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Regular Article

# Adsorption of polyelectrolyte-like proteins to silica surfaces and the impact of pH on the response to ionic strength. A Monte Carlo simulation and ellipsometry study

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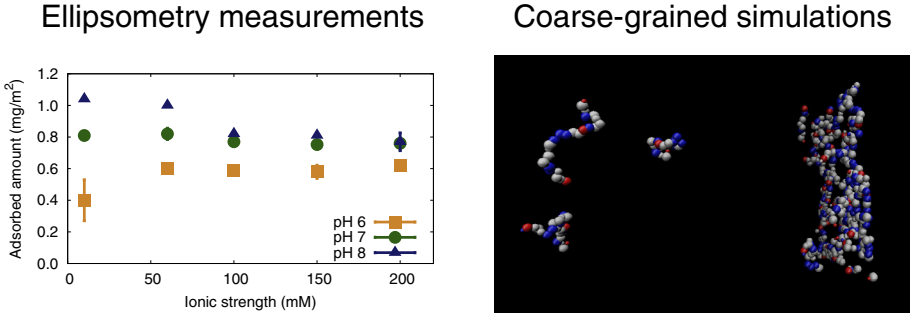
## GRAPHICAL ABSTRACT

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## ABSTRACT

*Hypothesis:* The adsorbed amount of the polyelectrolyte-like protein histatin 5 on a silica surface depends on the pH and the ionic strength of the solution. Interestingly, an increase in ionic strength affects the adsorbed amount differently depending on the pH of the solution, as shown by ellipsometry measurements (Hyltegren, 2016). We have tested the hypothesis that the same (qualitative) trends can be found also from a coarse-grained model that takes all charge–charge interactions into account within the frameworks of Gouy–Chapman and Debye–Hückel theories.

*Experiments:* Using the same coarse-grained model as in our previous Monte Carlo study of single protein adsorption (Hyltegren, 2016), simulations of systems with many histatin 5 molecules were performed and then compared with ellipsometry measurements. The strength of the short-ranged attractive interaction between the protein and the surface was varied.

*Findings:* The coarse-grained model does not qualitatively reproduce the pH-dependence of the experimentally observed trends in adsorbed amount as a function of ionic strength. However, the simulations cast light on the balance between electrostatic *attraction* between protein and surface and electrostatic *repulsion* between adsorbed proteins, the deficiencies of the Langmuir isotherm, and the implications of protein charge regulation in concentrated systems.



## 1. Introduction

Histatin 5 is a small protein (24 amino acid residues long) present in human saliva [2]. It does not have a well-defined

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three-dimensional structure under physiological conditions [3,4] and is thus considered to be an intrinsically disordered protein [5].

At neutral pH, histatin 5 has eight positive charges and three negative charges, which are rather evenly distributed over the protein [1]. This, in combination with the lack of a substantial amount of hydrophobic amino acid residues (histatin 5 only contains one phenylalanine and one alanine), makes histatin 5 resemble a polyelectrolyte [6].

Histatin 5 has attracted significant interest, mainly because of its capacity to kill the fungus *Candida albicans* [2,3,7–12] and the possibility of using it as the active substance in new peptide-based antifungal drugs [13,14].

Interestingly, histatin 5 is still effective against *Candida albicans* when adsorbed to hard surfaces present in the mouth [15]. It has been suggested that developing antimicrobial peptides that selectively stick to hydroxyapatite (the main material of the tooth enamel) could be a way to increase their therapeutic activity by reducing the proteolytic degradation that is otherwise a problem [16]. The rationale behind this idea is the observation that histatin 1 is able to resist proteolytic degradation by binding to hydroxyapatite [17], and the same is expected for histatin 5.

The present study is focused on investigating the pH- and ionic strength-dependence of the adsorption of histatin 5. Hopefully, a fundamental understanding of the adsorption of histatin 5 will contribute both to the understanding of polyelectrolyte adsorption in general and to the development of new antifungal peptides.

Earlier studies of adsorption of polyelectrolytes show no consensus regarding how the adsorption changes upon the addition of salt [18–26]. One reason could be that in many systems that have been studied, the charge density of the polyelectrolyte and/or the surface is too low for interactions between charged groups of the polyelectrolyte and the surface to be the dominant factor behind adsorption [27].

Our previous combined ellipsometry and Monte Carlo simulation study indicates that this is the case for the adsorption of histatin 5 to hydrophilic silica surfaces [1]. Therefore, a short-ranged potential was introduced between the surface and the amino acids in the simulations. It represents interactions such as van der Waals forces, hydrophobic interactions and hydrogen bonding, and corrects for possible overestimation of the entropy of the free histatin 5 chain.

We have observed experimentally (using ellipsometry) that the adsorbed amount of histatin 5 is generally dependent on ionic strength – but the trend is different depending on pH [1]. Our hypothesis was that this could be explained by the differences in the charges of the protein and the surface depending on pH (see Fig. 1 for an illustration). The argument was as follows:

When pH is lowered, the positive charge of the protein increases while the magnitude of the negative surface charge decreases. This would lead to an increase in the electrostatic repulsion between adsorbed protein molecules and probably to a decrease in electrostatic attraction between the protein molecules and the surface. When salt is added to the system, the primary effect would be the screening of the repulsion between the adsorbed proteins, inducing a higher adsorbed amount. At high pH, the protein is only weakly positively charged while the surface has a high negative charge. The addition of salt will then mainly screen the attraction between the proteins and the surface, and thus, the adsorbed amount will decrease.

In previous coarse-grained Monte Carlo simulation studies of the adsorption of histatin 5, only a single protein molecule was included [1,28]. In this infinitely diluted system, adsorption is always disfavoured when the ionic strength is increased. In the present study, we tested the hypothesis that by including several histatin 5 molecules (taking multi-protein effects into account) Monte Carlo simulations using the same coarse-grained model would give the trends observed experimentally.

## 2. Materials and methods

### 2.1. Simulations

Coarse-grained Monte Carlo simulations were performed using Faunus, a C++ framework for Metropolis Monte Carlo simulations [29].

#### 2.1.1. Model

In this coarse-grained model, each amino acid and the N-and C-terminals of the protein are represented as spheres (beads). Table 1 shows all the terms contributing to the system energy Hamiltonian, and the parameters of these terms are given and explained in Table 2. All interactions were assumed to be pairwise additive. The model has been described in detail elsewhere [1].

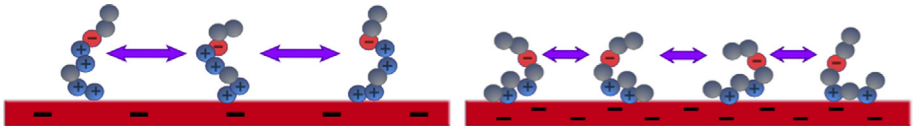
The protein–protein interactions were limited to those of the minimum image convention.

#### 2.1.2. Method

The protein molecules were simulated using the Metropolis Monte Carlo scheme [31] in the canonical (*NVT*) ensemble. The simulation box had periodic boundaries in the *xy*-directions and hard boundaries in the *z*-direction (normal to the surface). The side lengths were 150 Å in the *xy*-directions and 300 Å in the *z*-direction. A length of 300 Å corresponds to three times the contour length of histatin 5 and twenty times the radius of gyration [6]. An increase in the *xy* side lengths does not change the simulation results when the total concentration of histatin 5 in the box is kept constant.

Most simulations were performed with 20 protein molecules in the box ($N_{His} = 20$). When needed, more molecules were included. The constant number of histatin 5 molecules meant that the bulk concentration at equilibrium varied depending on the number of adsorbed molecules. This is not expected to affect the qualitative comparison with experimental results. The only effect will be that the observed changes in adsorbed amounts with increased ionic strength will be less pronounced than in a system with constant concentration.

When choosing a constant $N_{His}$ it is important that the number is high enough to obtain a bulk concentration, while still low enough not to give rise to unrealistic adsorption due to repulsion between the molecules in the bulk. The latter could lead to significant adsorption also to the surface constituting the uncharged boundary of the box in the *z*-direction. $N_{His} = 20$ was found to be



(a) Low pH (b) High pH

**Fig. 1.** A schematic illustration of how the protein net charge and the surface charge density depend on pH. At high pH, the adsorbed amount is higher than at low pH.