Substrate-Assisted Laser-Initiated Ejection of Proteins Embedded in Water Films

Yusheng Dou,[†] Nicholas Winograd,[‡] Barbara J. Garrison,*[,‡] and Leonid V. Zhigilei[§]

Department of Physics, Texas A&M University, College Station, Texas 77843, Department of Chemistry, Penn State University, University Park, Pennsylvania 16802, and Department of Materials Science and Engineering, University of Virginia, Charlottesville, Virginia 22903

Received: August 9, 2002

Molecular dynamics simulations have been employed to investigate laser initiated liftoff of the protein enkephalin embedded in a H_2O film adsorbed onto a gold substrate. The laser energy is deposited solely into the gold substrate at different heating rates. For fast heating rates on the picosecond time scale, the results show that large clusters of the H_2O molecules are ejected from the film entraining the enkephalin molecule away from the metal surface. For heating on the nanosecond time scale, the H_2O overlayer evaporates in the form of individual molecules and small clusters that are unable to entrain the enkephalin molecule.

Introduction

Matrix assisted laser desorption ionization (MALDI) mass spectrometry is a powerful technique for the analysis of large nonvolatile biological compounds.[1] The key to this technology is to find appropriate organic crystal hosts or matrixes that allow the biomolecules to be desorbed into the gas phase as charged molecular ions. Many of these matrixes have been found in recent years and their characteristics usually include high absorption of UV radiation, the ability to solvate the biomolecule, and the ability to transfer a proton during the ablation event. From a biological perspective, water would be a very attractive matrix for MALDI because it is a natural component of most biomaterials. One approach for using a water matrix has been explored with IR irradiation of an air-dried analyte solution,[2] where the energy is deposited through the strong O−H stretching mode of water near 3 μm. With this approach the mass spectrum of a lyophilized protein up to a molecular weight of 30 kDa was obtained. The low sensitivity encountered in the experiment, presumably due to the large penetration depth and concomitant large ablation yields, seems to have limited its use for mass spectrometry. An alternative approach for using water as a matrix is to place an ice film on a metal substrate and to use UV irradiation, absorbed mainly by the substrate. This technique was successfully employed to detect dissolved DNA with molecular weights up to 410 kDa.[3,4] Although this scheme is potentially valuable for DNA sequencing, the experimental results, obtained with 8 ns laser pulses, were not sufficiently reproducible for practical use.[5] In our own laboratory, we have demonstrated that when a UV laser of 100 fs pulse length is used to irradiate a metal substrate, the mass spectrum of biomolecules with a good signal-to-noise ratio can be recorded routinely.[6] Almost identical results are obtained when the experiments are repeated with different metal substrates including Au, Al, Si, and Cu. By comparison with the previous experiments,[3] the improvements in the reproducibility of the mass spectra can be attributed to the specifics of the ultrashort pulse laser irradiation. The advantage of using short laser pulses has been confirmed in recent experiments performed with a train of picosecond pulses generated by a mid-infrared free-electron laser.[7] Irradiation of frozen water samples containing angiotensin II has produced ion signals for the analyte molecule as well as for protonated water clusters of different sizes. The advantage of using UV radiation is that all the energy can be absorbed by the substrate if the systems are chosen correctly, thus avoiding heating the biomolecule via excitation of various vibrational modes.

These observations, taken under disparate conditions of laser fluence, wavelength, and time scale, are difficult to resolve using any unified theory. It is of fundamental interest, however, to examine the dynamics of energy flow from the substrate to an overlayer water film to see if the rate of energy deposition affects the prospects for desorption of intact biomolecules. Molecular dynamics (MD) simulations provide a tool from which insight into such energy transfer processes can be obtained. In previous publications,[8,9] we reported MD simulation studies of explosive boiling of H_2O films of different thickness adjacent to a heated Au surface. Potential applications of these calculations include eye surgery,[10] selective killing of cells by irradiation of incorporated adsorbing metal particles,[11] and laser assisted steam cleaning,[12] as well as mass spectrometry experiments of biological molecules.[3,4,6] The model proposed from the previous simulations provides the basis for understanding molecular-level processes that lead to the evaporation of a H_2O film from a hot substrate.

In this study, we investigate the liftoff of biomolecules embedded in H_2O films adjacent to a heated metal surface. The focus is on how the rate of heating the underlying metal substrate affects the liftoff process in H_2O films. A small biomolecule, enkephalin (574 Da), is chosen as the analyte molecule because the computations are tractable. Two different rates of heating the underlying metal substrate to 1000 K are simulated to mimic the deposition of laser energy into the system with two different laser pulse durations. The results of the present study show a dramatic difference in the liftoff of the enkephalin molecule embedded in a H_2O film depending upon the rate of heating of the underlying metal substrate. We expect that the study will provide guidance for optimization of the conditions for the efficient H_2O matrix-assisted volatilization of biomolecules in mass spectrometry experiments.

[†] Texas A&M University.
[‡] Penn State University.
[§] University of Virginia.

10.1021/jp0267306 CCC: $25.00
Published on Web 02/15/2003

Methodology

The basic prescription for modeling the dynamics of a water film due to heating of an adjacent metal surface has been described previously.[8,9] To incorporate the enkephalin molecule into the model, we utilize the MD program TINKER[13] rather than attempting to directly incorporate the interaction potentials for biomolecules into our own computer code. The many-body metal interaction potential and the heating algorithm were imported into the TINKER code.

Force Fields. In this study, all atoms of the system, including aliphatic and polar hydrogen atoms are included explicitly. Forces and interaction energies among atoms of the enkephalin molecule and water molecules are calculated using the OPLS All-Atom (OPLS-AA) force field.[14] The simple-point-charge (SPC) model developed by Berendsen et al.[15] is used to represent the H_2O-H_2O interactions. The force switch method[16] is employed to modify the long-range component of the potential. Cutoff distances of 0.85 and 0.95 nm are applied for the H_2O-H_2O and the OPLS-AA interactions, respectively. A detailed description of the potential and the treatment of electrostatic interactions for the H_2O-H_2O interaction have been described previously.[8] The Au−Au interactions are described by the many-body MD/Monte Carlo corrected effective medium (MD/MC−CEM) potential for fcc metals.[17] Of importance to this study is the fact that the MD/MC−CEM potential reasonably reproduces the experimental surface energy of Au.[18] The $Au-H_2O$ interaction is calculated by a modified version of the Spohr function.[19] All parameters in the potentials for the H_2O-H_2O and $Au-H_2O$ interactions are given elsewhere.[8] The interactions between the atoms in the enkephalin molecule and the Au atoms are ignored as the enkephalin molecule never gets closer than 1.2 nm to the substrate during the simulations.

MD Simulations. Simulations are performed for two systems. Each system contains a face centered cubic crystallite consisting of 1332 Au atoms in nine (111) layers of 148 atoms each. As described previously, the bottom half of the metal crystal is used for temperature control.[8] The H_2O film is composed of 897 H_2O molecules roughly arranged in 14 layers with an enkephalin molecule placed in the upper part of the film, as shown in Figure 1.

The Au substrate is heated to a designated temperature with the following protocol. The atoms in the bottom layer are held rigid. The next four layers of atoms experience stochastic forces evaluated by the generalized Langevin equation (GLE) method.[20] All metal atoms are subject to the $Au-H_2O$ and Au−Au interactions. Any required temperature of the whole substrate is achieved by adjusting the temperature in the GLE stochastic region. The time for the top four metal layers to equilibrate with the stochastic region is approximately 3 ps. The system is quenched to 0 K before any heating of the substrate.

To mimic the effects of the laser heating with different laser pulse widths, we have increased the temperature by 100 K increments in the stochastic region of the metal substrate every 5 ps for fast heating and every 100 ps for slow heating. For both schemes, the final temperature, 1000 K, is maintained for the remainder of the simulation after the heating period. The two rates correspond to heating the substrate in 50 and 1000 ps, respectively. The experimental laser pulse width would be shorter than these times, as time is required to convert the electronic energy from the laser absorption event into phonon modes of the lattice.

The velocity version of Verlet algorithm[21] is employed to integrate the equations of motion. The RATTLE constraint algorithm[22] is used to maintain the H_2O molecules rigid and the enkephalin C−H, N−H, and O−H bond lengths fixed in the simulations. The assumption that there are no vibrations in the high-frequency modes is justified because the partition function of the lowest frequency mode, the H−O−H bend, is only 1.02 at a temperature of 600 K, the maximum temperature reached by the ablated water film. Thus no energy should flow into these modes. Periodic boundary conditions are applied in the directions parallel to the metal surface. A time step of 1.0 fs is chosen on the basis of energy conservation tests.

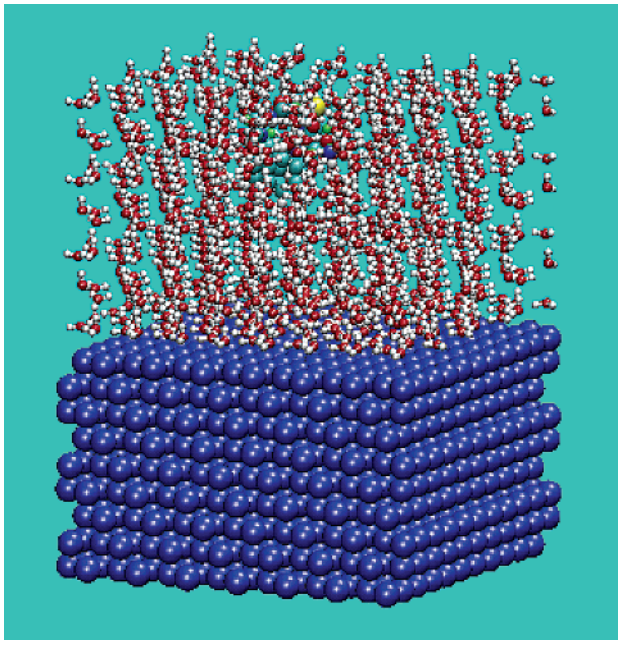

Figure 1. Initial configuration used in the simulations. The system consists of nine layers of Au atoms (blue), fourteen layers of H_2O molecules (red and white), and one enkephalin molecule (multicolored). The center of mass of the enkephalin molecule is located around 1.8 nm above the metal substrate.

Results and Discussions

In this section, we present the results from the MD simulations for the liftoff of the enkephalin molecule embedded in a H_2O film from a Au(111) surface heated to 1000 K with two different rates. Snapshots from the MD simulations at a time when the fate of the enkephalin molecule is determined are shown in Figure 2a,d for the fast and slow heating rates. The height of the center-of-mass of the enkephlin molecule as a function of time is given in Figure 2e,f. For the fast heating rate, the molecule starts to liftoff at about 60 ps whereas for the slow heating rate, the molecule starts to move toward the surface at about 300 ps.

The differences in the dynamics of the enkephalin molecule between the two different heating rates can be understood by examining the temperature evolution of the H_2O film. Parts b and c of Figure 2 show the temperature contour plots as a function of height above the surface and time for the fast and slow heating rates. The temperature is calculated using only the radial components of the kinetic energy as in our previous work because the axial direction can have a flow component of velocity in addition to the thermal motions.[8] The relevant color regimes in the temperature contour plots for the phase transition temperatures of water at 1 atm are teal for melting (273 K), green for boiling (373 K), and the pink/orange interface for explosive boiling (∼500 K).[23] As discussed in our previous work[8] for the fast heating rate, the temperature in the water film near the surface increases faster than it can thermally diffuse

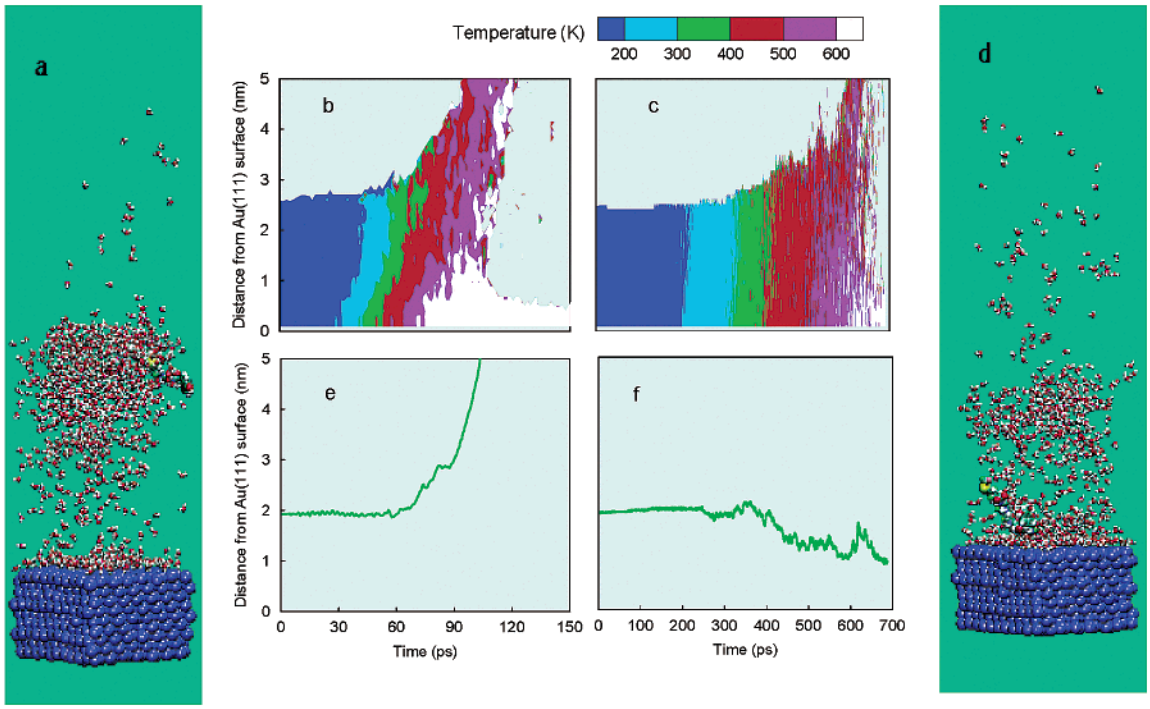

Figure 2. Simulation results for two rates of heating of the metal substrate, fast, 1000 K per 50 ps, heating (left part of the figure), and slow, 1000 K per 1 ns, heating (right part of the figure). (a) Snapshots from the MD simulations for the fast heating rate at a time of 105 ps. (b, c) Temperature contour plots as a function of distance from the surface and time for the fast and slow heating rates, respectively. (d) Snapshots from the MD simulations for the slow heating rate at a time of 630 ps. (e, f) Height of the enkephalin molecule above the surface vs time for the fast and slow heating rates, respectively.

through the remainder of the water film. Consequently, at about 90−100 ps, the water near the surface undergoes an explosive boiling phase transition. The water near the enkephalin molecule is only ~300 K at this time. The force from the explosive boiling pushes the outer part of the film including the enkephalin molecule away from the surface. For the slow heating process, the temperature of the water increases almost simultaneously throughout the depth of the film as shown in Figure 2c. Melting occurs at around 300 ps and intensive evaporation starts at around 400 ps. The melting is reflected in an increased mobility of the enkephalin molecule, whereas evaporation of water around the biomolecule leads to its gradual motion toward the surface, Figure 2f.

The calculations clearly show that for the entrainment of the biomolecule in a water film there must be a temperature gradient in the film such that explosive boiling can occur near the metal substrate. Hence, fast energy deposition in the substrate is essential.[6] Moreover, both the water film and the biomolecule should not absorb the laser radiation to achieve the maximum temperature gradient and to avoid fragmentation of the biomolecule. The nanosecond pulse width of the original DNA−ice experiments may, in fact, be the reason they were hard to reproduce.[3,5] Determining precise values of the laser fluence and the pulse width required to achieve the desired temperature evolution in the overlayer depends on the rate of the laser energy conversion to the thermal energy of the substrate, the thermal conductivity of the substrate and film, and the rate of energy transfer across the substrate-overlayer interface.

The final issue to be addressed concerns the conditions under which the biomolecule can survive to reach the detector intact. To understand the dynamics within the plume, we modeled the phase explosion of a system with an enkephalin molecule surrounded by water molecules. Energy corresponding to ~800 K was added to the system containing 1384 water molecules and one enkephalin molecule. Periodic boundary conditions were imposed until 30 ps when the sides of the box were removed. The system expanded outward due to the phase explosion and the temperature of the entire system decreased, as shown in Figure 3a. Concomitant with the cooling is evaporation of water molecules from the enkephalin molecule as shown in Figure 3b. The enkephalin molecule, however, never becomes completely free of water molecules. Solvation of the biomolecule by the matrix molecules was also found in atomistic simulations[24] of conventional UV MALDI and breathing sphere calculations[25] of UV MALDI. The issues of complete desolvation and ionization remain to be addressed.

Summary

In this work, we have investigated the liftoff of the enkephalin molecule embedded in a H_2O film for two different rates of heating the underlying metal substrate. The MD simulations demonstrate that for fast heating, a very thin layer of H_2O molecules near the metal surface undergo a phase explosion that provides acceleration for moving large clusters. The H_2O and enkephalin molecule embedded in a portion of the film are carried outward from the metal surface. For slow heating, the entire film is heated and the H_2O molecules vaporize constantly over the depth of the H_2O film. Slow heating is unable to produce enough force to lift the enkephalin molecule up from the metal surface. The simulation results clearly show that the lifted enkephalin molecule and associated H_2O clusters experience a cooling process during desorption due to the phase explosion and intensive vaporization of H_2O molecules. These simulation results provide a microscopic picture of how the rate of heating the metal substrate affects the liftoff of biological molecules embedded in the matrix.

It will be interesting to see if these calculations can be extended to help understand any of the observations discussed earlier.[3−6] There are many difficulties in making direct com-

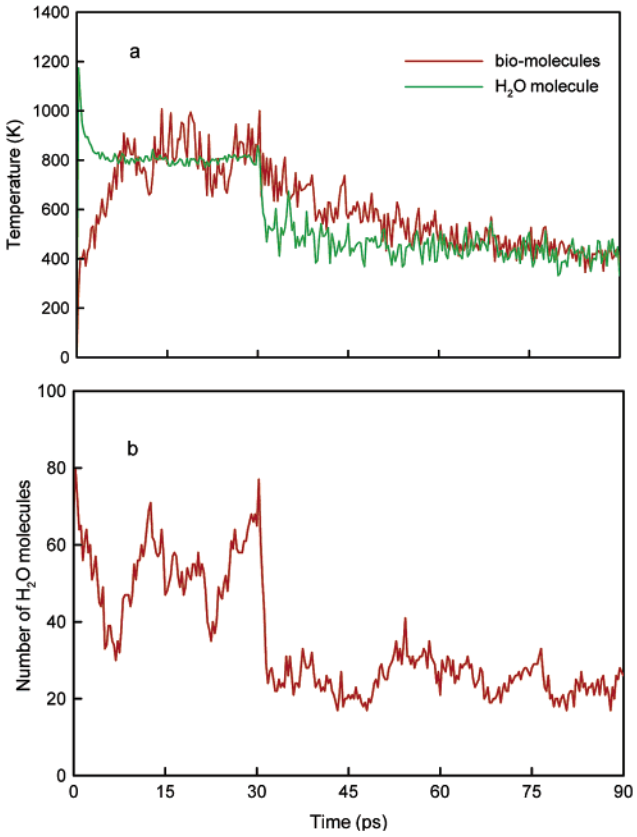

Figure 3. (a) Radial kinetic energies of the H_2O cluster containing the enkephalin molecule vs time. (b) Number of water molecules within 4 Å of the enkephalin molecule.

parisons to experiment due to the wide reported range of laser fluence and due to vagaries in the ionization process that inevitably must occur during desorption. There are, however, several interesting questions relevant to MALDI mass spectrometry experiments that arise from this work. For example, how do analyte molecules separate from surrounding H_2O molecules before they are actually analyzed by the mass detector? At what ratio of analyte molecules to H_2O molecules yield the most efficient liftoff conditions? In general, we hope to stimulate the development of novel modalities for laser ablation experiments that promise to expand the range of their already powerful applications.

Acknowledgment. The financial support of the Medical Free Electron Laser program from the Air Force Office of Scientific Research, the National Science Foundation, and the National Institute of Health. The Center for Academic Computing at Penn State University provided computational assistance.

References and Notes

(1) Hillenkamp, F.; Karas, M.; Beavis, R. C.; Chait, B. T. *Anal. Chem.* **1991**, *63*, 1193A.

(2) Berkenkamp, S.; Karas, M.; Hillenkamp, F. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 7003.

(3) Nelson, R. W.; Rainbow, M. J.; Lohr, D. E.; Williams, P. *Science* **1989**, *246*, 1585.

(4) Nelson, R. W.; Thomas, R. M.; Williams, P. *Rapid Commun. Mass Spectrom.* **1990**, *4*, 349.

(5) Williams, P. *Int. J. Mass Spectrom. Ion Processes* **1994**, *131*, 335.

(6) Berry, J.; Sun, S.; Dou, Y.; Winograd, N. Manuscript to be published.

(7) Baltz-Knorr, M. L.; Schriver, K. E.; Haglund Jr, R. F. *Appl. Surf. Sci.* **2002**, *11*, 197.

(8) Dou, Y.; Zhigilei, L. V.; Winograd, N.; Garrison, B. J. *J. Phys. Chem. A* **2001**, *105*, 2748.

(9) Dou, Y.; Zhigilei, L. V.; Postawa, Z.; Winograd, N.; Garrison, B. G. *Nucl. Instrum. Methods Phys. Res. B* **2001**, *180*, 105.

(10) Thompson, C. R.; Gerstman, B. S.; Jacques, S. L.; Rogers, M. E. *Bull. Math. Biol.* **1996**, *58*, 513.

(11) Lin, C. P.; Kelly, M. W.; Sibayan, S. A. B.; Latina, M. A.; Anderson, R. R. *IEEE J. Select. Topics Quantum Electron*. **1999**, *5*, 963.

(12) See, for example, Tam, A. C.; Leung, W. P.; Zapka, W.; Ziemlich, W. *J. Appl. Phys.* **1992,** *71*, 3515. She, M.; Kim, D.; Grigoropoulos, C. P. *J. Appl. Phys*. **1999,** *86*, 6519. Lu, Y. F.; Song, W. D.; Zhang, Y.; Low, T. S. *Proc. SPIE* **1998**, *7*, 3550.

(13) Ponder, J. W. TINKER: Software Tools for Molecular Design, Version 3.8, Washington University School of Medicine, 2000.

(14) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rivers, *J. Am. Chem. Soc*. **1996**, *117*, 11225.

(15) Berendsen, H. J. C.; Postma, I. P. M.; van Gunsteren, W. F.; Hermans, J. In *Intermolecular Forces*; Pullman, B., Ed.; Reidel: Dordrecht, The Netherlands, 1981.

(16) Steinbach, P. J.; Brooks, B. R. *J. Comput. Chem*. **1994**, *15*, 667.

(17) Kelchner, C. L.; Halstead, D. M.; Perkins, L. S.; Wallace, N. M. *Surf. Sci*. **1994**, *310*, 425.

(18) DePristo, A. E. In *Recent Advances in Density Functional Theory: Part 2 Methodology*; Chong, D., Ed.; World Scientific: River Edge, NJ, 1995.

(19) Spohr, E. *J. Mol. Liq*. **1995**, *64***,** 91.

(20) Garrison, B. J.; Kodali, P. B. S.; Srivastava, D. *Chem. Rev*. **1996**, *96*, 1327 and references therein.

(21) Swope, W. C.; Andersen, H. C.; Berens, P. H.; Wilson, K. R. *J. Chem. Phys*. **1982**, *76*, 637.

(22) Andersen, H. C. *J. Comput. Phys*. **1983**, *52*, 24.

(23) The temperature value for explosive boiling is estimated as 0.8 the critical temperature which is 640 K at 1 atm. Avedisian, C. T. *J. Phys. Chem. Ref. Data* **1985**, *14*, 695.

(24) Sadeghi, M.; Wu, X.; Vertes, A. *J. Phys. Chem. B* **2001**, *105*, 2578.

(25) Itina, T. E.; L. V. Zhigilei, L. V.; Garrison, B. J. *J. Phys. Chem B* **2002**, *106*, 303.