Role of Cranberry Juice on Molecular-Scale Surface Characteristics and Adhesion Behavior of *Escherichia coli*

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Abstract: Cranberry juice has long been believed to benefit the prevention and treatment of urinary tract infections (UTIs). As the first step in the development of infection, bacterial adhesion is of great research interest, yet few studies have addressed molecular level adhesion in this context. P-fimbriated *Escherichia coli* play a major role in the development of a serious type of UTI, acute pyelonephritis. Experiments were conducted to investigate the molecular-scale effects of cranberry juice on two *E. coli* strains: HB101, which has no fimbriae, and the mutant HB101pDC1 which expresses P-fimbriae. Atomic force microscopy (AFM) was used to investigate both bacterial surface characteristics and adhesion forces between a probe surface (silicon nitride) and the bacteria, providing a direct evaluation of bacterial adhesion and interaction forces. Cranberry juice affected bacterial surface polymer and adhesion behavior after a short exposure period ($<$3 h). Cranberry juice affected the P-fimbriated bacteria by decreasing the adhesion forces between the bacterium and tip and by altering the conformation of the surface macromolecules on *E. coli* HB101pDC1. The equilibrium length of polymer (P-fimbriae) on this bacterium decreased from $\sim$148 to $\sim$48 nm upon being exposed to cranberry juice. Highly acidic conditions were not necessary for the prevention of bacterial adhesion, since neutralization of cranberry juice solutions to pH = 7.0 allowed us to observe differences in adhesion between the *E. coli* strains. Our results demonstrate molecular-level changes in the surfaces of P-fimbriated *E. coli* upon exposure to neutralized cranberry juice.

Keywords: bacterial adhesion; urinary tract infection; cranberry; atomic force microscopy

INTRODUCTION

Urinary tract infections (UTIs) refer to the presence of microorganisms in the bladder, prostate, collecting system, or kidney (Johnson, 1991). UTIs are extremely prevalent, especially in females, the elderly, and infants. Approximately eight million people per year experience UTIs in the U.S. (Cohn and Schaeffer, 2004), resulting in annual estimated medical expenditures of $1.6 billion (Foxman, 2002). By age 24, one-third of women will have at least one physician-diagnosed UTI that was treated with prescription medication, and the total cost over 20 years (from 1995) of treating UTIs by antibiotics is estimated to be as high as $25.5 billion (Foxman et al., 2000). Certain groups, especially women, are more prone to repeated infections (Dwyer and O'Reilly, 2002). Recurrences frustrate the patient and may contribute to the development of bacterial antibiotic resistance.

UTIs are usually caused by Gram-negative bacteria, especially *Escherichia coli* (Johnson, 2003). *E. coli* remains the predominant uropathogen (80%) isolated in acute community-acquired uncomplicated infections (Ronald, 2003) and is the most prevalent pathogen associated with UTIs in young children (Sakran et al., 2003). As the first step of developing infections, bacteria must bind to the host cells and tissues, in most cases uroepithelial cells. For uropathogenic *E. coli*, Type 1 fimbriae (Bahrani-Mougeot et al., 2002) and P-fimbriae are proteinaceous macromolecules that facilitate the adhesion of *E. coli* to uroepithelial cells (Gunther et al., 2001; Mulvey, 2002).

Due to continuing concern over antibiotic resistance in numerous types of infections (Wilson and Gaido, 2004), growing research is directed at alternate solutions for infection treatment or prevention. Cranberry (*Vaccinium macrocarpon*) was being used as a medicine by Native Americans before 1620 and has been utilized as a urinary antiseptic for more than 200 years (Gunn, 1878).

Although only limited clinical studies have investigated the effects of cranberry product consumption on the presence of bacteria in the urine (bacteriuria) and/or the development of UTIs, promising results have been obtained in some cases, as reviewed in (Raz et al., 2004). For example, Avorn et al. supplied 300 mL doses (daily for 6 months) of cranberry juice cocktail vs. a placebo drink, in a study of 153 elderly women (Avorn et al., 1994). They found that bacteriuria and pyruria were significantly reduced in patients receiving the cranberry juice cocktail, compared to those receiving a placebo drink. Benefits have been found in clinical studies using cranberry juice cocktail (Avorn et al., 1994), cranberry mixed with water (Haverkorn and Mandigers, 1994), cranberry–lingonberry concentrate (Kontiokari et al., 2001), pure cranberry juice (Papas et al., 1966), and cranberry capsules and tablets (Stothers, 2002; Walker et al., 1997).

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Although cranberry has been observed to promote a healthy urinary tract, a detailed understanding of how cranberry benefits the body is still lacking. Initially, it was believed that the acidity of cranberry (due to benzoic acid that becomes hippuric acid in the urine) imparted the antibacterial activity (Blatherwick and Louisa, 1923). However, more recent experiments have shown that the pH of urine (after cranberry consumption) is only slightly decreased and that the effect is transient (Sobota, 1984; Walsh, 1992). Since the early 1980s, researchers began presenting alternative antibacterial mechanisms for cranberry. A possible treatment strategy is to use agents to prevent or decrease bacterial attachment to epithelial cells, as has been suggested for cranberry (Sobota, 1984). By impairing the adhesion step, the infection cannot develop.

The mechanisms by which cranberry alters the adhesion of *E. coli* are still poorly understood. Since the inhibition of adhesion of bacteria to eucaryotic cells in the presence of cranberry juice was first reported (Sobota, 1984; Zafriri et al., 1989), limited studies have addressed the effect of cranberry juice or its components on adhesion of *E. coli* to eukaryotic cells or other surfaces. Interestingly, Sotoba showed that the urine from mice and humans (after consumption of cranberry or cranberry juice compounds) still contained the materials that could make *E. coli* less adhesive to epithelial cells (Sobota, 1984). This suggests that the active compounds are not destroyed by the digestive system.

Very few in vitro studies have been performed to assess the adhesion of *E. coli* to a non-cell surface in the presence of cranberry. An extract from fresh cranberries decreased the strength of attachment of *E. coli* to glass coverslips when incubated together for 2 h (Allison et al., 2000). Preconditioning of the surface prior to biofilm formation also weakened the strength of attached cells (Allison et al., 2000). The adhesion behavior was only qualitatively observed, as attachment was inferred from counting the number of colony forming units (CFUs) that transferred from the glass slide to a plate with fresh media. The type of fimbriae expressed by this strain of *E. coli* was not discussed.

Many questions remain unanswered with regard to the role of cranberry in mediating the adhesion of *E. coli*. No study has addressed the molecular-level interactions between cranberry and the *E. coli* surface using a nanoscale tool such as atomic force microscopy (AFM). In the present study, AFM experiments and modeling were used to probe the nanoscale interactions between a model surface (silicon nitride) and carefully selected *E. coli* strains. The effect of cranberry on the conformation and adhesion properties of *E. coli* surfaces was quantified as a function of cranberry juice concentration.

MATERIALS AND METHODS

Cultures

E. coli HB101 was obtained from the American Type Culture Collection (ATCC 33694). *E. coli* HB101 is a plasmid-less, non-fimbriated bacterium (Goodacre et al., 1991). *Escherichia coli* mutant HB101pDC1 expresses P-fimbriae only (Connell et al., 1996). The mutant strain was kindly provided by Professor C. Svanborg from the Department of Medical Microbiology, Lund University. Cultures were grown in Tryptic Soy Broth (TSB) at 37°C and harvested in the mid-exponential growth phase. Bacterial cells were centrifuged for 15 min at 190*g* and resuspended in the desired media for the force measurements (described below). This low centrifugal force was chosen because previous results from our laboratory have shown that it is sufficient to pellet *E. coli*, but does so without causing any artifacts in cell surface characteristics and adhesion behavior (Bell and Camesano, 2005), as can be found when high forces are applied to bacteria (Pembrey et al., 1999).

Cranberry Juice

Consumer-grade cranberry juice cocktail (referred to hereafter as ''cranberry juice'') was purchased (Ocean Spray Cranberries, Inc., Lakeville-Middleboro, MA). To exclude the effects of low pH on adhesion, the pH of cranberry juice was adjusted to 7.0 by adding 1 M NaOH solution. Solutions were diluted to 5, 10, and 20 wt.% cranberry juice in ultrapure water (Milli-Q water, Millipore Corp.). Since cranberry juice cocktail contains 27 wt.% cranberry juice, we considered that to be an approximate upper limit to the cranberry concentration that a patient could be expected to consume, and we chose other concentrations below that value.

Bacterial Cell Preparation for AFM

E. coli were immobilized on cleaned glass slides using an EDC (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide Hydrochloride)/NHS (N-hydroxysuccinimide) crosslinking reaction, as described previously (Camesano et al., 2000). Slides were kept hydrated all the time before performing AFM work. When doing AFM experiments, bacterial-coated slides were immersed in ultrapure water, 5, 10, and 20 wt.% cranberry juice solutions. Typically, an AFM experiment lasted for <3 h. During this period, cells remain viable but are not growing. Previous work has shown that the chemicals used to prepare bacteria for the AFM experiment do not affect their viability (Camesano et al., 2000). Further, when the glass slide is placed in fresh bacterial growth media (TSB) after the conclusion of an AFM experiment, bacterial cells are seen to resume growth.

Force Measurements

Individual bacterial cells were probed by AFM (Digital Instruments Dimension 3100 with Nanoscope III controller). The imaging and selection of bacterial cells for force measurement has been described previously (Camesano and Logan, 2000). Measurements were carried out on five individual bacterial cells, 8 times per bacterium per chemical condition studied. Silicon nitride AFM tips were used

(DNPS, Digital Instruments), with an average spring constant of 0.13 ± 0.02 N/m, measured using the method of Cleveland et al. and the correlation equations given in the manufacturer's software. Before using the cantilevers, they were exposed to UV light for 10 min to remove any potential organic contamination.

Force Analysis

A force cycle yields 512 data points for each of the approach and retraction portions of the cycle. The data sets were converted to ASCII format and exported to a spreadsheet. Data were converted from deflection of the cantilever to forces using established procedures (Emerson and Camesano, 2004).

Modeling of AFM Data

A steric model has been developed to quantify the interaction forces between a surface of relatively high coverage of grafted polymers and a bare surface. This steric model also can be applied to the interaction force between a polymer-bearing bacterium and the AFM tip. Adaptation of the model of Alexander (Alexander, 1977) and de Gennes (De Gennes, 1987) to AFM data was performed (Butt et al., 1999), where the steric force, F_{st} is given by

$$F_{St} = 50 k_B T a L_0 \Gamma^{3/2} e^{-2\pi h/L_0} \quad (1)$$

where k_B is the Boltzmann constant, T is temperature, a is tip radius, Γ is polymer density, h is sample–substrate separation distance, and L_0 is the equilibrium polymer length, describing how far the polymers extend into solution.

From the AFM force measurements, we know the interaction forces and the distances between the bacterial cell surfaces and the tip. By fitting the steric model, the grafted polymer density and the equilibrium polymer length can be calculated. For *E. coli* mutant HB101 pDC1, the P-fimbriae are expected to be the largest proteinaceous structure on the cell surface, and so the equilibrium length and density from the steric model should correspond with the length and density of P-fimbriae on the bacteria. For *E. coli* HB101, which does not express fimbriae on its surface, other (smaller) structures such as mannose receptors on the surface (Wang et al., 1998) or lipopolysaccharides from the underlying cell membrane are expected to be demonstrated in the lengths and densities obtained from the steric model.

AFM Retraction Curve Analysis

Statistical analyses were used to analyze the AFM retraction profiles. After the AFM tip touches bacterial surface polymers, these molecules are compressed until they encounter the "compressing limitation." At this point, the AFM tip still can "approach" the bacterial surface further. But this "approach" is due to the deformation of the AFM cantilever instead of the compression of the biomacromolecules, since a very weak cantilever is used. During the process, some biomacromolecules absorb on the AFM tip. When retracting the AFM tip, the absorbed biomacromolecules exert adhesive forces. The AFM tip must surmount the adhesive forces (called pull-off forces or retraction forces). Retraction peaks corresponding to these adhesion events are observed. The retraction peaks can be considered to be independent and random events.

For each bacterium and solution studied, the retraction peaks were combined (without any averaging) and the distributions of pull-off forces and pull-off distances were independently calculated. Due to the natural heterogeneity and variability in the data, one retraction peak is not meaningful. Rather, the statistical distribution of the pull-off forces and pull-off distances for a whole population is used to explain the behavior of the bacterial system under each condition.

As a precaution, our general protocol is to make a force measurement on clean glass, make a measurement on the bacterium, and then return to the clean glass. Comparing the final and initial measurements on the glass allows us to ensure that biomolecules that adsorb to the tip during the retraction part of the force cycle are completely detached by the end of the cycle. Therefore, the tip is clean and a new cycle can begin.

RESULTS

Reproducibility of Force Cycle Data

Repeated force measurements on a given bacterium were reproducible under a single set of conditions. Figure 1A shows illustrative data for the approach curves on one representative cell of *E. coli* HB101pDC1, which show little variability. When the steric model was applied to the approach curve data, and correlation coefficients (R^2 values) were used to evaluate the goodness of fit, all were >0.95 (discussed below in more detail).

The retraction curves (Fig. 1B), show more variability, due to the dynamic nature of the bacterial surface polymers. In a single retraction curve, the tip may contact many biomolecules on the bacterial surface, and individual molecules may even contact the tip in multiple locations. Also, since these molecules are constantly moving and changing their conformations (due to Brownian motion and other intermolecular forces), it is not possible to contact the exact set of molecules in an identical conformation, even when a subsequent measurement is made a few seconds later. Despite the variability, statistical analyses were useful in combining and analyzing the retraction curve data from multiple force cycles. Multiple force cycles refer to repeated instances of the tip approaching and retracting from the bacterial surface.

Surface Molecules on *E. coli* Mutant HB101pDC1 (P-fimbriae)

Data from the 40 AFM force cycles per condition (eight measurements/cell, five cells/condition) were combined and

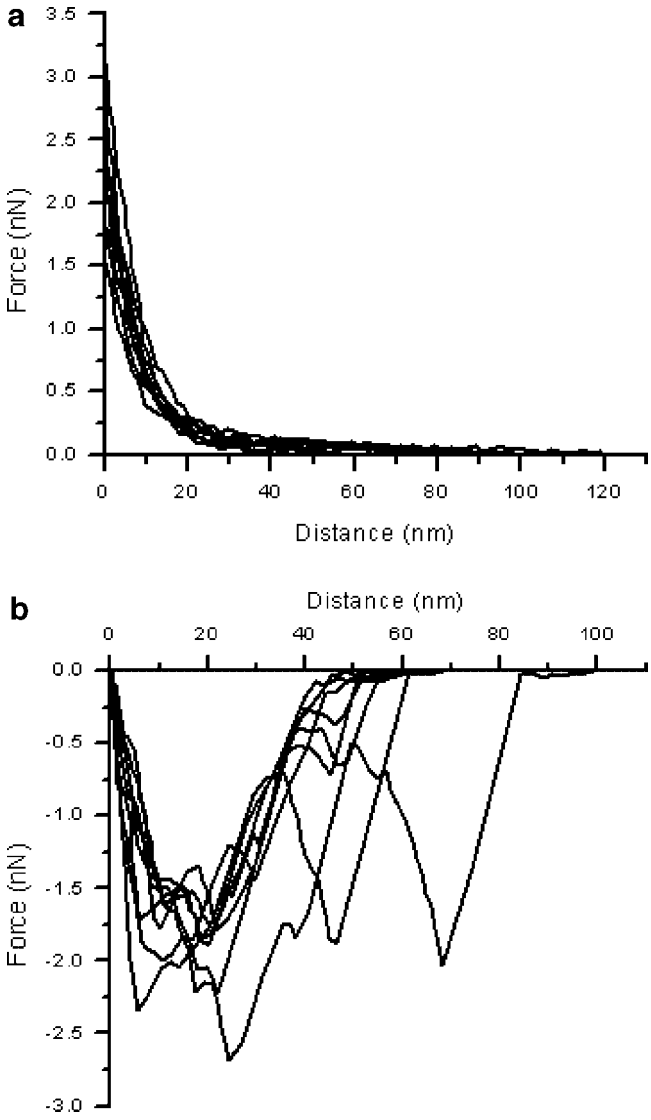

Figure 1. **A**: Representative approach curves on a single bacterium (*E. coli* HB101pDC1) in 5% cranberry juice. The eight measurements shown were performed on a single cell. For each condition, eight measurements were performed per cell, and five cells were examined. **B**: Representative retraction curves on a single bacterium (*E. coli* HB101pDC1) in 5% cranberry juice.

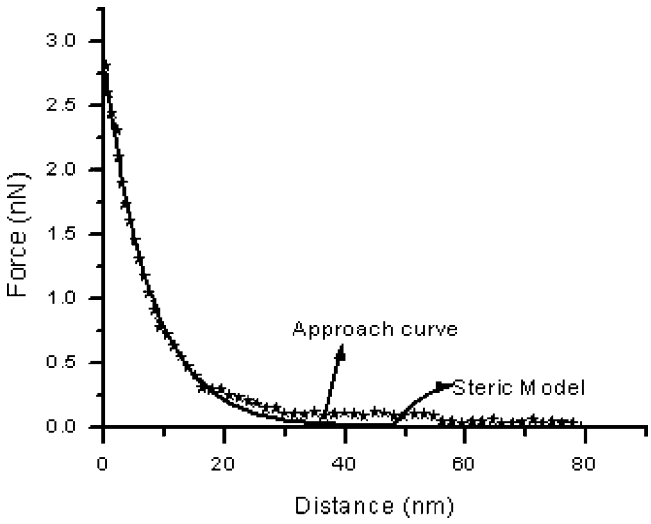

Figure 2. Representative example of a fit to the AFM approach curve data (symbols) with the steric model (solid line). *E. coli* HB101pDC1 in 5% cranberry juice. Based on the steric model fits, the equilibrium length is 48 ± 26 nm and the polymer density is $(8.01 \pm 2.36) \times 10^{15}\ m^{-2}$ ($R^2 = 0.99$).

the steric model was applied. The data for all 40 curves was used to determine average parameters for the equilibrium polymer length and polymer density. The equilibrium length of surface polymers, calculated from the steric model (representative data shown in Fig. 2) decreased from an average of 147 ± 125 nm in the absence of cranberry juice treatment to an average of 53 ± 21, 48 ± 26, and 48 ± 45 nm in 5%, 10%, and 20 wt.% cranberry juice solutions, respectively, when the P-fimbriated bacteria were in a solution cranberry juice.

The deviations reported are not indicative of "errors," but of the spread in the data due to natural heterogeneity and the complexity of these microbial systems. However, due to this scatter, we could not rely on average values alone to explain variations in model parameters. In order to detect differences in polymer behavior among the four chemical solutions and distinguish differences due to the random variation, a statistical test, the one-way repeated ANOVA test, was performed. This test was first applied to the water data as a "control" and to the cranberry juice data as test conditions. Our analyses demonstrated that there is a statistically significant difference between the equilibrium length in ultrapure water and each of the values in cranberry juice. In comparing water versus the 5 wt.% cranberry juice solution, the equilibrium length of the polymers decreased, but the decrease in the equilibrium length appeared to plateau as a function of cranberry juice concentration when higher concentrations were tested (Fig. 3A), since subsequent increases in the cranberry juice concentration did not further decrease the equilibrium polymer lengths. All of the cranberry juice concentrations had similar effects on the equilibrium length, and there were no significant differences among the equilibrium lengths in 5, 10, and 20 wt.% cranberry juice.

Fitting of the steric model to the approach curves also allowed us to calculate the polymer densities. In general, the density increased with a greater concentration of cranberry juice in solution (Fig. 3B) The polymer densities increased from an average of $(9.24 \pm 8.55) \times 10^{15}\ m^{-2}$ in the absence of cranberry juice treatment to an average of $(8.01 \pm 2.32) \times 10^{15}\ m^{-2}$, $(1.63 \pm 1.01) \times 10^{16}\ m^{-2}$, and $(2.16 \pm 1.86) \times 10^{16}\ m^{-2}$ in 5%, 10%, and 20 wt.% cranberry juice solutions, respectively, when the P-fimbriated bacteria were in a solution cranberry juice. Pure water and a cranberry juice concentration of 5 wt.% resulted in essentially identical values for the density. Further, statistical tests on the polymer densities revealed that there was no statistically significant difference between the grafted polymer density in ultrapure water and 5 wt.% cranberry juice, but that the densities in 5%, 10%, and 20 wt.% cranberry juice solutions were different

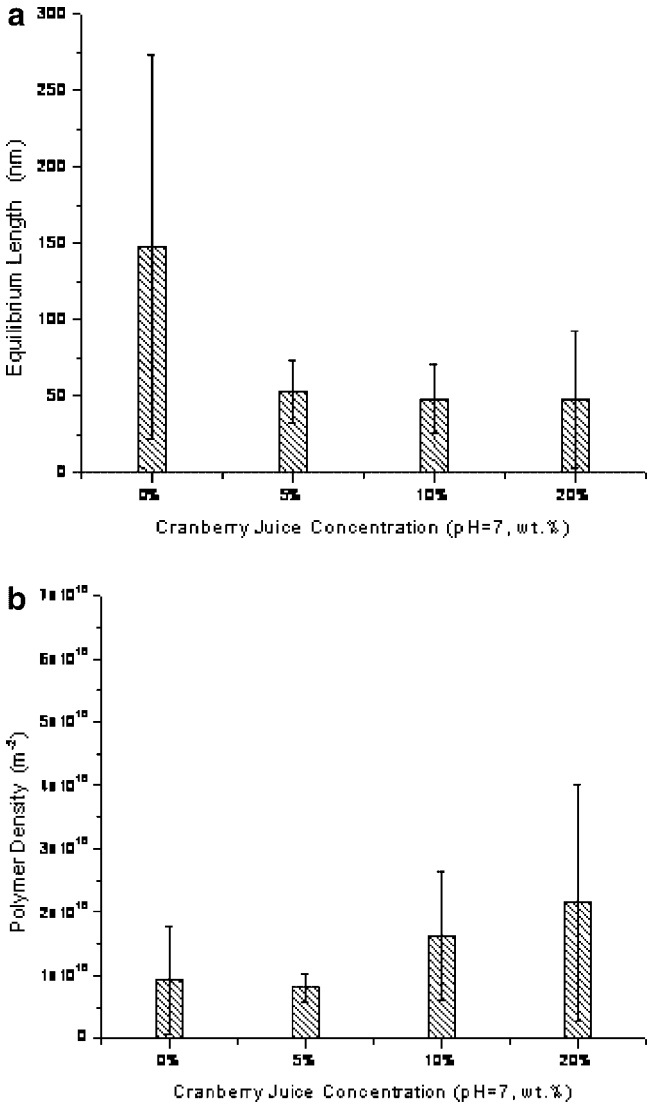

Figure 3. Steric model fits for *E. coli* HB101pDC1, as a function of cranberry juice concentration. $R^2 = 0.9$, 5 cells per concentration, 8 force measurements per cell, i.e. $n = 40$. **A**: The average equilibrium length L_o of *E .coli* HB101pDC1 as a function of cranberry juice concentration. **B**: The average polymer density Γ of *E. coli* HB101pDC1as a function of cranberry juice concentration. Error bars indicate one standard deviation.

from one another. Further, we note that the measurements in cranberry are completely reversible when the solution is then replaced with water.

In this case, the density of the molecules represents an apparent density, and is dependent on the conformation of the molecules. When the fimbriae are in their most extended conformation, as appears in water and 5 wt.% cranberry juice, the apparent density is lower because there is less biopolymer near the cell wall. However, cranberry juice in increasing concentrations changes the conformation of the surface molecules. When higher cranberry juice concentrations were used, the fimbriae appeared to become more compressed near the cell wall. Therefore, the density in that region would be higher.

Surface Macromolecules on *E. coli* HB101 (no Fimbriae)

The equilibrium length calculated from the steric model was not a function of the presence of cranberry juice or of the cranberry juice concentration for *E. coli* HB101. The equilibrium length of surface polymers, calculated from the steric model (representative data shown in Fig. 4A) varied from an average of 32 ± 10 nm in the absence of cranberry juice treatment to an average of 43 ± 27, 22 ± 5, and 30 ± 28 nm in 5%, 10%, and 20% cranberry juice solutions, respectively, In fact, the equilibrium length was nearly constant for all solutions studied (Fig. 4A). ANOVA statistical tests confirmed that with water as the control group, there were no significant differences in the equilibrium polymer lengths for any of the cranberry juice solutions. Likewise, the grafted polymer density of *E. coli* HB101 calculated from the

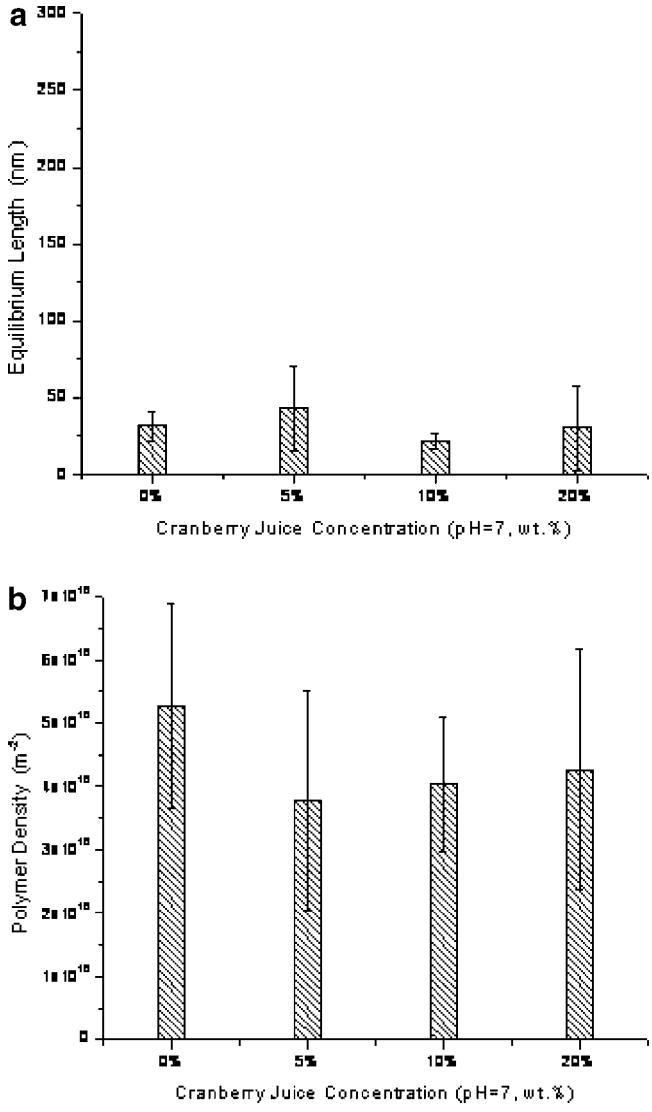

Figure 4. Steric model parameters fit for *E. coli* HB101 as a function of cranberry juice concentration. $R^2 = 0.9$, 5 cells per concentration, 8 force measurements per cell, i.e. $n = 40$. **A**: Equilibrium length L_o, (**B**) Polymer density. Error bars indicate one standard deviation.

steric model was not dependent on the cranberry juice concentration (Fig. 4B). The polymer densities varied from an average of $(5.27 \pm 1.62) \times 10^{16}$ m^{-2} in the absence of cranberry juice treatment to an average of $(3.78 \pm 1.74) \times 10^{16}\,m^{-2}$, $(4.03 \times 1.05) \times 10^{16}\,m^{-2}$ and $(4.26 \pm 1.89) \times 10^{16}$ m^{-2} in 5%, 10%, and 20 wt.% cranberry juice solutions, respectively and no statistically significant differences were observed in the grafted polymer densities for *E. coli* HB101 in any of the cranberry juice solutions.

Analysis of Retraction Curve Data: Pull-off Forces and Pull-off Distances *E. coli* Mutant HB101pDC1

Unlike the data from AFM approach curves, the retraction curves show more variability, even on a single bacterium. This is mostly due to the dynamic and heterogeneous nature of the biomolecules on the bacterial surface, which means that the AFM tip is likely to make contact with a different portion of the biomolecule or a different biomolecule in subsequent measurements (Camesano and Abu-Lail, 2002). While a single retraction curve is not very meaningful, reliable results can be expected by integrating many adhesion events over multiple cells. In our analysis, we examined 40 retraction curves in one cranberry solution (eight force measurements on five cells). The data were combined through histograms and statistical analyses to help their interpretation.

For *E. coli* HB101pDC1, adhesion forces were inversely correlated with the cranberry juice concentration (Fig. 5A). In ultrapure water or 5 wt.% cranberry, ~80% of the retraction forces were of an absolute magnitude >0.5 nN. In contrast, smaller adhesive forces were observed in the higher cranberry concentration solutions, with >40% of the retraction forces having an absolute magnitude <0.5 nN in the 20 wt.% cranberry juice.

The pull-off distances for *E. coli* HB101pDC1 decreased with increasing cranberry juice concentration (Fig. 5B.). More than 60% retraction peaks in the cranberry juice (5%, 10%, and 20 wt. %) showed up in the range <40 nm, while more than 60% retraction peaks in the ultrapure water appeared >40 nm.

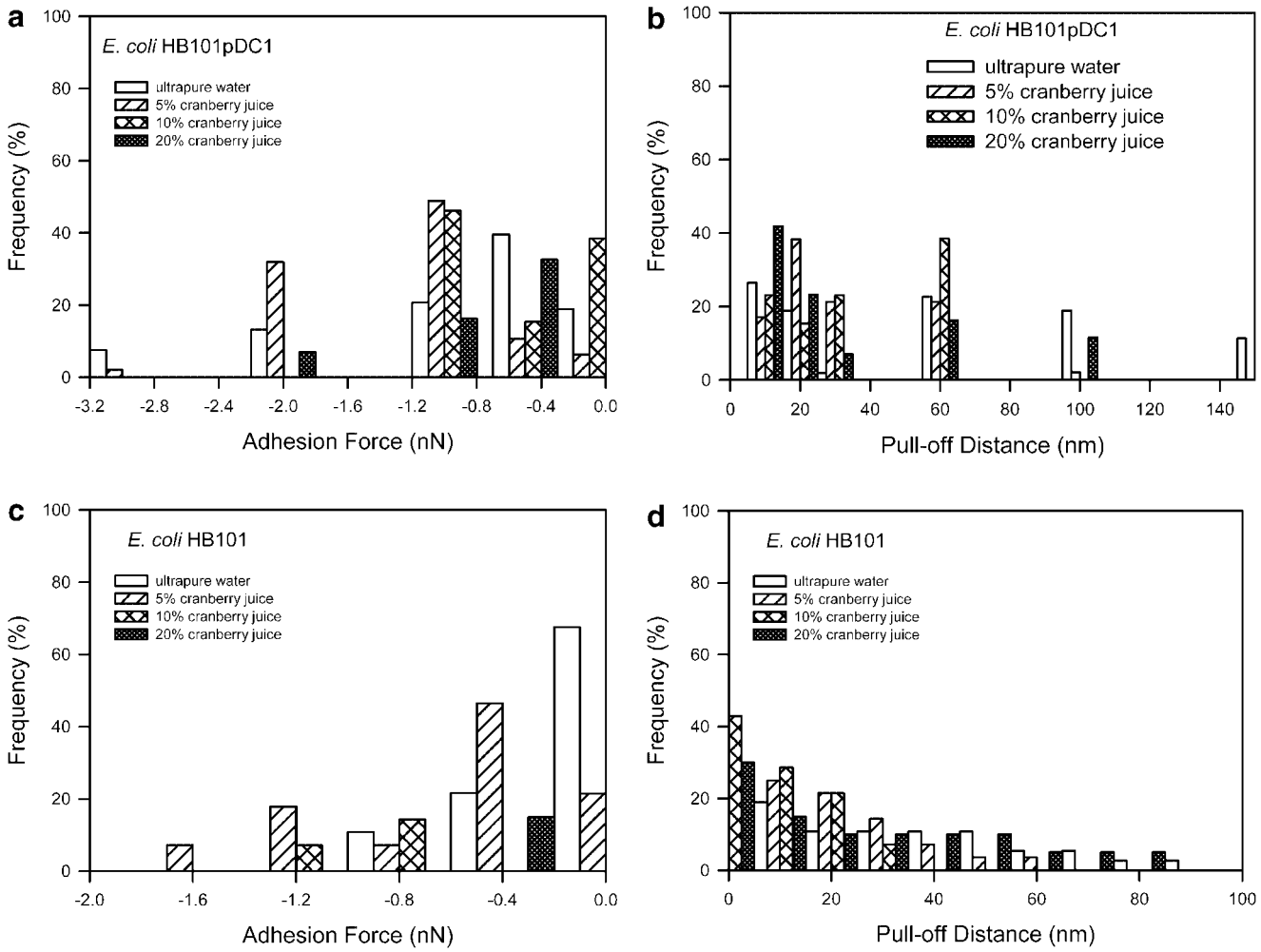

Figure 5. Distribution of parameters from AFM retraction curves. **A**: Retraction forces for *E. coli* HB101pDC1 as a function of cranberry juice concentration, n = 25; (**B**) Pull-off distances for *E. coli* HB101pDC1 as a function of cranberry juice concentration, n = 25; (**C**) Retraction forces for *E. coli* HB101 as a function of cranberry juice concentration, n = 25; (**D**) Pull-off distance for *E. coli* HB101 as a function of cranberry juice concentration, n = 25.

E. coli HB101

Clear trends were not present for the effect of cranberry juice on the adhesion forces and pull-off distances for the non-fimbriated bacteria. The adhesive forces in any solution between the probe and *E. coli* HB101 were somewhat low, with more than 80% of the adhesion forces between *E. coli* HB101 and the probe having absolute magnitudes between zero and 0.5 nN, irrespective of the cranberry juice concentration (0–20 wt. %) (Fig. 5C). Cranberry juice did not have much of an effect on the adhesive forces between the probe and this strain of *E. coli* HB101.

Similarly, the pull-off distances were not very sensitive to the cranberry juice concentration for HB101 (Fig. 5D). More than 80% of the pull-off distances measured in any of the solutions were <60 nm. Most pull-off distances of *E. coli* HB101 in the cranberry juice (5%, 10%, and 20 wt. %) and the ultra pure water occurred within the same range.

DISCUSSION

Mechanism of Cranberry Juice Action on P-fimbriated-*E. coli*

The biological role of fimbriae is to act as adhesins between bacteria and receptors on mammalian cells. While we did not study the binding between *E. coli* and mammalian cells, we showed that the *E. coli* itself can be affected by cranberry. Cranberry juice appears to affect fimbriae directly. Evidence for this is that cranberry juice affects the equilibrium lengths and density of the polymers on strain HB101pDC1, and the adhesion forces with a model surface, but these effects are not seen for the non-fimbriated strain, HB101.

We can consider several possible mechanisms to explain the interactions between cranberry juice and the surfaces of *E. coli*. These possibilities include (i) cranberry juice alters the conformation of the P-fimbriae; (ii) cranberry juice blocks the adhesive action of P-fimbriae; (iii) cranberry juice removes P-fimbriae from the cells, and (iv) cranberry juice causes genetic or phenotype-level changes in *E. coli* with P-fimbriae, causing non-expression of P-fimbriae. It is also possible that more than one mechanism is occurring simultaneously.

Role of Cranberry Juice on Conformation of P-fimbriae

Exposure to cranberry juice resulted in a decrease in the equilibrium length of the polymers on the surface of *E. coli* HB101pDC1 from ~148 to ~48 nm. This appears to be a conformational change in the surface fimbriae, indicating the proteins are becoming more compressed on the bacterial surface when cranberry is present.

Cranberry Juice Blocks Adhesive Action of P-fimbriae

Specific components in cranberry juice can bind to the P-fimbriae and inhibit the adhesion of P-fimbriaeted bacteria to a surface. The components were identified as non-dialyzable material (NDM) with a high molecular mass constituent (12000–15000 Da) (Burger et al., 2000). Howell et al. considered the components to be proanthocyanidin compounds that have both hydrophilic and lipophilic moieties (Howell et al., 1998). Although research is ongoing to further identify and characterize the key components (Lila, 2004; Lila and Raskin, 2005; Smith et al., 2002), the active components that can alter bacterial adhesion apparently are hydrophilic. Proteins, including fimbriae, would be hydrophobic in their unaltered states. After the hydrophilic components bind to the P-fimbriae, the adhesion force between the ''modified'' P- fimbriae and the AFM silicon nitride tip changes from the interaction between non-polar materials to that between the non-polar tip and the polar ''modified'' P-fimbriae. This results in a decrease in the adhesion forces between the model surface and the bacterium.

*Cranberry Juice Removes P-fimbriae From the **E. coli** Surface*

To reveal the relationship between the equilibrium lengths and the grafted polymer density, a further analysis was performed. According to mass conservation, the mass of surface polymers for a given strain should not change as a function of the cranberry juice concentration. The mass of the biomacromolecules on the outer surface of the cells is taken to be the product of the outer cell surface area, the equilibrium length, and the polymer density, respectively. Since the surface area does not change (verified by fluorescence microscopy experiments, data not shown), any variations in the equilibrium length and the polymer density should balance one another. Both in the presence and absence of cranberry juice, the product of the equilibrium length and the polymer density remains constant at $\sim 1 \times 10^9$ m^{-1}, and is independent of cranberry juice concentration. Further, the one way repeated ANOVA statistical tests showed that there were no statistically significant differences among the individual products from the three concentrations of cranberry juice. Therefore, the total mass of molecules on the bacterial surface does not change upon exposure to cranberry juice, and so it does not seem plausible that cranberry juice exposure is causing fimbriae to be removed.

Cranberry Juice Causes Loss of Expression of P-fimbriae

Consistent with the above mass balance analysis, cranberry juice exposure cannot cause P-fimbriae to not be expressed, at least not over the time scales studied here. However, growth of the cells in cranberry-containing media could potentially cause a different effect. Ahuja et al. reported P-fimbriated *E. coli* bacteria lost the P-fimbriae when growing in media mixed with liquid cranberry concentrate (unsweetened form of cranberry juice) (Ahuja et al., 1998). They hypothesized that some components in cranberry concentrate will interact with bacterial DNA and inhibit the expression of P-fimbriae.

Our results neither can prove nor disprove this possible mode of action of cranberry. However, we have shown that growth in cranberry-containing media is not necessary for affecting the *E. coli* surface, since growth was not a factor in our studies. Exposure to cranberry juice for even a short time period (<3 hrs. and during non-growth conditions) produces reversible yet important changes in surface properties.

Effects of Cranberry Juice pH on Bacterial Adhesion Behavior

Acidification of urine was speculated to be responsible for the anti-bacterial properties of cranberry for more than 100 years, but recent research showed that this was not the reason for the anti-bacterial properties of cranberry juice (Sobota, 1984; Zafriri et al., 1989). Therefore, we wanted to address whether the anti-adhesive response of *E. coli* to cranberry could be observed, even at non-acidic pH values. We adjusted the pH of the cranberry juice to 7.0 before the AFM experiments. The average equilibrium length of surface polymer, i.e. P-fimbriae on *E. coli* mutant HB101pDC1, decreased from ~148 nm in ultrapure water to ~48 nm in 20% cranberry juice. The cranberry juice after neutralization still has the capability to affect the adhesion and conformational behavior of *E. coli* mutant HB101pDC1. The finding that low pH is not necessary to prevent bacterial adhesion was verified by a direct approach.

Further, we can rule out electrostatic interactions as playing a dominant role in influencing the adhesion behavior between *E. coli* and the silicon nitride. Although water has the lowest ionic strength, all of the cranberry juice solutions have fairly low ionic concentrations. The cranberry juice cocktail (which is 27% cranberry) has an ionic strength of $\sim 9.55 \times 10^{-3}$ M. Therefore, the diluted juices have ionic strengths ranging from ~0.001 M (for the 5 wt.% solution) to ~0.007 M (for the 20% solution). If electrostatic forces were dominating the interaction between *E. coli* and the silicon nitride, we would expect to see decreased adhesion at the lowest ionic strength (i.e. pure water and 5 wt.% juice). Since the opposite trend was observed, it appears that non-electrostatic interactions are dominating in this system.

CONCLUSIONS

Cranberry juice components appear to affect P-fimbriae by altering their conformation and by binding of hydrophilic components. Both of these phenomena work to decrease the adhesion of P-fimbriated *E. coli* with a model surface. The AFM results show that cranberry juice has an immediate effect on the P-fimbriated *E. coli* bacteria. Some components in cranberry juice interacted with P-fimbriae directly, causing P-fimbriae to become compressed and less adhesive. A final note of interest is that most of the effects we saw occurred at a cranberry juice concentration between zero and 5%. Future work will be aimed at exploring the ''critical concentration'' of cranberry juice needed to alter the conformation and adhesion properties of P-fimbriae.

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