable in magnitude to the thermal energy scale. To estimate the Bjerrum length, we begin with the thermal energy scale, $k_B T$, where $k_B = 1.38 \times 10^{-23} \text{ J/K}$ is the Boltzmann constant and $T$ the absolute temperature ($T = 300 K$). The electrostatic energy between two elementary charges is:

$$U_{Ele} = \frac{e^2}{4\pi \varepsilon_0 \varepsilon_r T} \quad (8)$$

where $e$ is the elemental charge, $\varepsilon_0$ is the vacuum dielectric constant and $\varepsilon_r$ is the relative constant ($\varepsilon_r = 80$) of solvent (water). Considering the definition of $l_B$ and the equation (8), we obtain that:

$$k_B T = \frac{e^2}{4\pi \varepsilon_0 \varepsilon_r l_B} \quad (9)$$

This expression can be rewritten as:

$$l_B = \frac{e^2}{4\pi \varepsilon_0 \varepsilon_r k_B T} \quad (10)$$
at room temperature the Bjerrum length have a value $l_B \approx 0.71 \text{ nm}$. At this distance, two charged particles (elementary charges) have the electrostatic energy equal in magnitude to that of the thermal energy.

The criterion for the formation of an ionic pair between the protein and PE is based on the quantification of the distance $d_{rij}$ between a negatively charged monomer $i$ of the PE chain and an opposite charged residue $j$ of the protein. An ionic pair is considered as formed when this distance $r_{ij} < r_{cut} = 0.5\text{ nm}$, which is lower than the Bjerrum length. This value ensures that the attractive electrostatic energy of the ionic pair is greater than the thermal energy, since:

$$\frac{|U_{\text{Ele}}(r_{ij} = 0.5\text{ nm})|}{k_B T} \approx 1.42$$

which means that if the separation of ionic pair is lower, the magnitude of attractive electrostatic energy of ionic pair is at least 42% larger than the thermal energy of the system, and therefore a stable ion pair is formed.

In order to discuss how the conclusions would be changed by using different values of the cut-off radius, the average number of ionic pairs formed is calculated as a function of $r_{cut}$ for different PE cases.
Figure S1. Number of ionic pairs between protein-PE as a function of the pH for PE chains with A) $l_0 = 0.25 \text{ nm}$, B) $l_0 = 0.50 \text{ nm}$ and C) $l_0 = 0.75 \text{ nm}$. At different values of $r_{\text{cut}}$. 
Figure S1 shows the average ionic pairs formed in the interaction between a $\beta$-lactoglobuline (monomeric) and a PE chain as a function of pH with different values of $l_0$. The panels A, B, and C correspond to PE chains with $l_0 = 0.25 \text{ nm}, 0.50 \text{ nm}$ and $0.75 \text{ nm}$. Four different values of $r_{\text{cut}}$ are used (from 0.4 to 0.7). In each case as the $r_{\text{cut}}$ values increase, the number of ionic pairs formed increases. However, the curve has the same tendency for the different cut radius values. Thus, the qualitative conclusions are independent of the cut radius value chosen.

**SI3. The physical meaning of chosen values of $l_0$ and their relationship with the linear charge density of the PE.**

In order to cover a wide range lower to $l_B$, three different values of $l_0$ were chosen, since in this range ($l_0 \leq l_B$), non-linear effects due to the high charge density in strong polyelectrolytes are evident, such as the counterion condensation called Manning condensation. The phenomenon of counterion condensation now takes place when the dimensionless Coulomb coupling parameter:

$$\Gamma_{\text{Coul}} = \frac{l_B}{l_0} > 1$$

In this case, the Coulomb interactions dominate over the thermal interactions and counterion condensation is favoured in strong PE.

The values of Coulomb coupling parameter corresponding to each $l_0$ value are summarized in table SI1.

<table>
<thead>
<tr>
<th>$l_0$ [nm]</th>
<th>$\Gamma_{\text{Coul}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>2.84</td>
</tr>
<tr>
<td>0.5</td>
<td>1.42</td>
</tr>
<tr>
<td>0.75</td>
<td>0.98</td>
</tr>
</tbody>
</table>

*Table SI1. Different $l_0$ values and their corresponding Coulomb coupling parameters.*
The chain with \( l_0 = 0.25 \text{ nm} \) has the higher value of coulomb coupling parameter, therefore, it is expected that the electrostatic interaction plays an important role in the condensation of counterions, as well as in the ionization of the weak polyelectrolyte and in the interaction of the protein with the PE. In the limit when \( l_0 = 0.75 \text{ nm} \), the coupling parameter is close to 1. Therefore, the electrostatic interaction has a lower effect, as we will see next, studying the effect of \( l_0 \) on the ionization of weak PE.

The relationship between \( l_0 \) value and the linear charge density of a full charged PE, \( \tau^{FD} \), is the following:

\[
\tau^{FD} = -\frac{N_m}{(N_m - 1) \cdot l_0}
\]

where \( N_m \) is the polymerization index of chain. The linear charge density took values of \( \tau^{FD} \approx -1.37 \text{ nm}^{-1} \), \( \tau^{FD} \approx -2.05 \text{ nm}^{-1} \) and \( \tau^{FD} \approx -4.1 \text{ nm}^{-1} \) for the chains with \( l_0 \): 0.75 nm, 0.50 nm and 0.25 nm respectively. We can note that the denominator in the equation is the contour length of chain \( L_c \):

\[
L_c = (N_m - 1) \cdot l_0.
\]

When the PE chain is partially charged the relationship between \( l_0 \) value and the linear charge density of PE chain depends on the dissociation degree of monomers of the PE:

\[
\alpha^\text{MC}_{PE} = \frac{\langle m_i \rangle}{N_m}
\]

where \( m_i \) is the amount of dissociated monomers and \( N_m \) is the amount of monomers of the PE chain. In the Monte Carlo simulations, the linear charge density of PE chain was calculated as:

\[
\tau^{MC}_{PE} = -\frac{\langle m_i \rangle}{L_c}
\]
The dissociation degree takes values from 0 to 1, where the higher value means that the entire anionic PE chain was deprotonated. We can note that the $\tau_{PE}^{MC}$ is directly proportional to $\alpha_{PE}^{MC}$:

$$\tau_{Al}^{Id} = -\frac{N_m}{L_c} \alpha_{Al}^{Id} = \tau^{FD} \alpha_{Al}^{Id}$$

where $\tau^{FD}$ is the linear charge density of PE chain when is fully dissociated. In the following figures are depicted the dissociation degree and linear charge density both as a function of $pH$.

![Graphs showing dissociation degree and linear charge density as functions of pH](image)

Figure S2. A) Average dissociation degree of isolated PE chain as a function of the pH B) Linear charge density of the isolated PE chain as a function of the pH. In both figures three different values of $l_0$ were used: 0.25nm, 0.50 nm and 0.75 nm; for a PE chain of $N_m = 40$ and $c_{satt} = 10 mM$.

We studied the effect of varying the equilibrium bond length on the isolated PE chain. Figure S2b depicts the linear charge density ($\tau$) of the PE chain as a function of the solution $pH$. The ideal linear charge density is depicted in dotted line and the results obtained from the simulations in full symbols. The PE chain with $l_0 = 0.25nm$ has the higher value of $\tau$ and $l_0 = 0.75nm$ the lowest value. As the $pH$ increases, the linear charge density increases in the three cases. A tendency of the three PE chain to follow the same line of linear charge density can be observed (black continues line) until each chain
reaches its maximum value at $\tau$. There is a point, at $pH = 2.5$, in which the three PE chain have the same value of linear charge density. In order to analyse this behaviour, we studied the dissociation degree of the PE chain as a function of the solution $pH$, figure S2A. At $H = 2.5$, figure S2A also shows that the dissociation degree takes different values in the three cases, from $\alpha = 0.3$ for a PE chain with $l_0 = 0.25nm$ to $\alpha = 0.8$ for $l_0 = 0.75nm$. Since the distance between the monomers of the PE, chain is shorter in the first case, the electrostatic repulsion increases. Therefore, the electrostatic energy change has a more positive $\Delta U_{el}$ value, which reduces the probability of dissociation of neutral monomers. Analysing these two figures, it is possible to discern between the linear charge density and the effect of separation of adjacent charged monomers.

**SI4. Comparison between different coarse-grained models of the polyelectrolyte.**

The coarse-grained model chosen to represent the polyelectrolyte chain is the simplest possible to study the coupled effects of linear charge density, dissociation of titratable monomers and interaction between polyelectrolyte and protein. This model is characterized by maintaining the distance $l$ between neighboring monomers near an equilibrium value $l_0$, by a strong harmonic potential. This scheme ensures that we maintain a fixed linear density of titratable groups in the chain, which in combination with the degree of ionization of the titratable groups will cause the linear charge density.

From the extensive observation of the animations obtained from the simulations with $l_0 = 0.75$ nm we do not observe phenomena such as counterions to sit along the bond or penetrate the chain molecule or intra-chain crossing. However, we carried out an extensive series of Monte Carlo simulations where the original coarse-grained model was modified to take into account the phenomena mentioned above.
The original and modified coarse-grained models are shown in figure S3, where the titratable and neutral monomers are depicted as red and green particles respectively. The original model shown titratable groups bonds for harmonic potential with equilibrium length of $l_0 = 0.50 \text{ nm}$ and $l_0 = 0.75 \text{ nm}$. The modified model 1 have a neutral monomer between two consecutives titratable groups bonds with harmonic potential with $l_0 = 0.50 \text{ nm}$. This new neutral monomer is located on the line that joins the centers of the titratable groups, at an equal distance from the two groups ($\approx 0.25 \text{ nm}$). The equivalent form, for the modified model 1, the chain with $l_0 = 0.75 \text{ nm}$ have two additional neutral monomers between two consecutives titratable groups.

FIGURE S3. Original and modifies models for PE chain. The original coarse grain model for polyelectrolyte chain with $l_0 = 0.50 \text{ nm}$ and $l_0 = 0.75 \text{ nm}$ are shown in first column. The modified model 1 has an additional neutral monomer between two titratable monomers with $l_0 = 0.50$ and two additional neutral monomers between two titratable with $l_0 = 0.75 \text{ nm}$. The modified model 3 has the same types of particles, but the bonds between two neighbors particles have $l_0 = 0.25 \text{ nm}$. The titratable and neutral monomers are represented by red and green particles respectively. The blue and pink particles represent the small cations and anions respectively.
The modified model 2 have the same composition of particles, but two consecutives particles (Regardless if they are titratable or neutral groups) are bond for an harmonic potential with $l_0 = 0.25 \text{ nm}$ (Kuhn length has a value close to 0.25 nm).

In order to compare the original and modified models, the ionic pair formed between PE and protein are showed as a function of pH.

Figure S4. Number of ionic pairs formed between PE and protein as a function of $pH$. Original and modified coarse-grained models for PE are depicts as solid and empties symbols. The PE chain have 40 titratable monomers with A) Chain with $l_0 = 0.50\text{ nm}$, original and modified model with one neutral monomer between two titratable monomers. B) $l_0 = 0.75\text{ nm}$ original and modified model with two neutral monomers between two titratable
Figure S4 shows the interaction between the protein and polyelectrolyte, the results obtained using the original and modified coarse grained models for PE were compared.

The Panel A shows the results obtained for the PE chain with $l_0 = 0.50$ nm. The original PE model results are depicted in solid square symbols. The modified model 1 results (which is added a neutral monomer between two titratable groups) are depicted with empty square symbols. The modified model 1 shows lower values of $n_{IP}$ than the original one. However, in general the results are close to the original model, additionally the difference between models is lower than the standard deviation. The modified model 2 shows number ionic pairs very similar to the original model.

The Panel B depicts the comparison between the results of original and modified models PE chain with $l_0 = 0.75$ nm. The original and modified 1 model show an excellent agreement in the number of ionic pairs formed as a function of pH. The modified model 2 shows a slight increase in the value of ionic pairs, but very close to the results of the original model.

With these results we can ensure that the original model is an approximation to the modified models.

References
