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Adsorption of the intrinsically disordered saliva protein histatin 5 to silica surfaces. A Monte Carlo simulation and ellipsometry study

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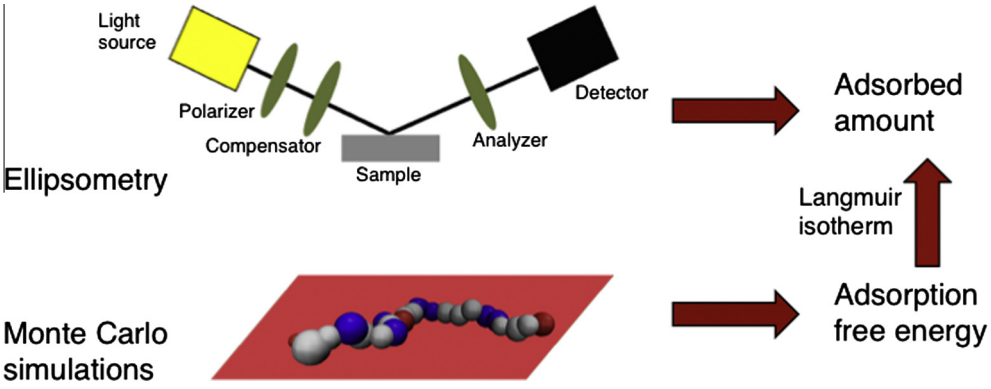
GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: The adsorption of histatin 5 to hydrophilic silica surfaces is governed by electrostatic attractive forces between the positive protein and the negative surface. Hence pH and ionic strength control the adsorbed amount, which can be described by coarse-grained Monte Carlo simulations accounting for electrostatic forces and charge regulation of the protein.
Experiments: The amount of histatin 5 adsorbed to hydrophilic silica surfaces at different pH and ionic strengths was measured using null ellipsometry. The results were compared with coarse-grained Monte Carlo simulations of a single histatin 5 molecule and a surface with a fixed, smeared charge set according to experimental values for silica. The Langmuir isotherm was used to calculate the surface coverage from the simulation results. The effect of charge regulation of the protein was investigated.
Findings: Even though electrostatic attractive forces are important for the investigated system, a non-electrostatic short-ranged attraction with a strength of about 2.9 k_BT per amino acid was needed in the simulations to get surface coverages close to experimental values. The importance of electrostatics increases with increasing pH. Charge regulation of the protein affected the results from the simulations only at high surface charge and low ionic strength.

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1. Introduction

Histatin 5 is a short, basic protein containing 24 amino acid residues, of which seven are histidines (sequence: Asp-Ser-His-Al a-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-S er-His-Arg-Gly-Tyr [1]). It is present in human saliva and belongs to the family of intrinsically disordered proteins [2], as shown by CD spectroscopy [3] and NMR [4]. It has both antifungal [5] and antibacterial [6] properties, primarily against the fungus *Candida albicans*. Histatin 5 adsorbs to the cell wall/membrane of *Candida albicans* and enters and kills the cells. The mechanism by which the protein is transported into the cell may be a combination of transporter-mediated uptake across the cell wall, direct transfer across the membrane, and endocytosis [7]. Direct transfer across a liposome membrane has been observed [8]. The anticandidal activity has received much attention [1,3,7,9–12] and it has been shown that a conjugate of histatin 5 with spermidine is a potential drug against oral candidiasis [13]. Histatin 5 is also a constituent of the protective film called the enamel pellicle and offers some defence against acid-induced degradation of enamel [14].

Adsorption to negatively charged surfaces is crucial for the function of histatin 5 both as a part of the pellicle and as an antimicrobial agent. We have investigated the surface adsorption of histatin 5 to hydrophilic silica surfaces using ellipsometry and coarse-grained Monte Carlo simulations with the aim to answer three questions. The questions and the motivations behind them follow here:

Question 1: How does the adsorbed amount of histatin 5 depend on pH and ionic strength?

Motivation: In human whole saliva, the pH varies between approximately 5.7 and 7.8 [15], while the ionic strength of saliva from the parotid glands varies between 30 and 100 mM [16]. This natural variation makes it interesting to study how the adsorption of histatin 5 is affected by pH and ionic strength. Furthermore, the killing activity of histatin 5 has been shown to be dependent on these conditions. The ability of histatin 5 to kill *Candida albicans* decreases with increasing ionic strength [9,12], and while Xu et al. found no significant pH-dependence [9], Kacprzyk et al. found that histatin 5 killed *Candida albicans* more effectively at pH 7.4 than at pH 5.5 [17].

Question 2: Can we describe the adsorption of histatin 5 with a coarse-grained model with a single protein molecule?

Motivation: Coarse-grained models provide a way of investigating properties of large molecules at a reduced computational cost. The surface adsorption of histatin 5 has been studied previously using coarse-grained Monte Carlo simulations, where one coarse-grained histatin 5 molecule was adsorbed to a flat surface with a smeared charge [18]. The results were compared with atomistic simulations and density functional theory calculations. We want to extend previous work on the coarse-grained model by performing a corresponding experimental study to verify the simulation parameters and gain further insight into the adsorption mechanism by comparing experiments and simulations. By using the Langmuir adsorption isotherm to convert the adsorption free energies found from simulations into surface coverages, the results from simulations and experiments can be directly compared.

Question 3: Is charge regulation important for the adsorption of histatin 5 in a physiologically relevant system?

Motivation: The high content of histidines, which have a pK_a of ~6, makes it possible for histatin 5 to regulate its charge depending on the environment at physiological pH. The positive net charge of histatin 5 increases when the protein approaches a negatively charged surface. Earlier coarse-grained Monte Carlo simulations have shown that this mechanism increases the surface adsorption under some conditions [18]. In order to gain further knowledge on the importance of charge regulation, we use simulations to investigate whether this is the case also for the conditions studied here.

2. Materials and methods

2.1. Experimental

2.1.1. Materials

Chemicals. Synthetic histatin 5 was bought from American Peptide Company, Inc., U.S.A. (lots no V11131T1, 1312047T, and 1303040T). The peptide content was 59.7% and the purity of the peptide 95.8%. In order to remove excess salt and other impurities, the protein was purified using size exclusion chromatography, according to the procedure described in the Methods section below.

All buffer solutions were prepared using MilliQ water. Buffers containing 10 mM tris (Saveen Werner AB, Sweden, lot no. 2Z007904) were used at pH 7, 8 and 9. The pH was adjusted using 1 M hydrochloric acid. When a higher ionic strength was needed, sodium chloride (Scharlau, Spain, Prod. no. SO02271000) was added to the buffer. For the measurements at pH 6, a 10 mM bis-tris (Merck Millipore, U.S.A., lot no. XA27K) buffer was prepared in the same way. At pH 4 and 5, a 10 mM buffer made from acetic acid (Scharlau, Spain, batch 34335) and sodium acetate (Sigma–Aldrich, U.S.A., lot no. BCBH6230V) was used.

Substrates. Silicon wafers which were oxidized to give an approximately 300 Å thick silica (silicon dioxide) layer were purchased from Semiconductor Wafer, Inc., Taiwan. The wafers were cut into pieces and cleaned in an alkaline solution with hydrogen peroxide, followed by cleaning in an acidic solution with hydrogen peroxide, according to the procedure described by Landgren and Jönsson [20]. This procedure has been shown to effectively remove organic contaminants and metal ions [21]. The slides were then stored in ethanol (Solveco AB, Sweden). Before the measurements, the surfaces were rinsed in three steps with water, ethanol and water, dried with nitrogen and put in a plasma cleaner (model PDC-3XG, Harrick, U.S.A.) for 5 min at a pressure of approximately 0.06 mbar. The plasma cleaning was done to remove any remaining organic contaminants.

Approximate surface charge densities of silica under different conditions can be found in Table 1. Note that the surface charge varies substantially with pH and ionic strength.

2.1.2. Methods

Size exclusion chromatography. Surface chemistry experiments are very sensitive to surface active impurities, and to ensure the purity of the histatin 5, size exclusion chromatography was used. The separation range of the column (Superdex 75 10/300 GL, GE Healthcare, Sweden) was 3–70 kDa. The column was filled with a 10 mM tris buffer with 140 mM NaCl at pH 7. NaCl was included in the buffer to reduce undesired electrostatic interactions between the column material and the protein.

Table 1
Approximate surface charge densities of silica particles at different pH-values and ionic strengths (I) adjusted with KCl, taken from Samoshina et al. [19].

pH	Approximate surface charge density (μC/cm^2)	
	I = 10 mM	I = 100 mM
6	−0.25	−0.50
7	−1.00	−2.00
8	−2.75	−5.75
9	−7.00	−12.50

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