Variation of Surface Charge along the Surface of Wool Fibers Assessed by High-Resolution Force Spectroscopy

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ABSTRACT

In this study, we have mapped the surface charge of wool fibers using chemically specific high-resolution force spectroscopy in order to better understand the dispersion of amino acids in relation to fiber morphology. The inter-surface forces between standard atomic force microscopy (AFM) probe tips (tip radius ~ 50 nm) functionalized with COOH and NH_3 terminated alkanethiol self assembling monolayers and the wool surface were used to estimate the surface charge per unit area using linear Poisson-Boltzmann-based electrostatic double layer theory. The positional measurement of nano-scale surface charge showed a correlation between the surface charge and fiber morphology, indicated that basic amino acids are located near the scale edges.

INTRODUCTION

Wool fibers used in textiles typically undergo processing to increase resistance to shrinkage [1]. These fibers have a scale-like cuticle on the cortex along the fiber length (*Figure 1*). These scales cause the ratcheting effect leading to "felting shrinkage" observed subsequent to washing of woolen fabrics [2]. To reduce the felting shrinkage, manufacturers can use a chlorination finishing procedure that involves the application of organic halides to remove the wool scales and subsequently increase the shrink-resistance of fabrics made from these fibers [3,4]. This treatment, however, results in high levels of absorbable organic halides discharged in wastewater, an environmental concern. Both market pressure and ecological legislation have hastened the need for alternative methods for mitigating this shrinkage such as enzyme processing [5]. The enzymes break down the peptide chains within the wool scale; however, they are also capable of attacking the protein throughout the fiber.

Results from several studies on the application of enzymes for fiber processing have shown that enzyme treatments can be used to modify wool fibers by breaking down the fiber protein and thus have potential as anti-felting agents and/or scouring agents [2, 6]. Unfortunately, the mechanical properties of enzyme-treated fibers tended to be reduced, because the selected enzymes attacked proteins along the wool fiber surface uniformly. In addition, the enzyme treatments decreased the fiber diameter. A potential method for increasing the effectiveness of these enzyme treatments would be to determine the distribution along the fiber surface of the seventeen amino acids comprising wool fibers.

Although the amino acid components of wool fibers have been established by analysis of protein assays, x-ray photoelectron spectroscopy, as well as Fourier transform Raman spectroscopy [7-10], these results cannot be used for surface studies. Previous surface characterization of the zeta potential of wool fibers using the streaming potential method presented the potentials at a range of pH values. At a pH of 6, similar to the pH at which the experiments in this paper were carried out, the zeta potential for the wool fibers was -24 mV [11]. This indicates a slightly negative charge over the entire surface of the wool. The functional groups of several of the amino acids that make up proteins are charged, including negatively charged aspartic and glutamic acids and positively charged histidine, lysine, and arginine. The results of this study will narrow the distribution possibility by using chemically specific high-resolution microscopy to map the charges along the wool surface [11-18] at a high resolution. High-resolution force microscopy as used in this work differentiated between types of amino acids with nanometer resolution based on charge interactions on the surface of wool fibers.

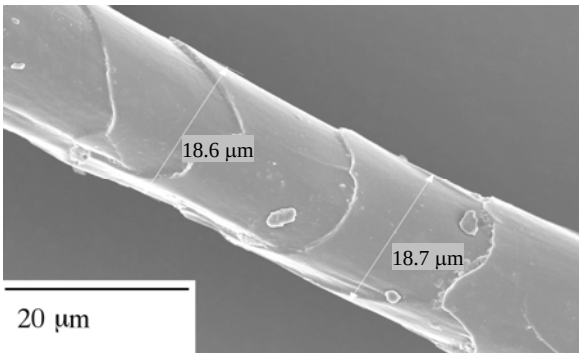

FIGURE 1: Scales along a merino wool fiber were shown to occur at relatively regular intervals in this SEM image. The scale ridges are approximately 500 nm in height and each scale is spaced around 20 μm apart.

EXPERIMENTAL METHODS

Tip Preparation and Force-Distance Experiments

High resolution force spectroscopy experiments were performed using Au-coated Si_3N_4 cantilevers (Veeco Probes, nominal spring constant, k ~0.12 N/m, and tip radius, R_{tip} ~50 nm) that were chemically functionalized with a self assembled monolayer (SAM) of 10mM 11-mercaptoundecanoic acid or 11-amino-1-undecanethiol to create negatively charged carboxy-terminated and positively charged amine-terminated probes. The probes were then rinsed with and stored in ethanol until use. Tips were rinsed with 0.15M Phosphate Buffered Saline (PBS) just prior to use.

Merino wool fibers were scoured according to standard industrial procedures and mounted onto atomic force microscope (AFM) pucks with cyanoacrylate epoxy. The samples were then placed in 0.0015M phosphate buffer solution with a fluid cell of a Dimension 3100 Veeco AFM. A total of ten fibers were prepared, with each experiment performed on a single scale on each of the ten fibers. Contact-mode imaging scans over an area of 20 μm × 5 μm with the long axis of the scan along the fiber axis at constant displacement velocity of 1 μm/sec were used to image the fiber morphology. Using the "Point and Shoot" feature of Veeco Nanoscope controller software, normal force versus tip-sample separation distance was measured every 250 nm in an evenly spaced 5 μm x 5 μm grid centered over the scale edge. Three individual approach curves were taken at each grid location to be able to obtain a good average value for the charge at that location. This gave 1,200 force-distance curves per scale for each of the 10 scales analyzed. The spring constant for each cantilever was measured using the thermal tune feature of the Veeco Nanoscope software. In addition, the probe end-radius for each tip was verified for the AFM tips using scanning electron microscopy (SEM) and was found to be ~50 nm for both tips used in this study.

Estimation Of Surface Charge Density From Force-Distance Curves

The electrostatic interactions across each scale were calculated from the individual force-distance curves (*Figure 2*) using the linearized Poisson-Boltzmann equation with constant charge boundary conditions [11, 13]. This application of the linearized Poisson-Boltzmann equation does stretch the assumptions of the linearized model due to the low ionic strengths and small size of the tip [14]. However, since the goal of this study was to understand the trends in the distribution of charge over the surface of the fibers rather than the exact magnitude, the linearized PB equation was used to minimize computation time on the 12,000 individual force curves analyzed. Using the linearized Poisson-Boltzmann theory, the electrostatic force, F, calculated between the hemispherical tip and the wool surface has the form [19]:

$$F = \frac{4\pi R_{tip}\sigma_{tip}\sigma_{wool}}{\varepsilon_w \kappa} e^{-\kappa D} \quad (1)$$

where σ_{wool} is the charge density on the wool, σ_{tip} is the charge density of the tip, ε_w is the permittivity of the fluid (6.923×10^{-10} C^2/Nm^2), κ^{-1} is the Debye length, and D is the tip-sample separation distance. From previous experiments on self-assembled monolayer coated control substrates, the surface charge density of the carboxyl-terminated and amino-terminated tips were estimated to be -0.01 C/m^2 and +0.02 C/m^2, respectively [13]. The Debye length at 0.0015M NaCl is ~8 nm; therefore, force-distance curves were fit for distances between 8 and 40 nm to minimize any short range non-electrostatic components of the interaction (including van der Waals forces) [13].

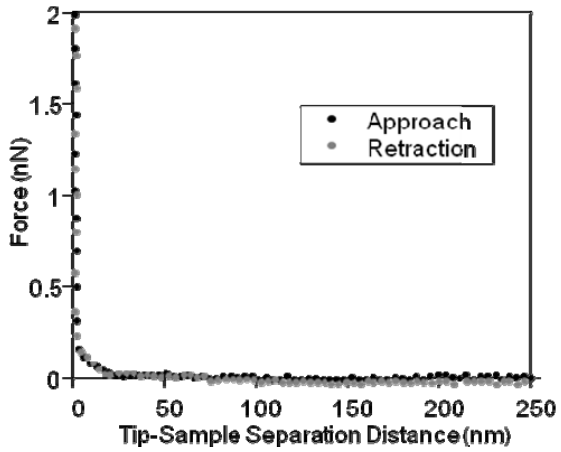

FIGURE 2: Sample force-distance curve showing normal force between the NH^+ terminated SAM probe tip and wool fiber surface in a 0.0015M phosphate buffer solution as a function of separation distance between the probe tip and fiber.

Labeling Of Carboxyl And Amine Groups With Gold Nanoparticles

To further corroborate the AFM results, the carboxyl or amine groups on the wool fibers were labeled using gold nanoparticles. Gold nanoparticles (Sigma, 20 nm diameter) were functionalized with carboxyl groups using 10mM 11-mercaptoundecanoic acid or amino groups using 11-amino-1-undecanethiol. These particles were then treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and N-hydroxysulfosuccinimide and allowed to incubate on cleaned wool fibers for 2 hours at 37ºC. Carboxyl-terminated particles were then bound to the primary amine groups on the wool surface while, in separate samples, amine-terminated particles were bound to the carboxyl groups on the wool surface. These labeled fibers were then imaged by backscatter scanning electron microscopy (SEM).

Treated wool fibers were dried, sputter coated with a thin coating of platinum (approximately 4 Å), and placed in a Hitachi S4800 Ultra High Resolution Field Emission SEM for backscatter electron (BSE) imaging. Since gold has a high atomic number compared to the background wool composition, the gold nanoparticles are easy to identify and appear much brighter than their surroundings when viewed with the backscatter detector.

RESULTS AND DISCUSSION

Topography of fiber scales was measured by contact mode AFM and showed that wool scales had curved ridges. The scales were fairly consistent in their morphology and were found to be 565 +/- 124 nm in height and about 20 μm long, that is, the distance from one scale ridge to the next.

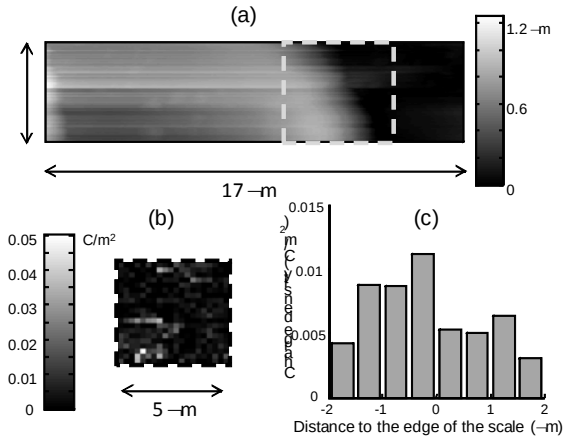

FIGURE 3: a) A contact mode AFM height image of a merino wool fiber scale. The overlaid square shows the specific location probed with high-resolution force spectroscopy (HRFS). b) The corresponding charge map calculated from the HRFS data measured with the positively charged probe tip. c) Averaged surface charge density as a function of distance to the edge of the scale calculated from the charge map. The distance from the scale edge was calculated so that the mid-point of the scale ridge was taken as 0. Positive charge areas are located closer to the edge of the scale.

The surface charge of the wool fiber calculated using the NH_3 functionalized tip and measuring the repulsion (*Figure 3*). The data were compiled to show the placement of positive-charged moieties on the fiber surface as a function of the fiber topography (*Figure 3 a & b*). Within 3 μm of the scale edge, the average surface charge density due to the amine groups was estimated to be 0.0055 +/- 0.0012 C/m^2. Although there is variation in the measured surface charge density, clear trends were observed. As shown in *Figure 3* for a sample scale, there were more positive charge groups within 0.5 μm of the edge of the scale than further away (*Figure 3c*).

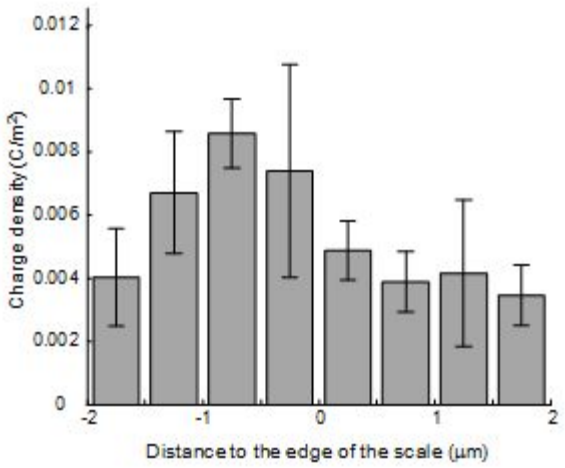

FIGURE 4: Average of surface charge per unit area from the HRFS data on five different scales using NH_3 SAM. The distance from the scale edge is normalized so that 0 is the middle of scale ridge.

This is shown as a trend in the averaged data over all scales (*Figure 4*); although the standard deviation is large due to variance between fibers, the average charge due to positive groups at distances greater than 2 μm from the edge of the scale was significantly lower than that within 1 μm from the edge ($p = 0.019$, Paired t-test). Given the known amino acid distribution of wool fibers as a whole, these surface measurements indicate that there is a higher concentration of either Lysine or Arginine near the scale edge. This non-uniform distribution of primary amine groups (Lysine) near the scale edge was further confirmed by observation of the SEM images of wool fibers treated with functionalized nanoparticles. As shown in *Figure 5*, the backscatter EM revealed that, although some nanoparticles were attached in the middle of the scales, the nanoparticles were predominantly found near scale edges.

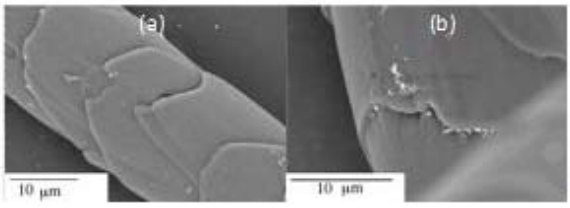

FIGURE 5 a) Backscatter SEM images of wool fibers with gold nanoparticles bound to Lysine residues. b) Particles were found near scale edges.

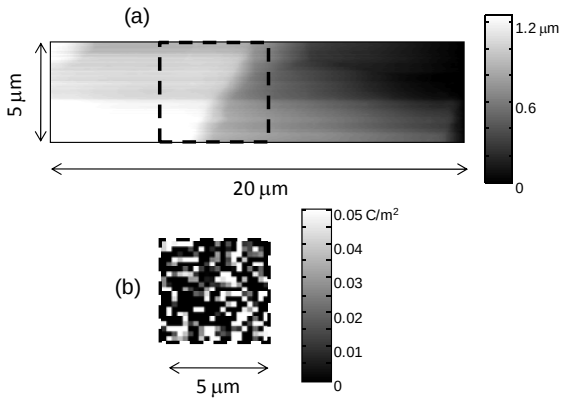

FIGURE 6: a) A contact mode AFM height image of a merino wool fiber scale. The overlayed square shows the specific location probed with high resolution force spectroscopy (HRFS). b) The absolute value of the corresponding charge map calculated from the HRFS data measured with the negatively charged probe tip. This shows a random uniform distribution of negative charged areas over the surface of the scale edge.

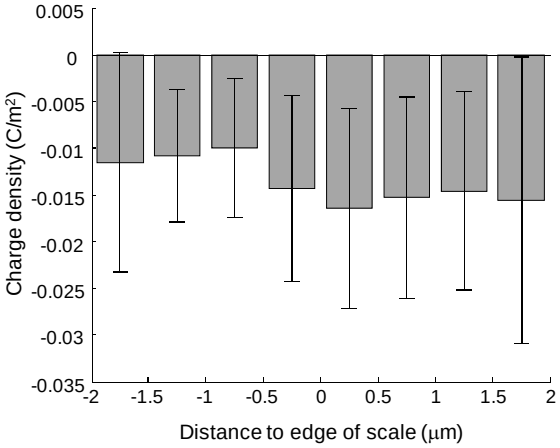

FIGURE 7: Approximation of the magnitude of the surface charge per unit area from the HRFS data across five scales using COOH SAM. The distance from the scale edge is normalized so that 0 is the middle of the scale ridge. This shows an even distribution of acidic amino acid groups across the wool fiber surface.

The estimated negative surface charge measured from the COOH functionalized tip force curves (*Figure 6*) indicates where there are negative charge moieties distributed somewhat evenly on the fiber surface. The force-distance curves also show that these negative charge moieties are not located preferentially along the scale ridge (*Figure 7*). Considering the amino acid composition of wool, these results would indicate an evenly spaced distribution of Aspartic or Glutamic acids along the wool surface. In addition, the average surface charge density due to negative charged groups was -0.011 +/- 0.0059 C/m^2. This uniform distribution of acidic carboxyl groups was further confirmed by the

backscatter EM images of the gold labeled wool. As shown in *Figure 8*, the gold particles functionalized to attach to the carboxyl groups were uniformly distributed over the wool fibers. In addition, the density of particles is higher in these images (*Figure 8*) than in the images of the particles bound to amine residues (*Figure 5*). This further confirms that the net surface charge is negative.

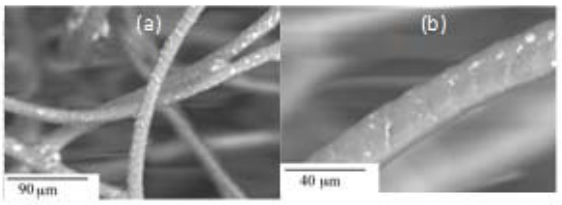

FIGURE 8: a) Backscatter SEM images of wool fibers with gold nanoparticles bound to Glutamic Acid and Aspartic Acid residues.

The average net surface charge density over the surface of a scale was found to be about -0.006 C/m^2. Zeta potential, ζ, can be estimated from a charge density using the linearized Poisson-Boltzmann formation. Briefly, ζ is the potential at the slip plane, which is approximately the potential one Debye length, κ^{-1}, away from the surface. Thus, since the potential decays exponentially away from the surface in the linear Poisson-Boltzmann formulation, the relationship between the surface charge density and the zeta potential is as follows:

$$\sigma_{wool} \approx \varepsilon_w \kappa \phi_{surface} \approx \frac{\varepsilon_w \kappa \zeta}{e} \qquad (2)$$

where $\phi_{surface}$ is the potential at surface calculated using the linear Poisson-Boltzmann equation. Using the above equation, a net average charge density over the surface of the wool fiber, -0.0055 C/m^2, will translate to a measured zeta potential of -23 mV, which is consistent with the previously published results on zeta potentials of -24 mV [11].

CONCLUSIONS

This study determined charge density by means of AFM, which has previously demonstrated adequate force sensitivity in an electrolytic fluid to permit the measurement of electrostatic interactions between the tip and the sample [13]. AFM was used to identify localization trends in charge affiliated with the scales on the surface of wool so that the position of particular amino acids could be mapped. The fiber surface shows different charge values at selected locations, which presumably correlates with different chemical composition. In addition, the surface is fairly hydrophilic with very little evidence of hydrophobic regions. Mapping of specific charge groups using functionalized tips indicates that although all scales have a net negative surface charge, there is variability and certain scales seem to have more negatively charged groups on their surface than do others. Negative charges seem uniformly distributed over the scale surface. This is a relatively high surface charge density on the same order as some self assembled monolayers [20]. This result likely correlates to the distribution of acidic amino acid residues. Given the known amino acid composition of wool, these AA residues can be only Aspartic acid or Glutamic acid. From the surface charge density measurements, the approximate density of acidic residues was estimated to be about 1 group per 15 nm^2 over the surface of the wool fiber. However, as observed in both the AFM and SEM data, the surface of the wool is not homogeneous in the density of acidic groups. Although there was no prevalence of acidic groups at the edge of the scales or other topographical feature the fibers, the acidic groups were always found in small clusters as seen in the AFM charge maps and in the clustering of nanoparticles in the SEM images. Mapping did reveal the presence of positively charged domains near the scale edges. This result would indicate a localized concentration of basic amino acids. The backscatter SEM results further confirm these results: carboxyl acid groups are uniformly distributed over the surface of wool fibers while amine groups are concentrated near the edge of scales. Overall, the density of groups resulted in a net average negative charge over the surface of the fiber. These results are consistent with the previous measurement of wool zeta potentials, which implied that wool was negatively charged.

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