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A coarse-grained model for flexible (phospho)proteins: Adsorption and bulk properties

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ABSTRACT

Protein adsorption is a complex process that it controlled by several different mechanisms, for example: (i) electrostatic interactions between the protein and the surface, and (ii) between adsorbed proteins; (iii) dispersion interactions; (iv) hydration effects; and (v) structural rearrangements of the protein to balance conformational chain entropy with energetics.

The aim of this study was to develop a simple model for the adsorption of intrinsically disordered proteins onto surfaces at a mesoscopic level of detail, while retaining protein integrity. Monte Carlo simulations were used in order to study the thermodynamical and structural properties of the flexible phosphoprotein β-casein, in bulk and adsorbed to hydrophilic silica surfaces, in order to evaluate the effect of varying pH, monovalent salt concentration, and degree of serine phosphorylation. Experimental evidence from our previous study, published in this Journal, was used to set up and tune the Hamiltonian of the model.

Our simulations show that protein-surface electrostatic interactions are, indeed, not the main driving force behind adsorption under the simulated conditions. Despite its importance, when taken alone, this type of interaction is not enough to promote the adsorption of β-casein at any salt concentration. Adsorption is only possible through the inclusion of a protein-surface short-ranged attractive interaction potential with a minimum interaction strength of 2.25 k_BT. This represents *the lowest interaction strength* required to mimic experimental adsorption results. An equally important finding is that considerable protein net charge fluctuations, due to phosphorylated serine saturation, have a negligible contribution to the free energy of adsorption.

1. Introduction

In 1906, Phoebus Levene identified phosphate in the protein vitellin (phosvitin) at the Rockefeller Institute for Medical Research (Levene & Alsberg, 1906) and, by 1933, together with Fritz Lipmann, he had detected phosphoserine in casein (Lipmann & Levene, 1932). Phosphoproteins are a group of proteins, which are chemically bonded to one or several phosphate groups, and/or to any other molecule through a phosphate group. Serine is the most commonly phosphorylated amino acid, followed by threonine. Phosphorylation of any site on a given protein can change its structure and function dramatically, especially when electrostatic interactions are important for its interaction(s) and function(s).

β-casein (together with αS1-casein) is the most abundant milk protein and belongs to the intrinsically disordered protein (IDP) family (Dunker et al., 2001). Its primary structure is proline-rich and consists of 209 amino acid residues, including five phosphorylated serines. The molecular mass is ~24 kD and the protein has an amphiphilic character with one hydrophilic and one slightly hydrophobic domain. The N-terminal region of the sequence is rich in polar and negatively charged amino residues, including all five phosphorylated serines (located at positions 15, 17–19, and 35), while the majority of the hydrophobic and positively charged residues are located near the C-terminal. β-casein is considered to be a calcium-carrier, with calcium binding to the phosphorylated regions. Therefore, it is regarded as an important protein for human health both in terms of nutrition as well as for the remineralization of the skeleton and teeth enamel. Numerous studies have been devoted to β-casein adsorption onto hydrophobic surfaces. At neutral pH, β-casein adsorbs through the hydrophobic C-terminal, which anchors to the hydrophobic surface, while the hydrophilic N-terminal protrudes into the solution, forming a brush-like structure

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from a macroscopic point of view (Atkinson, Dickinson, Horne, & Richardson, 1995; Brooksbank, Davidson, Horne, & Leaver, 1993; Dalgleish & Leaver, 1991; Dickinson, Horne, Phipps, & Richardson, 1993; Fragneto, Su, Lu, Thomas, & Rennie, 2000; Mackie, Mingins, & North, 1991; Murray & Cros, 1998). It has been shown that the adsorbed amount increases as pH is lowered towards the isoelectric point, and that the presence of calcium ions modifies the structure of the β-casein monolayer, reducing the hydrophilic layer thickness and the adsorbed amount (Atkinson et al., 1995). Theoretical studies using self-consistent field theory qualitatively confirm these findings, and significant effects of ionic strength and pH have been found (Dickinson, Pinfield, Horne & Leermakers, 1997; Leermakers, Atkinson, Dickinson, & Horne, 1996;), in line with experimental results (Kull, Nylander, Tiberg, & Wahlgren, 1997; Lee, Park, Chung, & Kim, 2004; Velev, Campbell, & Borwankar, 1998). However, for hydrophilic surfaces, such as negatively charged silica, a few available studies show that adsorption can be both strengthened (Kull et al., 1997) and weakened (Lundin, Elofsson, Blomberg, & Rutland, 2010) by increasing ionic strength. Despite the contradiction, the influence of ionic strength points out towards the importance of electrostatic interactions for β-casein adsorption. In fact, a recently published study by our group (Svensson, Kurut, & Skepö, 2014), clearly shows that electrostatic interactions are, indeed, important for the adsorption of β-casein to silica surfaces, having direct influence on the adsorbed amount and saturation of the surface. It was then suggested that this is a result of a delicate counterbalance between: (i) electrostatic repulsion between the surface and the protein; (ii) electrostatic attraction between positively charged amino acids in the protein and the negatively charged surface; and (iii) electrostatic repulsion and excluded volume effects between adsorbed proteins at the surface. The hypothesis is that positively charged amino acids serve as anchoring points to the surface. Another interesting finding of the aforementioned study was that the addition of urea greatly affects β-casein adsorption, with the addition of 1 M urea causing a decrease in the adsorbed amount by as much as 40%. This was interpreted as a sign that non-electrostatic interactions between the protein and the surface, such as hydrogen bonding, dispersion forces and hydrophobic effects (eg. hydrophobic dehydration), should not be overlooked and may actually be as important as electrostatics.

In sum, from what was mentioned above, it is legitimate to assume that electrostatic interactions are of great importance, despite the existence of diversity within experimental results, whose discrepancies may be due to several factors such as impurities in the samples, by both proteins and small ions, such as calcium, or other monovalent/divalent salts. Another possibility is the intactness and degree of phosphorylation of β-casein.

Thus, the aim of the work presented here is to develop a general coarse-grained model applicable to a wide range of intrinsically disordered proteins. For this purpose we have further developed our computational model and method for the simulation of IDPs (Evers, Andersson, Lund, & Skepö, 2012; Kurut, Henriques, Forsman, Skepö, & Lund, 2014; Skepö, 2008; Skepö, Linse, & Arnebrant, 2006), which now includes titrating sites for phosphorylated serines and a refined Lennard-Jones-based short-ranged attractive protein-surface potential, tuned from our recent experimental results (Svensson et al., 2014).

2. Material and methods

2.1. Model

A coarse-grained model of β-casein was used to study its bulk properties in solution and its interaction with charged surfaces. The protein is represented as a flexible chain of soft spheres connected by harmonic bonds. Salt and counterions are implicitly described, and the solvent is treated as a dielectric continuum. The amino acid radii were determined from their molecular weight as described elsewhere (Evers et al., 2012), assuming a common density of 1.4 g/mL, and the 209 amino acid sequence was obtained from UniProtKB (The UniProt Consortium, 2014) (ID: P02666). The N- and C-terminals are represented as individual spheres, independent from their respective residues. Table 1 comprises all terms contributing to the overall system energy Hamiltonian.

Amino acid residues Ala, Ile, Leu, Met, Phe, Pro, Trp and Val are considered hydrophobic and are thus the only ones considered for the short-ranged attraction summation between residues, ie. intramolecular hydrophobic interactions, see equation (3) in Table 1. The titration scheme employed here depends on the system's electrostatics, pH and the intrinsic acid dissociation constants, $pK_{a,i}$. We used the same pK_a values as in reference (Evers et al., 2012), except for the phosphorylated serine cases (residues 15, 17–19 and 35), where we used a pK_a value of 5.8 (Zachariou, Traverso, Spiccia, & Hearn, 1996). When explicitly specified that we assume saturation of the phosphorylated serines by calcium, it is implied that these residues will be considered net-neutral and will not titrate.

Non-electrostatic protein-surface interactions such as van der Waals (dispersion) forces, hydrogen bonding and hydrophobic (dehydration) effects are compiled in a simple Lennard-Jones potential (equation (6) in Table 1), of interaction strength ε_s. It is pairwise additive and encompasses all protein amino acid residues. Both hydrogen bonding and van der Waals forces are short-ranged and seem well described by the choice of potential, which also holds for hydrophobic dehydration, which is obviously short ranged by nature. Since only van der Waals forces are ubiquitous throughout the protein sequence, our rationale is that hydrogen bonding and hydrophobic dehydration, which are mutually exclusive, may be considered approximately similar in magnitude, therefore yielding a similar ε_s for all amino acids, independently of their nature. Protein-surface electrostatics are modeled according to Gouy-Chapman theory (equation (7) in Table 1). The reader is encouraged to find more about protein adsorption and alternative approaches to ours in (Malmsten, 2003; Norde, 1986; Roth, Neal, & Lenhoff, 1996).

Table 2 comprises all input parameter values used in this work.

2.2. Method

A typical simulation of a single protein (being it in bulk or near a charged surface) is done using the Metropolis Monte Carlo (MC)

Table 1
Individual terms of the system energy Hamiltonian.

Inter-residue interactions		
(1)	Harmonic bonds	$\sum_{ij}^{b} k_b (r_{ij} - r_{eq})^2$
(2)	Pauli repulsion	$\sum_{ij}^{all} 4\varepsilon_{rep} \left(\frac{R_i+R_j}{r_{ij}}\right)^{12}$
(3)	Short-ranged attraction	$\sum_{ij}^{h} \varepsilon_h$ for $r_{ij} \le r_h$
(4)	Debye-Hückel electrostatics	$\sum_{ij}^{el} \frac{z_i z_j k_B T \lambda_B}{r_{ij}} e^{-\kappa r_{ij}}$
(5)	Titration	$\sum_{i}^{tit} k_B T(pH - pK_{a,i}) \ln(10)$
Surface-residue interactions		
(6)	Lennard-Jones	$\sum_{i}^{all} \varepsilon_s \left[\left(\frac{R_i}{r_{s,i}}\right)^{12} - 2\left(\frac{R_i}{r_{s,i}}\right)^{6}\right]$
(7)	Gouy-Chapman electrostatics	$\sum_{i}^{el} 2 z_i k_B T \cdot \ln\left\{\frac{1+\Gamma_0 e^{-\kappa r_{s,i}}}{1-\Gamma_0 e^{-\kappa r_{s,i}}}\right\}$, with $\Gamma_0 = \tan h\left[\frac{1}{2}\sin h^{-1}\left(\rho\sqrt{\frac{\pi\lambda_B}{2I}}\right)\right]$