J|A|C|S
COMMUNICATIONS

Published on Web 02/12/2003

Adhesion between Molecules and Calcium Oxalate Crystals: Critical Interactions in Kidney Stone Formation

Xiaoxia Sheng,[†] Michael D. Ward,*[,†] and Jeffrey A. Wesson[‡]

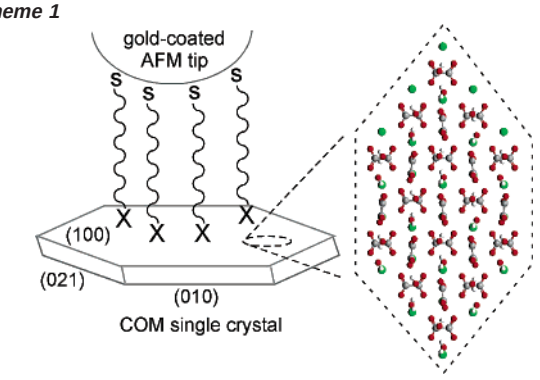
Department of Chemical Engineering and Materials Science, University of Minnesota, 421 Washington Avenue SE, Minneapolis, Minnesota 55455, and Nephrology Division, Department of Veteran Affairs Medical Center and the Medical College of Wisconsin, 5000 West National Avenue, Milwaukee, Wisconsin 53295

Received December 3, 2002; E-mail: wardx004@umn.edu

Kidney stone disease, which occurs in approximately 10% of the U.S. population, causes substantial suffering and occasional renal failure, but the disease mechanism is poorly understood. Kidney stones are crystal aggregates, most commonly containing calcium oxalate monohydrate (COM) crystals as the primary constituent, and often are found attached to epithelial cells at the tip of renal papilla. Calcium oxalate dihydrate (COD) is less common in kidney stones,[1,2] even though it is found in the urine of asymptomatic individuals.[3,4] This suggests that aggregation and attachment to renal cells are particularly important for COM. Notably, in vitro studies have suggested that *anionic* molecules or macromolecules with substantial anionic functionality (e.g., carboxylate)[5−10] play an important role in selectivity toward COM and COD, crystal habit and growth rate, aggregation, and attachment to cells. Furthermore, kidney stones contain measurable amounts of carboxylate-rich proteins that may serve as adhesives and promote aggregation of COM crystals. The microscopic origins underlying aggregation and attachment to cells, however, have not been examined directly. Because these processes are surface-related, the different behaviors of the two crystal forms are likely correlated with the distinct structures of their exposed crystal faces. We describe herein preliminary atomic force microscopy (AFM) measurements of adhesion forces between tip-immobilized molecules and the COM (100) surface that reveal the effect of functional groups on adhesion and support an important binding role for carboxylate groups. These results suggest a feasible methodology for identifying the most important crystal surface-macromolecule combinations related to stone formation.

AFM has been used for direct measurement of the adhesion force between various molecular and biomolecular surfaces,[11,12] but studies involving single-crystal surfaces have been rather limited.[13,14] Using a well-established approach, we anchored various organosulfur compounds to a gold-coated Si_3N_4 AFM tip through the Au−S bond to create surfaces equipped with specific functional groups. These tips were brought into contact with the large (100) face of COM crystals submerged in an aqueous solution saturated with 0.10 mM calcium oxalate (CaOx) at pH = 7.05 (Scheme 1). AFM images of the crystal surface revealed 200-nm wide terraces separated by 6.3 Å steps (Figure 1), in agreement with the *a* lattice parameter (a = 6.290 Å).[15] A total of 1000 individual force−distance curves were collected for each modified tip at 20 different locations on the (100) surface. The unbinding forces from the retraction portion of these curves were tabulated into histograms, and the mean adhesion force and standard deviation were determined from the normal distribution curve.[16] The histogram for a

Scheme 1



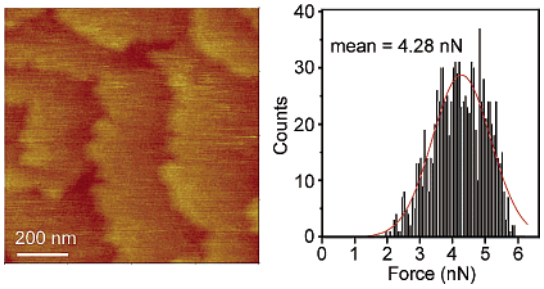


Figure 1. (a) AFM image of the COM (100) surface in 0.10 mM CaOx. (b) Histogram of adhesion forces measured for a Au:S$(CH_2)_{10}COO^-$ tip contacting the (100) surface.

Au:S$(CH_2)_{10}COO^-$ tip is illustrative; the mean adhesion force is 4.28 ± 0.41 nN.

Figure 2 depicts the adhesion forces measured for various modified tips and the COM (100) surface in the saturated CaOx solution. Two classes of organosulfur reagents − arenethiols and alkanethiols − were examined to delineate the contribution of steric factors, which can affect the number density of adhesive molecules on the tip (molecules per unit area) as well as local binding between a terminal functional group and a COM (100) crystal site. The data reveal that within each class the adhesion forces for carboxylate-terminated tips are larger than those of the other tips. For example, the mean adhesion force for the Au:S$(CH_2)_{10}COO^-$ tip was approximately 5 times that observed for Au:S$(CH_2)_{10}CH_2OH$ or Au:S$(CH_2)_{10}CH_3$ (0.96 ± 0.20 and 0.74 ± 0.08 nN, respectively). Similar trends were observed in the arene series, with the mean adhesion force for the Au:S$(C_6H_4)COO^-$ (2.06 ± 0.35 nN) greater than those of the Au:S$(C_6H_4)OH$ and Au:S$(C_6H_4)NH_2$ tips (1.49 ± 0.18 and 0.89 ± 0.16 nN, respectively). These data suggest an important role for the carboxylate group in processes responsible for kidney stone formation, specifically macromolecule-mediated adhesion of COM crystals to cells and crystal aggregation.

[†] University of Minnesota.
[‡] Department of Veteran Affairs Medical Center and the Medical College of Wisconsin.

10.1021/ja029575h CCC: $25.00

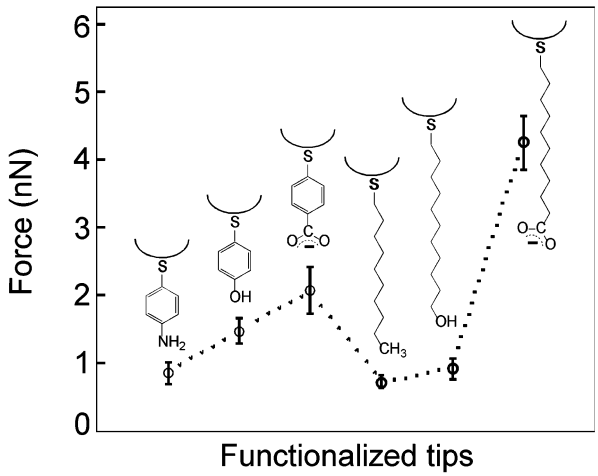


Figure 2. Adhesion forces measured by tips functionalized with various molecules. Measurements were made in 0.10 mM CaOx solution.

The pH of the medium (pH = 7.05) favors ionization of the carboxylate groups.[17] The strong adhesion exhibited by the carboxylate tips suggests an important role for binding to calcium ions (green atoms in Scheme 1) on the (100) surface, possibly in a specific manner that mimics oxalate binding with these calcium sites. The smaller binding force observed for Au:S(C_6H_4)COO^- may reflect (*i*) the lower number density of carboxylate groups on the tip within the contact area for arenes compared with alkanes, (*ii*) greater steric interactions introduced at the crystal binding site by the arene group, (*iii*) conformational rigidity (compared with the alkane chain), which would prevent proper alignment of the carboxylate group at the crystal binding site. The slightly larger adhesion forces observed for the hydroxyl-terminated tips, relative to their corresponding amine- and methyl-terminated analogues, may reflect the participation of weak hydrogen bonding of the hydroxyl groups with the oxalate groups on the surface. The higher force exhibited by the phenol, which is a better hydrogen-bond donor than the aliphatic alcohol, supports this suggestion. The low adhesion force for the amine-terminated tip is particularly noteworthy. Previous experiments have demonstrated that amine-functionalized polymers have negligible effect on CaOx crystal growth and the selectivity toward COM and COD, consistent with weak binding between amines and CaOx surfaces.

Recent studies performed in our laboratories have revealed that poly(aspartic acid) (polyD) strongly inhibits COM growth and shifts the selectivity of newly formed crystals to COD. Real-time in situ AFM investigations demonstrated that polyD strongly attenuated the growth of COM, reflecting adsorption of polyD on the COM crystal surface. Growth was suppressed preferentially along the [001] direction (the long axis of the COM crystal), suggesting specific binding to COM crystal sites on the (021) apical planes. These measurements, however, did not permit direct examination of polyD binding to the (100) surface. Force measurements performed here with a Au:S$(CH_2)_{10}COO^-$ tip revealed a progressive decrease in the adhesion force upon addition of polyD to the force measurement medium (0.15 mM CaOx; pH = 6.90). Over the course of 1 h, the mean adhesion force decreased from 3.75 ± 0.44 to 1.90 ± 0.19 nN (Figure 3). Parallel measurements revealed that the adhesion force did not decrease in the absence of polyD. Apparently, the carboxylate-rich polyD adsorbs on the (100) surface and blocks the binding of the tip-immobilized carboxylate ions, possibly to specific crystal binding sites. The reduced adhesion force indicates that the tip-immobilized carboxylate ion binds less strongly to the polyD adsorbate than to COM crystal binding sites. This

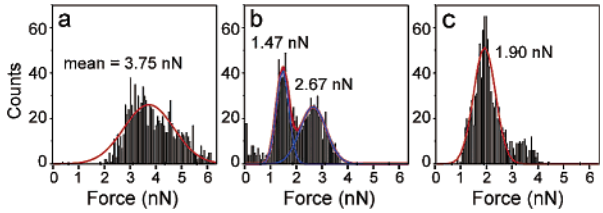


Figure 3. Histograms of adhesion forces measured for a Au:S$(CH_2)_{10}$-COO^- tip in contact with the COM (100) surface in 0.15 mM CaOx solution; pH = 6.90: (a) in the absence of polymer, (b) and (c) 30 and 60 min after addition of 4 μg/mL of polyD.

competitive binding behavior mimics the known reduction in attachment of COM crystals to renal epithelial cells in the presence of carboxylate-rich urinary macromolecules.

The observations described above demonstrate further the utility of AFM for probing binding of specific functional groups to crystal surfaces. Adhesion forces can be affected by surface energy terms associated with the creation of new surfaces during an unbinding event.[18] The similar surface energies expected for the tips in Figure 2, however, are expected to minimize the contribution of these effects toward differences in adhesion force (except for the methyl-terminated alkanethiol). The results are consistent with the growing evidence that carboxylate-rich synthetic macromolecules and native urinary proteins play important roles in regulating aggregation of COM and its attachment to renal epithelial cells. We anticipate that measurements of adhesion forces between small molecules or proteins and various faces of COM and COD crystals will identify the most relevant macromolecule-crystal surface combinations related to kidney stone formation.

Acknowledgment. The authors gratefully acknowledge the support of the Jacob Lemann, Jr. Endowment at the Medical College of Wisconsin, the Department of Veterans Affairs, and the McKnight Foundation (University of Minnesota).

References

(1) Mandel, N. S.; Mandel, G. S. *J. Urol.* **1989**, *142*, 1516.
(2) Prein, E. L. *J. Urol.* **1963**, *89*, 917.
(3) Elliot, J. S.; Rabinowitz, I. N. *J. Urol.* **1980**, *123*, 324.
(4) Dyer, R.; Nordin, B. E. *Nature* **1967**, *215*, 751.
(5) Lieske, J. C.; Huang, E.; Toback, F. G. *Am. J. Physiol. Renal Physiol.* **2000**, *278*, F130.
(6) Millan, A. *Cryst. Growth Des.* **2001**, *1*, 245.
(7) Tunik, L.; Addadi, L.; Garti, N.; Furedi-Milhofer, H. *J. Cryst. Growth* **1996**, *167*, 748.
(8) Wesson, J. A.; Worcester, E. M.; Kleinman, J. G. *J. Urol.* **2000**, *163*, 1343.
(9) Guo, S.; Ward, M. D.; Wesson, J. A. *Langmuir* **2002**, *18*, 4284.
(10) Lieske, J. C.; Deganello, S.; Toback, F. G. *Nephron* **1999**, *81*, Suppl 1, 8.
(11) Noy, A.; Vezenov, D. V.; Lieber, C. M. *Annu. Rev. Mater. Sci.* **1997**, *27*, 381.
(12) Takano, H.; Kenseth, J. R.; Wong, S.; O'Brien, J. C.; Porter, M. D. *Chem. Rev.* **1999**, *99*, 2845.
(13) Abendan, R. S.; Swift, J. A. *Langmuir* **2002**, *18*, 4847.
(14) Danesh, A.; Davies, M. C.; Hinder, S. J.; Roberts, C. J.; Tendler, S. J.; Williams, P. M.; Wilkins, M. J. *Anal. Chem.* **2000**, *72*, 3419.
(15) Tazzoli, V.; Domeneghetti, C. *Am. Mineral.* **1980**, *65*, 327−334 ($P2_1/c$, a = 6.290 Å, b = 14.580 Å, c = 10.116 Å, β = 109.46°).
(16) The spring constant of the gold coated Si_3N_4 cantilever (0.074 ± 0.03 N/m) was measured by a previously reported method: Cleveland, J. P.; Manne, S.; Bocek, D.; Hansma, P. K. *Rev. Sci. Instrum.* **1993**, *64*, 403.
(17) (a) Smith, D. A.; Wallwork, M. L.; Zhang, J.; Kirkham, J.; Robinson, C.; Marsh, A.; Wong, M. *J. Phys. Chem. B* **2000**, *104*, 8862−8870. (b) Vezenov, D. V.; Noy, A.; Rozsnyuai, L. F.; Lieber, C. M. *J. Am. Chem. Soc.* **1997**, *119*, 2006.
(18) Skulason, H.; Frisbie, C. D. *Anal. Chem.* **2002**, *74*, 3096.

JA029575H