Mechanism of Sputtering of Large Biomolecules by Impact of Highly Ionizing Particles

Peter Williams
Department of Chemistry, Arizona State University, Tempe, Arizona 85287

and

Bo Sundqvist
Tandem Accelerator Laboratory, University of Uppsala, S-751 21 Uppsala, Sweden
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We describe a model for the ejection of large biomolecules from surfaces by impact of high-energy (~1 MeV/nucleon) heavy ions. The model involves the rapid vibrational excitation of the biomolecule by low-energy electron irradiation arising from the incident particle track. The excitation breaks most hydrogen bonds binding the molecule to the surface and causes the molecule to expand on a subpicosecond time scale; the expanding molecule pushes against the substrate and generates sufficient momentum to escape.

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Ejection of very large molecular ions from surfaces by megaelectronvolt ion bombardment has had a major impact on biomolecular mass spectrometry since the pioneering work of Macfarlane and co-workers over ten years ago,[1] but no model has yet explained all details of the ejection process. The energy deposited into electronic excitation by fast heavy ions in solids ($\sim 10^3$ eV/Å for 100-MeV ^{127}I) is far greater than the relatively small binding energy between a large biomolecule and its neighbors or a substrate. However, it has not been clear how this excitation is coupled to molecular motion resulting in desorption of massive molecular ions. The present paper describes a new approach to this problem.

Desorption by fast heavy-ion impact involves nuclear motion that is totally decoupled from that of the primary particle; numerous experiments have shown that at high energies ion yields scale solely with the energy deposited into electronic excitation.[2,3] Desorbed ions leave the surface with translational kinetic energies ~1-2 eV[4] (measurements exist[5] for ion masses up to 1182 u); fragmentation of bovine insulin (mass ~5733 u) suggests that the ejected insulin ions are vibrationally excited by some hundreds of electronvolts.[6,7] Recent measurements indicate that sputtering yields of intact neutral molecules are very high: 1180 intact molecules of leucine [molecular weight (MW) 131] or ~580 intact molecules of a peptide [luteinizing hormone releasing hormone (LRHR) MW 1182] per incident high-energy ion; only ~1 molecule in 10^4 is ionized.[8,9]

The ejection process either must transfer enough kinetic energy to the molecule to overcome the surface binding, or must break the surface bonds selectively, leaving structural bonds in the molecule intact. We assume here that the surface bonding is that of a protein to other proteins—a mixture of hydrogen and ion-pair bonds (~0.2 eV/bond[10]) and much weaker van der Waals interactions. We consider a protein molecule of mass 10000 u with something over 1000 constituent atoms. Deposited on a surface, such a molecule is approximately spherical,[11] ~20 Å in diameter, and contains roughly 100 amino acid residues. If some 20-30 residues are accessible to form hydrogen bonds with the substrate, the binding energy is ~4-6 eV. An ejection model must explain how these bonds are selectively broken and stay broken for an extended period. A particle of mass 10000 u with kinetic energy 2 eV moves away from the surface slowly—only 2 Å/psec. Bond formation involving either electron or proton rearrangement must occur on a much shorter time scale, so that either a continuing excitation must exist, or the entire molecule must be given enough kinetic energy—5-8 eV—to overcome the surface binding and escape with 1-2 eV excess energy. Although this translational energy appears trivial compared to the *energy* deposited by a megaelectronvolt projectile, for a heavy molecule, it represents an enormous *momentum*, $(2mE)^{1/2}$, and accounting for this momentum is a major problem that has not hitherto been addressed.

The ion "track" in the solid has been subdivided into two regions[12]: the "infratrack" within 5-10 Å radius of the ion trajectory (the Bohr adiabatic radius) in which the Coulombic perturbation due to the incident ion is strong enough to cause ionization, and the "ultratrack," a region irradiated by secondary electrons from the infratrack, which may extend 100-1000 Å from the ion path. From the massive sputtering yields measured by Sundqvist *et al.*,[8] and the ejection of very large (~30 Å diameter) molecules,[13] it seems clear that intact molecule ejection must occur from the ultratrack.

Two classes of models have been discussed. Hedin *et al.*[14] discuss the possibility of ejection arising from surface-bond cleavage by secondary electrons in the ultratrack. Bond fission following electronic excitation or ionization can impart kinetic energies of several electron-

volts to the dissociating atoms. However, if a molecule of mass ~ 10000 u is attached to the surface by only 20–30 bonds, breaking all these simultaneously produces far less than the momentum required to eject the entire molecule with an energy of a few electronvolts. In addition the authors do not explain how hydrogen bonds can be broken selectively, leaving structural bonds in the molecule intact. A thermal model treats ejection as evaporation from a small zone rapidly heated to a temperature $\sim 2\times 10^4$ K for a period $\sim 10^{-12}$ s.[15] This model does not address the mechanism of electron-atom coupling or the microscopic details of ejection. If equilibrium is assumed, an energy of 1–2 eV in one translational mode corresponds to the storage of 3–6 *keV* in vibrational energy in the ~ 3000 normal modes of a 1000-atom molecule. Such large internal energies should lead to dissociation. King *et al.*[6] and Chait[7] estimate that the fragmentation observed for bovine insulin (~ 800 atoms) results from vibrational energies almost an order of magnitude smaller—no more than 600–800 eV. Lucchese[16] has recently argued that vibrational-translational equilibrium need not exist in desorption, on the basis of computer studies of desorption of small molecules such as CO. However, it is not yet clear how extrapolation from CO to large biomolecules can be justified. No current model explains in detail how energy, initially deposited into electronic excitation, is transferred preferentially into translational motion of a very large entity. The rationalization of this feature is a major objective of the model that we now describe.

We begin by specifying that ejection must occur from the ultratrack. The energy distribution of secondary electrons produced in the infratrack peaks at zero energy[12]; the relatively few high-energy electrons lose energy rapidly to excitation and ionization (inelastic mean free paths for electron energies ~ 20–1000 eV are 5–20 Å) until their energy drops below excitation thresholds, so that most secondary electrons traversing the ultratrack have energies ~ 1 eV. We note that *direct* coupling between electronic and nuclear motion can be remarkably efficient in this "subexcitation" energy regime through the excitation of molecular vibration in polar bonds,[17,18] and we suggest that the abundant low-energy electrons irradiating molecules in the ultratrack can excite with high probability most, if not all, of the intramolecular bonds in these molecules to the first vibrational level or higher. This excitation will result in a rapid expansion of each molecule. An expanding molecule pushing against its subsurface neighbors (which are themselves excited), or against a rigid substrate, can generate sufficient momentum to leave the surface.

We need to estimate both the degree of expansion possible, and the kinetic energy resulting from that expansion. It appears reasonable to use thermal expansion coefficients for high-molecular-weight polymers to guide this estimate. As will be seen below, the requisite kinetic energy is achieved for our model molecule with ~ 3–5% linear expansion. For many polymers, coefficients of linear expansion at room temperature are $\sim (3$–$5) \times 10^{-5}$/K.[19] For a 3%–5% change in length, even assuming no increase of the expansion coefficient with temperature, we thus require excitation equivalent to a temperature increase no more than ~ 1000 K. For the covalent bonds in a protein molecule, $h\nu_0 \sim 0.1$–0.3 eV; thus the expansion corresponds to excitation of such bonds only to the $v=1$ level, which does not require violation of selection rules (cross sections for $\Delta v = 1$ are significantly greater than for $\Delta v = 2$ or higher[17]). Data are lacking for vibrational excitation cross sections in large molecules, but we can gain insight from small-molecule data. For electron energies of a few tenths to one electronvolt, and for heteronuclear diatomic molecules (e.g., H_2O), cross sections for excitation of the $v=1$ states, for both "hard" (stretching) and "soft" (bending) modes, can be several square angstroms[17,18] as a result of electron interaction with the bond dipole. Short-range as well as long-range interactions occur[18]; the former will not be screened in the dielectric solid so that cross sections should be similar to gas-phase values. In a large molecule, a 1-eV electron clearly could lose 0.1-eV increments to more than one bond so that a single electron could rapidly transfer most of its energy to a single protein molecule. It is evident that nearly simultaneous electron-impact excitation of most of the normal modes of a large biomolecule to low-lying vibrational levels can occur for a large fraction of the molecules irradiated in the ultratrack.

To calculate the kinetic energy available from expansion, we need the time scale on which the expansion occurs. We first note that the molecule cannot respond on the time scale of the excitation. A 1-eV electron traverses a 20-Å-diameter molecule in $\sim 10^{-14}$ s, but the rate at which the molecule as a whole can be pushed away from the surface must be limited by the fundamental vibrational frequency of the molecule. McCammon[20] notes that, because proteins are densely packed structures, in their large-scale motion they can be pictured as behaving like a continuous elastic material. Suezaki and Go[21] state that the frequency of the fundamental "breathing" mode of an elastic sphere of radius r is $(1/r)(E/\rho)^{1/2}$, where ρ is the density and E the Young's modulus of the material. For globular proteins these authors assume a value for E of 10^{10} N/m^2, as intermediate between the typical value $\sim 10^9$ N/m^2 for bulk polymers and a value $\sim 10^{11}$ N/m^2 determined spectroscopically for the α helix of poly-L-alanine.[22] They calculate a fundamental frequency for the proteins α-chymotrypsin and pepsin ($r \sim 20$ Å) in good agreement with low-frequency peaks (~ 30 cm^{-1}) seen in the Raman spectra of these proteins.[23] For our model molecule $r \sim 10$ Å; the period is 6×10^{-13} s. Thus for a free molecule the expansion is complete in 3×10^{-13} s. For an ex-

pansion of 3%–5%, pushing against a rigid substrate, or against similarly expanding neighbors, the center of mass of the molecule moves 0.3–0.5 Å in this time. Assuming a constant acceleration over this period,[24] the final velocity of the center of mass is 200–300 m/s, which, for a molecule of mass 10000, corresponds to a kinetic energy of 2–6 eV.

The expansion coefficient over a 1000-K temperature rise must be considerably greater than the room-temperature value, and so energies greater than the 4–6-eV surface binding energy are clearly accessible, even for less than complete excitation of the molecule. However, it is also clear that if all the atoms involved in hydrogen bonds have energies of a few tenths of an electronvolt, most hydrogen bonds will be ruptured. The vibrational excitation may thus supply a microscopic low-energy bond-breaking process, *operating selectively for hydrogen bonds* over a large portion of the ultratrack, in addition to the macroscopic expansion which supplies the gross kinetic energy, and momentum, of the molecule as a whole.

To calculate a sputtering yield we note that ~25% of the energy lost by the incident ion is deposited as secondary electrons in the ultratrack.[25] For LHRH molecules this corresponds to ~1.8 keV over the 7-Å thickness of a single molecular layer for 1000-eV/Å energy loss. We assume that the whole of this residual energy is efficiently coupled into vibrational excitation, and that all molecules so excited can be ejected. If we assume excitation by 0.1 eV/mode, each 150-atom LHRH molecule stores ~45 eV, and ~40 molecules can be sputtered from each layer. The ion yield for LHRH saturates at a sample thickness ~70–80 Å.[9] Taking this to be an ejection depth we calculate a sputtering yield for LHRH of 400–460 molecules/incident ion, in reasonable agreement with the measured value of 580.[8]

The model described here is consistent with observed features of the ejection process. It rationalizes the ejection of intact molecules, despite the necessity to overcome significant surface binding energies, and is consistent with estimates of sputtering yields and the internal energy of desorbed molecular ions. Exciting the ~2400 normal modes of the bovine insulin molecule by ~0.1–0.3 eV would give an internal energy of a few hundred electronvolts, somewhat lower than the estimates (600–800 eV) of King *et al.*[6]; it is now known that ion-induced fragmentation is less than observed in the data cited by King *et al.*, so that their internal-energy estimates should be revised downward. Finally, we can address the distribution of translational energies. It seems clear that molecules near the infratrack may be ejected with quite high energies (several electronvolts) if they survive dissociation. Molecules far from the infratrack will leave with very low energies. The energy spectrum (of ejected *neutral* molecules) will be skewed to low energy (there are more molecules far from the track, and molecules near the track are more likely to be dissociated), but may extend to energies of several electronvolts.

In applying the model of ejection of smaller molecules, it seems reasonable to treat these as assemblages. One might therefore expect that a projectile capable of ejecting several hundred 100-atom molecules might equally well eject several thousand 10-atom molecules which would occupy a similar volume. This assemblage will be connected to its surroundings by about the same number of bonds as the larger molecules, but Young's modulus in such an assemblage would be lower than in large protein molecules, so that the ejection efficiency should be lower. Given the significant internal energy of the assemblage, dissociation to individual molecules would rapidly occur. The data of Sundqvist *et al.*[8,9] seem to confirm the idea that ejection is more efficient for larger molecules: their measured mass yield for leucine molecules (MW 131) is $\sim 1.5\times 10^5$ amu/(incident ion),[8] whereas for LHRH (MW 1182) the estimated yield is $\sim 7\times 10^5$ amu/ion.[9]

Although the infratrack in our model does not eject any intact molecules, it may play a role in ionization. The infratrack is a rich source of electrons and light ions which could attach to the desorbed molecules from the ultratrack. The observed low degree of ionization may then reflect the small spatial and temporal overlap between the plume of fast charged particles ejected from the infratrack and the slower molecules ejected from the ultratrack.

In general, the large cross sections for vibrational excitation of polar bonds by low-energy electrons appear to offer a rapid and efficient coupling between electronic and nuclear motion which could account for many "electronic sputtering" effects, and may play a role, for example, in fast ion erosion of ices[26] or in adhesion enhancement in thin-film couples with large electronegativity differences.[27] Because coupling between vibrational modes in large molecules is rapid, it may be acceptable to speak of a partial equilibrium in these modes. The present model then explains how a relatively low degree of vibrational excitation can lead to a significant translational kinetic energy in a large molecule. The efficacy of fast heavy ions for large-molecule ejection is seen to be due to the capability of these projectiles to cause near-instantaneous multiple vibrational excitation over large volumes.

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