# Particle-Induced Desorption Mass Spectrometry of Large Involatile Biomolecules: Surface Chemistry in the High-Energy–Short-Time Domain

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Insulin is a good model for a large involatile, thermally labile biomolecule. It contains 51 amino acid units, consists of two peptide strands connected at two points by delicate disulfide links, and has a diameter of a few nanometers. The molecular weight of human insulin is 5803.6378 corresponding to an elemental formula of $C_{257}H_{383}O_{77}N_{65}S_6$. These data were *calculated* from the known composition of this peptide. Until recently, no method in mass spectrometry had been able to volatilize and ionize such a large and fragile molecule.

The first mass spectrum of insulin containing the molecular ion plus fragment ions has now been reported.[1] Gas-phase molecular ions of insulin were desorbed by irradiating a thin film of bovine insulin with 90-MeV $^{127}I$ ions from a nuclear accelerator. The spectra, recorded by the time-of-flight (TOF) method, are shown in Figure 1. This result is but the latest in a series of impressive gains made in the mass spectrometry of large biomolecules. They are due to the development of several related desorption–ionization methods referred to here collectively as particle-induced desorption.

They began with the introduction of californium-252 plasma desorption mass spectrometry ($^{252}Cf$ PDMS) in 1974, a method which uses the interactions of hundred MeV heavy ions (from the spontaneous fission of $^{252}Cf$) in a solid matrix to induce desorption and ionization.[2] After the initial report, it was soon realized that the essential feature of $^{252}Cf$ PDMS was that the deposition of energy was highly concentrated and the excitation lasted for only a very short period of time. Under these conditions, large thermally labile molecules were able to survive intact and to sublime from the surface as ionized species.

Other means of producing the same effect were sought and found. Benninghoven, in 1976, showed that *keV* ions impinging on the surface of a thin film of biomolecules induced the same desorption–ionization process observed in $^{252}Cf$ PDMS.[3] This method (secondary ion mass spectrometry, SIMS) had been used routinely in surface analysis of inorganic species but had never been applied to thermally labile biomolecules. In 1978, Meuzelaar demonstrated that excitation by short duration laser pulses (<10 ns) produced patterns of desorbed molecular ions and fragment ions essentially the same as in $^{252}Cf$ PDMS and SIMS.[4] This third variant of particle-induced desorption is called laser desorption (LD). The fourth variation introduced by Barber et al. in 1981 uses a keV beam of neutral atoms as the vehicle for energy transfer and is called fast atom bombardment (FAB).[5] What is significantly different about FAB is the medium from which desorption–ionization occurs—a liquid solution matrix. The FAB method is illustrated along with the other three methods in Figure 2.

Molecular ion yields are high for the simple biomolecules (several tenths per incident ion in $^{252}Cf$ PDMS) but decrease dramatically with increasing mass above $m/z$ 1000. The molecular yields of the peptide $\beta$-endorphin and the marine toxin palytoxin (2681 u)[6] are $10^{-3}$ to $10^{-4}$ per incident ion in $^{252}Cf$ PDMS. The effect of the matrix on the desorption–ionization process is more acute for the larger biomolecules: exposure of a thin film of $\alpha$-cyclodextrin to the summer Texas air for a few hours prior to analysis reduces the molecular ion yield from $10^{-2}$ to less than $10^{-4}$ per incident ion in $^{252}Cf$ PDMS. A sample of an antibiotic from one laboratory will give excellent mass spectra while a sample of the same molecule from another laboratory will give *no* detectible molecular ions.

Despite these problems and a low-level feeling of insecurity that desorption–ionization is under the influence of "hidden variables", all of the variants of particle-induced desorption have had some measure of success in the high molecular weight realm.

The megavolt $^{127}I$-labeled insulin work is currently the record for a biomolecule in its natural form. A method for sequencing synthetic chemically blocked deoxyoligonucleotides up to the 14-mer ($m/z$ 6957), which makes use of $^{252}Cf$ PDMS, has been reported,[7]

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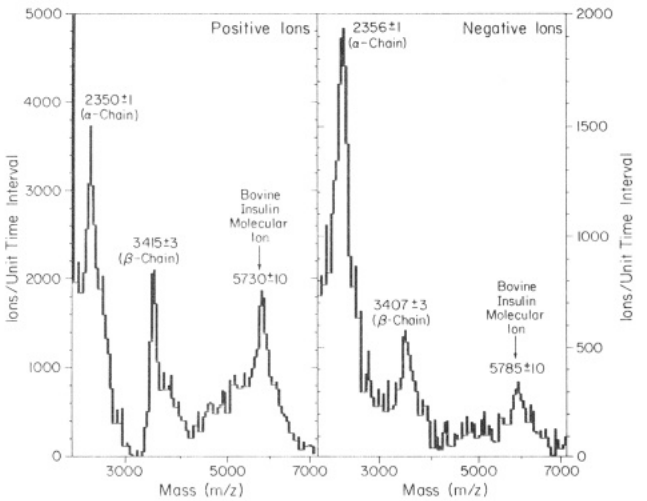


**Figure 1.** Mass spectra of bovine insulin. These molecular ions were desorbed from the surface of a thin film excited by 90-MeV $^{127}I$ ions (20+) from a nuclear accelerator. The particle current was 2000 $s^{-1}$ and the spectra were accumulated for 1.5 h with a low-resolution TOF mass spectrometer optimized for high mass sensitivity.[1]

and a dimer of a similar molecule (a 12-mer) has been detected at $m/z$ 12637.[8] By means of SIMS, synthetic chemically blocked deoxyoligonucleotides at the trimer level ($m/z$ 2079) have been desorbed by 25-keV $Cs^+$ ions.[9] The FAB method has been used to obtain mass spectra of glucagon (3481 u) and of the oxidized bovine insulin $\beta$ chain (3494 u).[10]

High molecular weight mass spectrometry of biomolecules is one of the frontier areas of analytical chemistry. It is replete with challenge in every segment of the total effort: fundamental studies to identify and control the "hidden variables" relating to the desorption–ionization process, determination of the best medium for the mass separation of high molecular weight ions (e.g. high field magnets, time of flight, ion cyclotron resonance), and detection of massive, low-velocity ions. This Account focuses on just one of these areas—fundamental studies related to the desorption–ionization process.

## The Initial Interactions

One of the features of particle-induced desorption is that in the initial interaction of the incident particles, a large amount of energy is deposited into a highly localized region of the matrix of biomolecules. Some of this energy is transformed into internal vibrational modes and into molecular translation/rotation of the surface molecules, initiating fragmentation and desorption. The mechanism of energy deposition is quite different when the bombarding projectile is a collection of coherent infrared photons or a megavolt heavy ion or a keV atom, yet the spectrum of desorbed molecular and fragment ion species is essentially the same.[11] This suggests that at some stage in the transfer of energy to the surface molecules some sequence of events is common to all the methods, at least for those events that have an influence on the mass spectrum of desorbed ions. This is an important distinction because many of the changes that are produced in the matrix by the incident particle may not be related to the desorption–ionization process. For SIMS, FAB, and $^{252}Cf$ PDMS,

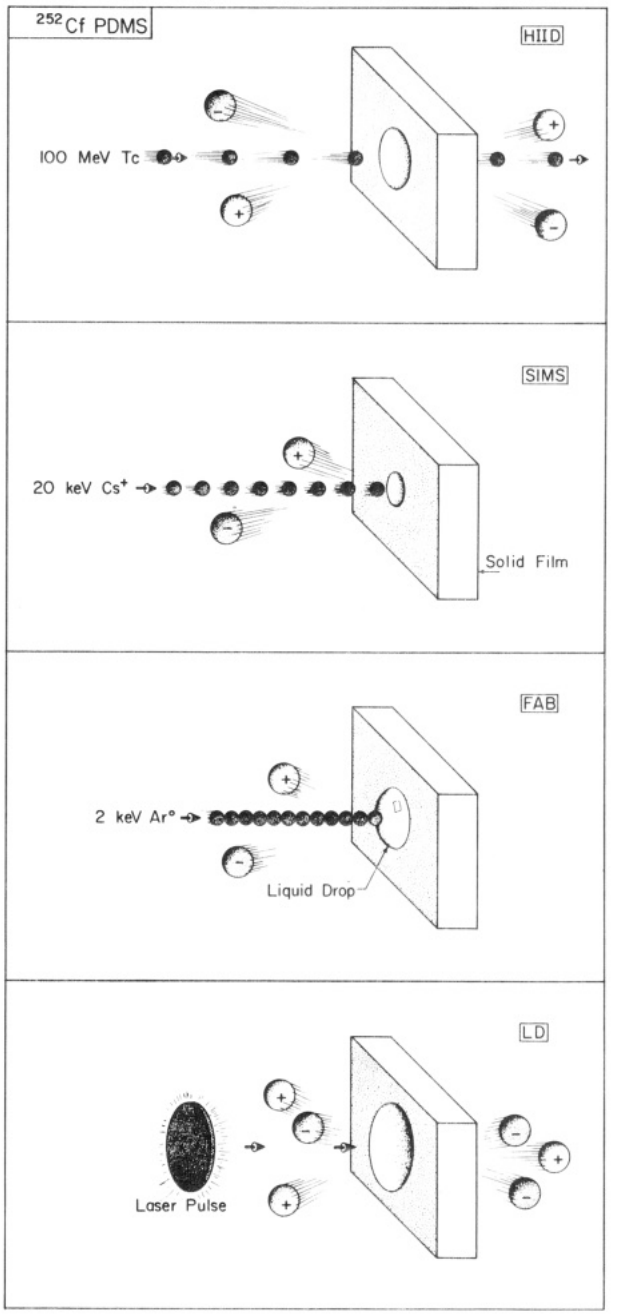


**Figure 2.** Schematic representation of the four variations of particle-induced desorption mass spectrometry: californium-252 plasma desorption ($^{252}Cf$ PDMS and heavy ion induced desorption (HIID), which employ weak currents (2000 $s^{-1}$) of hundred megavolt heavy ions to inititate the desorption–ionization process.[2] Ions pass through thin films (<10 $\mu$m) and ions are desorbed from both sides of the film. The second variation, secondary ion mass spectrometry (SIMS), uses keV ion currents (up to $10^6$ $s^{-1}$), and ions are desorbed from the front surface.[3] Fast atom bombardment (FAB) utilizes still higher particle currents of neutral keV atoms ($\sim 10^{10}$ $s^{-1}$) impinging on the surface of a droplet of a viscous liquid solution (e.g., glycerol) from which molecular ions are desorbed.[5] The fourth variant laser desorption (LD) excites a solid surface with a single short duration laser pulse (<10 ns), $10^8$ W $cm^{-2}$. The desorbed ion yield is so high that a complete mass spectrum can be obtained with a single laser pulse.[4]

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it is the action of a single incident particle that establishes the conditions for desorption–ionization of a large involatile biomolecule. The probability that a given incident particle desorbs an involatile molecular ion is very small, but each incident keV atom deposits energy and generates some form of collision cascade, and each megavolt ion penetrating a dielectric layer produces an ionization track and surface crater.

Energy transfer in solids and liquids involves "particles" that mediate the energy transfer but that do not exist outside the solid. A phonon is a quantum of intermolecular vibrational energy which can be generated by the recombination of a positive ion and electron formed in the primary energy deposition event or by the collision cascade triggered by the impact of a SIMS particle. The desorption of an involatile molecule may be the result of a multiphonon excitation of the bonds holding the molecule to an aggregate.[12] Phonons can also transform their energy into unstable internal vibrational modes of the molecules in the matrix resulting in molecular dissociation.

The molecular exciton is a second fundamental quasi-particle that mediates energy transfer in condensed media.[13] This is a migrating excitation center whose properties depend upon the specific coupling between molecules and account for the collective excitation of an aggregate of molecules (free exciton). Molecular excitons are also transformed into the vibrational and rotational excitations of individual molecules.

In particle-induced desorption, whether a particular primary excitation will generate the spectrum of molecular excitons and phonons required to desorb a large tightly bound molecular ion at a specific site could very well depend on the local properties of the aggregate of molecules from which the molecular ion emerges, their relative orientation, and the purity of the aggregate. Perhaps these are some of the hidden variables in particle-induced desorption.

## Nuclear Excitation–Primary Energy Deposition in SIMS and FAB

Nuclear excitation is an energy loss process that dominates for incident ions and atoms at keV energies. At these energies, the incoming projectile perceives an aggregate of insulin molecules as a collection of C, H, O, N, and S atoms. The struck atom dissipates its energy to the surrounding molecules through collision-like processes.[14] For systems of molecular aggregates, at some stage the atomic collision cascade process transforms to molecular kinematics. High energy atomic ions are observed in the SIMS spectra of desorbed species, and these are presumably ejected in the initial stages of the collision cascade.[15] But for an aggregate of large molecules the energy must ultimately partition into the bonds that set molecules in motion (phonons) and into internal degrees of freedom (molecular excitons) in order to generate the molecular ions that are observed in SIMS and FAB. Molecular ion emission occurs in the latter stages of the energy delocalization process. This is reflected in the low kinetic energy (<5 eV) of the desorbed molecular ions.[15]

The top half of Figure 3 is an attempt to give a graphical presentation of the energy transfer process for SIMS and FAB. The energy scale for the sequence of events is derived from computer simulations of Garrison.[16] The impact of a 5-keV $Xe^+$ ion on a collection of molecular aggregates establishes time zero for the sequence of events. The effect of incident energy on molecular ion yield suggests that only the energy deposited to a depth of ~8 nm is contributing to the process.[17] The surface area excited by a 14-keV $Cs^+$ ion is 10 times larger than that of a 14-keV $K^+$ and the molecular ion yield is largest when the incident ion is at a grazing angle (~15°), enhancing the deposition of energy in the surface region.[17] The initial stages of the collision cascade develop within $10^{-15}$ s, ejecting atomic species with energies in excess of 10 eV. In the final stages, excited molecular aggregates at the surface dissociate and desorb at low energy, giving the collection of molecular ions and fragment ions observed in SIMS and FAB mass spectra. A representative kinetic energy spectrum for a SIMS process that includes the contribution of neutrals and ions[18] is also shown in Figure 3. The spectrum is divided into two sections to portray the high-energy atomic component and the low-energy molecular ion portion.

## Electronic Excitation–Primary Energy Deposition in $^{252}Cf$ PDMS

The nuclear excitation component of the linear energy transfer ($dE/dx$) for a $^{35}Cl$ ion peaks at ~20 keV where $dE/dx$ is ~800 eV $nm^{-1}$. With increasing $^{35}Cl$ energy, $dE/dx$ slowly drops to a minimum of 500 eV $nm^{-1}$ at ~100 keV. Above that energy $dE/dx$ increases to a second maximum at 35 MeV, with a $dE/dx$ value of 4500 eV $nm^{-1}$.[19] This is the region of electronic excitation. The $dE/dx$ due to electronic excitation varies with the effective charge of the incident ion. The 20+ charge state, 100-MeV fission fragments from $^{252}Cf$ decay have a $dE/dx$ of 10000 eV $nm^{-1}$.[20] The electronic excitation is initially in the form of separated positive ions (holes) and electrons (e–h pairs) and electronically excited atoms. As these species deexcite, the e–h pairs recombine and the energy is dissipated to the surroundings.

When e–h pair formation occurs in metals, the recombination is extremely fast (~$10^{-17}$ s) and the energy is carried away by electron conduction.[20] No intense phonon spectrum is generated, and unlike the keV ion interactions of SIMS, the ion desorption yield from thin metal films excited by megavolt ions is small.[21] If the matrix is an insulator, the desorption yield from electronic excitation is extremely high. A single fission fragment can desorb as many as 1000 molecules, leaving a crater visible by electron microscopy 20 nm in diameter and 15 nm deep.[22] Chemically reactive tracks 15–20 μm long and 10 nm in diameter are formed in

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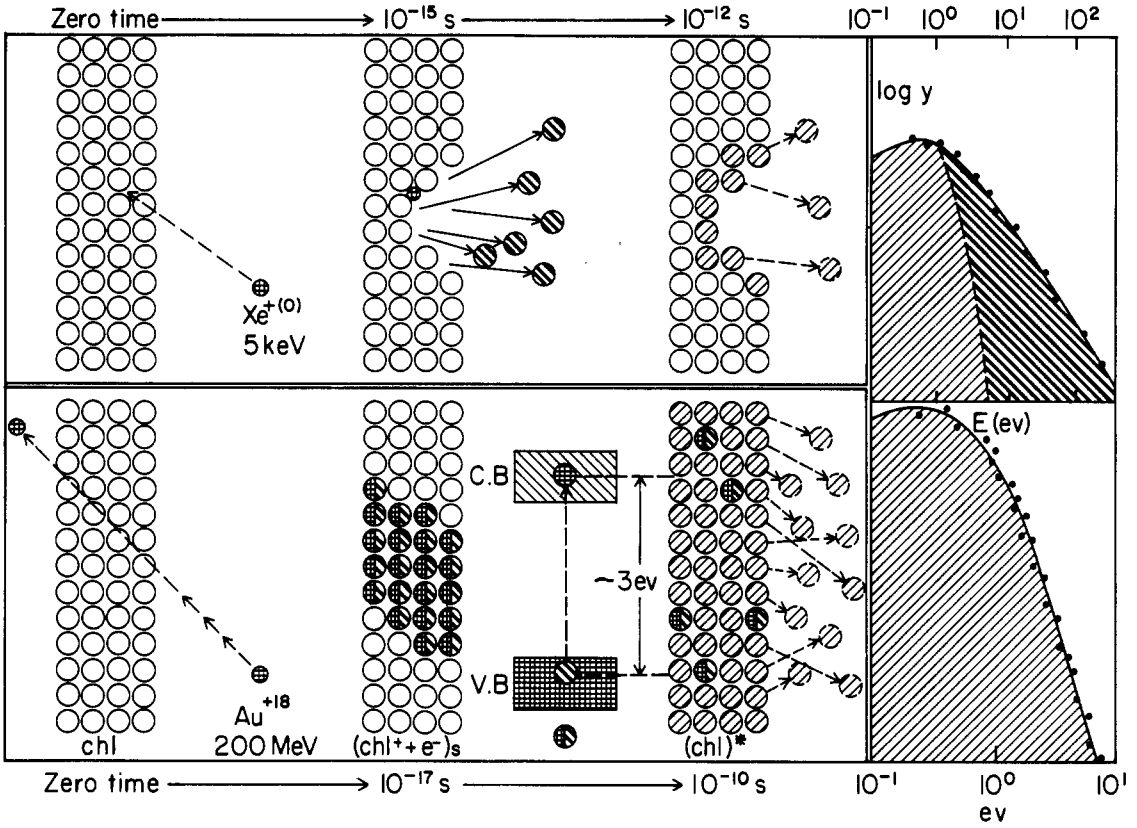


**Figure 3.** A portrayal of the time sequence of events for the particle-induced desorption process. The top portion refers to the nuclear excitation process (SIMS, FAB) where the incident particle initiates a collision cascade releasing first high energy atomic ions and in the later stages low energy molecular ions and fragment ions that form the structurally significant component of SIMS–FAB mass spectra. The lower portion illustrates the electronic excitation process in $^{252}$Cf PDMS (HIID). The ultimate particle, a 200-MeV $Au^{18+}$ ion, passes through more than a thousand molecular layers, producing an intense ionization track consisting of separated positive ion electron pairs. These recombine, and the energy release is transferred to phonon vibrations which propagate to the surface and induce desorption of low energy molecular ions. The low kinetic energy molecular ion component of SIMS is similar to that for $^{252}$Cf PDMS (HIID). The heavy-lined balls denote high energy atomic ions and the lighter-lined balls low energy molecular ions.

mica,[20] and up to 15 electrons are ejected from the surface of any solid excited by a fission fragment.[23] Yet despite the occurrence of these catastrophic events, they do not appear to be directly related to the desorption–ionization process. A monolayer deposited on a surface gives the same ion yield as does a deposit of 10 or 100 layers.[24] The desorption–ionization process for megavolt ion excitation clearly selects only molecules that are at or near the surface of the matrix.

In contrast to keV particle-induced desorption, the dynamics of molecular ion desorption by megavolt ions is completely decoupled from the incident ion trajectory; emission occurs with equal probability in the forward and back hemispheres and the kinetic energy distribution of molecular ions is an energy-shifted Maxwell–Boltzmann distribution ($\sim 10^4$ K).[25]

The surface area excited by an incident 100-MeV fission fragment may be as much as 200 times larger than for the keV ions of SIMS. This conclusion is derived by comparing relative molecular ion yields from a common matrix excited by keV and MeV ions.[19] The $^{252}$Cf PDMS work has thus far produced the largest molecular ions of biomolecules. This may be indicative that the area of excitation is important in the desorption of the very large species like insulin.

The development of a fission track can be described by using the lower portion of Figure 3 as a graphical representation of the sequence of events. Time zero is established with the impact of a 100-MeV heavy ion on the surface of the matrix. The nuclide, $^{252}$Cf, is a convenient source of these ions, but heavy ions in the same mass-energy range from a nuclear accelerator have now been employed (heavy ion induced desorption, HIID).[26] The insulin work is one of the important results to come from these studies.[1] The molecular ion yield is 35 times higher when the projectile enters at a 20° grazing angle than when it is normal to the surface demonstrating, as in SIMS (FAB), energy deposition in the surface region is an essential component of particle-induced desorption.[27] A bare 20-MeV $^{16}O^{8+}$ ion desorbs eight times more molecular ions than a 20-MeV $^{16}O^{2+}$ ion whose nucleus is shielded by six electrons and a 50-MeV $^{127}$I ion has a 25 times larger desorption yield than a 5-MeV $^{12}$C ion.[27]

The initial set of molecular exciton sites is schematically represented in Figure 3 at $10^{-17}$ s for a chlorophyll matrix. In the Ritchie–Claussen model, it is the migration and decay of these molecular exciton states that are the energy source for the generation of a thermal spike around the ionization track.[28] The ex-

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citon energy is transformed into intermolecular vibrations of the matrix (phonons) that propagate to the surface and contribute to the desorption process. An experimental kinetic energy distribution of desorbed species characteristic of megavolt ion desorption is also shown in Figure 3. This includes neutrals and ions.[28] It does not have the high-energy component featured in SIMS.

Although molecular ion desorption is essentially complete within $10^{-10}$ s, other processes continue. Alkali metal ion emission is sustained for periods up to a few microseconds,[29] and gross permanent changes develop in the matrix around the track: melting, lattice defects, and surface cratering.[19,21] If molecular ion formation were connected with crater formation, each fission fragment would produce many more molecular ions than are observed.

## The Desorption Process for Involatile Molecular Species

The molecule to be desorbed (the adsorbate) is bound to other molecules near the surface through the myriad of polar interactions that make the molecule involatile. These forces are so strong that it is very difficult to prevent aggregation from occurring. In $^{252}$Cf PDMS and SIMS, the aggregates comprise groupings of the involatile biomolecule, whereas in FAB, the aggregate is the involatile biomolecule solvated by molecules of the liquid polar matrix (e.g., glycerol).

Chlorophyll (Chl) has been a good model for studying molecular aggregation. Aggregation of Chl is enhanced in nonpolar solvents (e.g., dry octane), and this can be detected by energy shifts in the visible absorption spectrum. Thin films of Chl *a* prepared from these solutions produce molecular ion aggregates of Chl *a* up to the 7-mer in $^{252}$Cf PDMS;[30] the aggregates are sufficiently stable that even fragmentation of the aggregate (loss of a phytyl tail from one of the constituents) is observed.

There is evidence that molecular aggregation at the surface may be important for the desorption–ionization process. In $^{252}$Cf PDMS, although the probability is low that a given fission fragment desorbs a molecular ion or fragment ion of a complex species if it does desorb a molecular ion, one or more fragment ions are also desorbed with high probability in the same fission track.[25] Such correlations suggest that although a molecular aggregate is excited with low probability, when it is excited it "dissociates" into monomers and fragments of the monomer. Thin films consisting of small random aggregates made by electrospraying very dilute solutions give much lower molecular ion yields but equivalent fragment ion yields compared to deposits of the same thickness composed of larger, more ordered aggregates prepared from more concentrated solutions.[29] Sample impurities can quench molecular ion yields in $^{252}$Cf PDMS and SIMS; these may inhibit the formation of large ordered molecular aggregates for the same reason that impurities interfere with crystal formation. The FAB method, for which the molecular aggregates are solute–solvent clusters, seems not to experience this impurity–molecular ion quenching effect. This suggests that a solvated solute in FAB serves the role of the large ordered aggregate in solid films in influencing desorption and ionization.

If the molecular aggregate is the fundamental unit for the particle-induced desorption of strongly interacting biomolecules, then the molecular exciton may play a key role in energy transport and delocalization, just as it does in funneling solar energy through Chl aggregates to the reaction centers in photosynthesis.[13] Desorption and molecular dissociation might then be a consequence of the decay of molecular excitons to phonons within the cluster. For highly polar species (e.g., large unprotected oligonucleotides above the pentamer) where none of the particle-induced desorption methods have yielded molecular ions, it may be that the binding energy of a monomer unit in the aggregate is much larger than the available phonon energy that can be put into the desorption process.

In FAB, the solvated molecular cluster may serve an additional role by modifying the complex strong interactions of other monomer molecules.[10] The binding energy is effectively reduced and the monomer is more readily dissociated from the aggregate when it is excited. Furthermore, if solvated clusters are dissociated by the action of the incident beam, they can readily re-form in the liquid state whereas in SIMS of solid films high particle total currents result in molecular ion quenching.[31] A similar effect is observed in the $^{252}$Cf PDMS of UV-irradiated solid films.[30] Perhaps in the solid state large ordered aggregates can be irreversibly dissociated into small random aggregates by extensive energy deposition at the surface leading to a quenching of desorption–ionization as if impurities were introduced into the sample. The genius of FAB may not reside so much in the neutral charge state of the projectile but rather in the isolating, self-healing, powers of the solvated monomer in a mobile medium.

## Chemistry and the Ionization Process

The dynamics of the desorption process do not distinguish ions from neutrals, but it is the ionic component of the spectrum of the desorbed species that is detected in mass spectrometry. This is usually small. For example, the molecular ion yield in the $^{252}$Cf PDMS of $UO_2$ is only 0.3% of the total yield[32] of all desorbed U-containing species ($\sim$1500 per fission fragment).[22] Only rarely is the major component of the molecular ion yield simply that of the parent molecule ($M^{\pm}$). Generally, at some stage in the excitation process, the adsorbate has acquired or lost a hydrogen $(M \pm H)^{\pm}$, attached a cation $(M + Na)^+$ or anion $(M + Cl)^-$ or undergone hydrogen-exchange reactions $(M + 3Na - 2H)^+$. The large involatile molecules participate in these reactions. Palytoxin forms $(M + Na)^+$ ions ($^{252}$Cf PDMS); when it is deposited over a layer of CsI, it produces $(M + Cs)^+$ ions as well.[6] Molecular ions of large peptides observed in FAB are generally $(M \pm H)^{\pm}$.

Part of the recent research in particle-induced desorption has focused on the role of the matrix in enhancing molecular ion formation, on the incorporation of acidic components (e.g., toluenesulfonic acid) and

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cationic species (alkali metal halides) to increase the concentration of available reactant ions, and on sample pretreatment (e.g., low temperature annealing) to diffuse reactant ions to sites close to the adsorbate.[33]

There can be only two places where chemical transformations during particle-induced desorption can occur—on the surface and in the gas phase.[34] The gas-phase processes should resemble the well-established principles of chemical ionization, but surface chemistry ionization is a little beyond our present level of understanding. Benninghoven has recently shown that molecular ion yields from amino acid monolayer films in SIMS are sensitively dependent on the nature of the substrate.[35] This is a key observation since it not only proves the contributions of surface phenomena but also suggests a new variable in the development of particle-induced desorption for large molecules: the substrate–adsorbate interaction. Most experiments in particle-induced desorption have involved multilayer deposits, which means that the substrate and adsorbate are generally the same molecular species. Since the role of molecular aggregates has been suggested in the energy dissipation process, they may also influence the chemical changes that take place during the period of localized excitation. Large ordered molecular aggregates such as are formed in Chl may protect their constituents from high-energy internal excitation by providing a mechanism for energy delocalization via the molecular exciton. Evidence for this comes from the $^{252}Cf$ PDMS of Chl monomers isolated in a paraffin matrix where, in contrast to the normal spectrum, only fragment ions are observed.[36]

For the application of particle-induced desorption to large biomolecules like insulin, it is fortunate that some chemical reactions occur during the brief period of localized high excitation that produce the cationized or anionized molecular ions that appear in the mass spectrum. The term "preformed" ion has been used to suggest that these species already exist in the matrix prior to excitation. Except for FAB, where a solvated ion in the liquid matrix is an essential component of enhanced desorption–ionization, the particle-induced desorption methods use solid films in which, in the absence of a high dielectric polar fluid medium, charged species exist as ion pairs. A FAB experiment when the glycerol "runs dry" collapses to the more difficult problem of separating ion pairs from the solid state in the excitation process.

## A Model for the Ionization Process

A microscopic model is now proposed to account for the formation and desorption of large molecular ions from the surface of a matrix of molecules in a condensed medium. Because of local variations in the properties of surfaces comprised of molecular clusters of variable size, order, and composition, it is impossible to develop an exact analytical model based on the fundamental variables of surface ionization—Fermi surfaces, ionization potentials, quantities that have meaning for clean single crystals with specific orientation and well-prepared adsorbate states. The model presented here is an attempt to provide a conceptual basis for understanding ion formation and what might be done to improve or control the ionization process, particularly for the large biomolecules.

The basic premise in this model is that for the large involatile biomolecules surface ionization is the dominant mechanism; the final charge state of the desorbed species is determined by the substrate–adsorbate interaction during the desorption process. Many theories of surface ionization have been proposed in recent years. None deal with large organic molecular species per se, but a recent theoretical treatment of Nørskov and Lundqvist has some new features that are particularly attractive.[37]

Although formulated for simple adsorbate systems (atoms, small molecules) and single crystal substrates with well-defined electron states near the surface, the model can be at least conceptually cast within the framework of surface ionization of a collection of molecular aggregates. For a particular aggregate, the molecule to be desorbed is the adsorbate, and the rest of the aggregate constitutes the substrate. Intermolecular interactions within the aggregate couple the electronic states of each molecule, forming exciton bands. The multiplicity of the exciton band is dependent on the number of molecules in the aggregate, and the energetics of the exciton band is dependent on the relative orientation and degree of alignment of molecules within that particular aggregate.[13]

In the Nørskov–Lundqvist model, if the adsorbate is chemically identical with the substrate constituents (e.g., Chl *a* adsorbate on a Chl *a* aggregate), it will be electrically neutral. Further, there is a set of states in the adsorbate molecule that is most likely to receive an electron in the surface ionization, forming $M^-$ ion (lowest unoccupied molecular orbital states, LUMO), and a set of states where an electron is most likely to come from, forming an $M^+$ ion (highest occupied molecular orbital, HOMO). When the adsorbate is contained in the aggregate, these states coalesce into the exciton band structure. As the adsorbate recedes from the surface in the desorption process the HOMO, LUMO states of the desorbed species change energy, approaching that of an isolated molecule at infinite separation where they correspond to its electron affinity and ionization potential.

According to the Nørskov–Lundqvist model, the electron transfer takes place in a zone 0.2–0.3 nm from the surface.[37] This is schematically represented at the top of Figure 4. The adsorbate emerges from the zone as a positive or negative ion or as a neutral. The direction and probability for electron transfer are dependent on the interaction between adsorbate–substrate electronic states and the velocity component of the adsorbate normal to the surface. A generalized representation of the partitioning of the kinetic energy spectrum in surface ionization is shown in Figure 4. The neutral molecule distribution obtained from kinetic energy data on $UF_4$ + 5 MeV F is taken to be a "universal molecular desorption curve" for particle-induced desorption (with the high momentum atomic ion component of SIMS filtered out). Superimposed on

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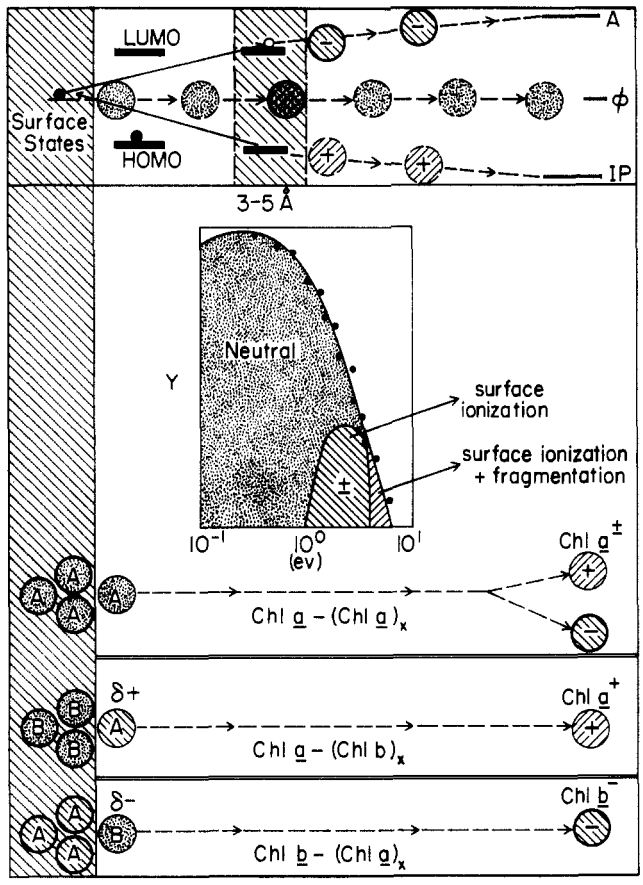


**Figure 4.** Surface ionization–desorption of molecular ions. The top portion models the Nørskov–Lundqvist mechanism[37] where the adsorbate bound to the substrate with a work function $\phi$ contains electron-donating and -accepting states (HOMO, LUMO) that become the ionization potential (IP) and affinity (A) states at infinite separation. The desorbed species passes through an ionization window 3–5 Å from the surface where the adsorbate is excited by interaction with the substrate and emerges as a positive or negative ion or neutral. The center insert represents a "universal" kinetic energy spectrum for particle-induced desorbed *molecular* species[29] partitioned by the surface ionization process into an ion kinetic energy component[25] and a neutral component. The lower section portrays the surface ionization of chlorophyll a (Chl a) from different aggregation states comprising pure Chl a, (Chl b)$_x$ – Chl a, where the Chl a adsorbate has a positive partial ionic character ($\delta^+$), and (Chl a)$_x$ – Chl b, where Chl b adsorbate has a negative partial ionic character ($\delta-$).

this is a "typical" ion kinetic energy spectrum which dominates the higher energy component plus an addition component (surface ionization–fragmentation) which is suggested here as a possibility since the excitation induced by the adsorbate–substrate interaction could involve vibrationally unstable molecular states.

While the dissociation of a "pure" molecular aggregate like Chl *a* leads to $M^{\pm}$ formation with near equal probability in $^{252}$Cf PDMS, a more general condition is that the adsorbate molecule is slightly different from the other molecules in the molecular aggregate but is still coupled to the electronic states of the substrate. This condition is explicitly treated in the Nørskov–Lundqvist theory. In this case, there is a net transfer of electron density to or from the adsorbate depending on the position of the affinity and ionization states of the adsorbate relative to the exciton states of the aggregate substrate, giving the adsorbate within the aggregate a net positive or negative charge. This influences the final charge state in the surface ionization when desorption occurs. These concepts have been tested by studying Chl *a* and Chl *b* aggregates and observing molecular ion yields by $^{252}$Cf PDMS. Chl *b* is slightly more electronegative than Chl *a* because it contains a formyl group in the C-3 position in place of a methyl group but its overall structure is sufficiently similar that mixed aggregates form. Chl *a* in a molecular aggregate of Chl *b* would be expected to have a positive partial ionic character ($\delta^+$). In the $^{252}$Cf PDMS of a 1:1 Chl *b*:Chl *a* aggregate, the intensity of the $M^+$· ion of Chl *a* is indeed significantly larger than for Chl *b* and more $M^-$ ions of Chl *b* than of Chl *a* were produced. These findings are summarized graphically in the lower half of Figure 4.

As speculation on how cationized [$(M + H)^+$, $(M + Na)^+$] or anionized species [$(M - H)^-$, $(M + Cl)^-$] could be formed in the framework of this model, one can visualize the following sequence of events. Some molecular aggregates contain molecules that have reactant interstitials such as alkali metal–halide ion pairs or residual solvent molecules associated to them through dipole–dipole interactions. Excitation of the aggregate initiates chemical reactions of these interstitial species, with some of the members of the aggregate giving to them a net preformed partial ionic character; thus addition of $Na^+$ can give $(M + Na)^{\delta+}$, hydrogen abstraction can give $(M - H)^{\delta+}$, and reaction with $CHCl_3$ can give $(M + Cl)^{\delta-}$. This is followed by the desorption process in which the species pass through the zone of surface ionization and emerge into vacuum as positive or negative ions or neutrals.

The molecular ion spectrum that is observed in particle-induced desorption is then a representation of the population of molecular species at the surface with varying degrees of partial ionic character filtered by the selectivity of the surface ionization process. This process chooses from among all desorbed species the fastest, those with energetically favorable affinity or ionization states, and those with the strongest adsorbate–substrate electronic interaction to form the mass spectra observed in particle-induced desorption. Inspection of recent FAB mass spectra of large molecules taken in high resolution clearly show that although an $(M + H)^+$ or $(M - H)^-$ ion may dominate the spectrum in the molecular ion region, small components of $M^+$, $(M - H)^+$, $M^-$, $(M + H)^-$ are generally present.[10] This indicates that the outcome of the surface ionization process for a particular adsorbate may not be totally predetermined but is, like any other quantum-mechanical process, governed by transition probabilities.

It certainly is easier to form molecular ions of a molecule like insulin than to understand *how* they are formed in particle-induced desorption. But when the pieces of the desorption–ionization mechanism are finally put together, we may be able to do more than helplessly accept the low or nonexistent molecular ion yields presented to us by a randomly prepared and ill-defined matrix.

## Concluding Remarks

The impetus for extending mass spectrometry to high molecular weight biomolecules is that it will find a function in analytical molecular biology for the estimation of molecular weights, complementing gel permeation chromatography and sedimentation equilibrium but enabling much more accurate determinations

to be made. The difference between human or bovine insulin or even the presence of one genetic error in its 51 amino acid structure can *now* be detected by mass spectrometry. The insulin work gives encouragement that higher molecular weight proteins will give interpretable mass spectra. If one were able to determine the molecular weight of an unknown protein in the 10 000 to 20 000 range *prior* to sequence analysis, one would then be in a position to design a more efficient protocol for its sequence determination.

To extend these methods to higher molecular weights, the size of the molecule relative to the area of excitation will eventually become an important consideration. The chemically protected oligonucleotides are a convenient molecular gauge to study this question since each residue is ~0.5 nm long and the protecting groups foster a linear tertiary structure. The largest studied thus far by $^{252}Cf$ PDMS is a 14-mer, a 7-nm-long molecule with the same long dimension as hemoglobin, which is a medium-sized globular protein with a molecular weight of approximately 65 000. Whether science would be enlightened by knowing the molecular weight of hemoglobin more accurately is not immediately obvious. Perhaps these kinds of measurements would provide answers for which there are no questions. Nevertheless the history of molecular biology has demonstrated that higher resolution generally leads to deeper levels of understanding.

So, if these be the goals of particle-induced desorption mass spectrometry, the space yet to be covered includes energy transport in molecular solids, surface chemistry in the high-energy–short-time domain, and the dynamics of surface-ionization desorption for large organic molecules—the fundamental ingredients of the process, which, considered separately, constitute interesting fundamental studies in physics and chemistry.

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# Nuclear, Electronic, and Frequency Factors in Electron-Transfer Reactions

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Electron-transfer reactions are distinguished by their ubiquity and by their essential roles in many physical, chemical, and biological processes. Thus, understanding the factors which determine electron-transfer rates is of considerable importance.

Although a number of theories have been proposed,[1-15] there is general agreement that the crux of the electron-transfer problem is the fact that the equilibrium nuclear configuration of a species changes when it gains or loses an electron. In the case of a metal complex this configuration change involves changes in the metal–ligand and intraligand bond lengths and angles as well as changes in the vibrations and rotations of the surrounding solvent dipoles.

These configuration changes are similar to those that result from the electronic redistribution that occurs when a molecule absorbs or emits a photon. In view of the similarity of the nuclear configuration changes resulting from electron transfer and photon capture (or emission), and, more importantly, because the two processes occur rapidly on the nuclear time scale, the rates of thermal electron transfer, electronic energy transfer, and a variety of nonradiative processes can be understood in terms of a common theoretical framework.[15] Within this framework the rate constants can be expressed as a product of a nuclear, an electronic, and a frequency factor.

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