DEVELOPMENT OF LASER-ION BEAM PHOTODISSOCIATION METHODS

Progress Report
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During this report period our research efforts have concentrated on the continued development of the tandem magnetic sector (EB)/reflectron-time-of-flight (TOF) instrument, preliminary experiments with the tandem TOF/TOF instruments, developing the methodology for performing photodissociation with pulsed lasers, experiments with laser ionization of aerosol particles, fundamental studies on matrix-assisted laser desorption ionization (MALDI), and ion-molecule reaction chemistry of ground and excited state transition metal ions.

The "PROJECT PROGRESS REPORT" is divided into three (3) sections: (I) PHOTODISSOCIATION, (II) MATRIX-ASSISTED LASER DESORPTION IONIZATION STUDIES (including MALDI of aerosols), and (III) ION CHEMISTRY FUNDAMENTALS. The instrument development in support of each of these areas is included with that particular section.

I. PHOTODISSOCIATION STUDIES

Our photodissociation work is aimed toward developing structural mass spectrometry methods using pulsed lasers. The long-term objectives are to develop new structural characterization methods for complex molecular systems, including, but not limited to biomolecules and clusters. Pulsed lasers provide the highest level of sensitivity and the greatest amount of experimental versatility. The studies address the fundamentals of photodissociation and the development of experimental hardware to perform such studies.

TOF instrument development is a highly active research area because new mass spectrometry methods have opened new research frontiers. The performance of TOF instruments is now comparable to that of the highest quality analytical instruments and low-cost, high reliability electronics make this a highly desirable instrument for environmental and biological research.

Development of tandem EB/R-TOF instrumentation. The development of instrumentation for performing tandem mass spectrometry with TOF detection has rapidly progressed. The construction of a second generation EB/R-TOF instrument was completed in the previous report period, and our efforts over the past year have focussed on using this instrument for a variety of tandem mass spectrometry experiments. Papers on correlated ion-neutral detection (included in last years report) and signal-to-noise enhancement for ion-neutral correlation (listed "in press" in last years report) have been published. In addition, a review chapter was prepared as a part of an ACS symposium organized by Prof. R. J. Cotter (Johns Hopkins University) describing both the instrumentation and applications of the instrument for photodissociation. Current research is focussed on demonstrating
ultra-high sensitivity tandem mass spectrometry experiments for characterizing trace level water soluble organics. The water-soluble organics project is a collaborative program with Texaco, and tandem mass spectrometry is one of the methods used for this research.

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Development of TOF/TOF Instruments for Photodissociation Studies. A direct spin-off of the EB/RTOF research has been the development of a prototype TOF/TOF instrument for photodissociation studies of ions formed by laser ionization. Structural characterization of large molecules by tandem MS methods is difficult for compounds with molecular weights greater than 1000-1500 da. The limitation arises due to the low cross-sections for collision-induced dissociation (CID) of large ionic systems coupled with the low ion transmission of sector type instruments. TOF instruments have much higher transmission efficiencies, especially for truly large molecules (MW > 5000), and are more compatible with pulsed lasers for performing photodissociation. Therefore, it appears feasible to combine tandem TOF instruments with matrix-assisted laser desorption ionization (MALDI) and pulsed-laser photodissociation for structural mass spectrometry of large molecules. The prototype instrument developed for this research is novel and consist of two linear TOF instruments (see Figure 1). TOF-I is 50 cm in length and the ions are accelerated to 10 keV (from 20 keV to 10 keV), the ions drift through the laser interaction region, and are then accelerated by an additional 10 keV (10 keV to ground). The two-stage acceleration allows parent ions (denoted m\textsubscript{1}) to be separated from product ions of dissociation (either metastable ions, CID fragment ions, or photofragment ions denoted m\textsubscript{2}). In addition, because ions receive the full acceleration and neutrals (formed by metastable dissociation reactions) formed in region 1 only receive the initial acceleration, ions and neutrals are dispersed in time.

We have performed preliminary experiments on several model compounds to evaluate the potential utility of the TOF/TOF experiment for analytical photodissociation studies. Figure 2 contains ion/neutral (lower trace) and photodepletion spectra (upper trace) for three model peptides. Note that the ion
Examples of the photodepletion response have also been observed for bradykinin, mellitin, and bovine insulin. It appears that even ions as large as bovine insulin may be photodissociated by 193 nm photons using the approach described.
signal attenuation when the laser is fired indicates that some fraction of the \( m_1 \) ions have been converted to fragment ions, and attenuation of the neutral signal indicates that some fraction of the neutrals have been ionized, presumably by and MPI type process. The photodepletion spectra suggest an unexpectedly high sensitivity for this type of experiment. For instance, ion/neutral spectra such as that shown can be obtained on a few femtromoles of samples, thus we anticipate comparable spectral quality for photofragment ions.

I want to stress two points about this work: (i) the studies clearly demonstrate photodissociation with femtomole level detections is feasible, and (ii) molecules as large as bovine insulin (MW 5735) can be photodissociated using 193 nm radiation. Our prior work on photodissociation of large molecules has been hampered by the transmission efficiency of the EB/R-TOF instrument, but we feel confident that these objectives are feasible with TOF/TOF instruments.

We are continuing the work on photodepletion of large molecules and ionization of the the neutrals in order to understand various aspects of the MALDI process. A reflectron for TOF-2 is currently being built. The reflectron is needed in order to improve the mass resolution of the photofragment ions and the MPI of neutrals.

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The work described above is not yet ready for publication, but preliminary results were presented at recent meetings. Before this work is published I want to be able to show a high quality photofragment ion spectrum of either melittin or bovine insulin. I project that this paper will be ready for submission by October 1993.

We have collaborative projects with two biochemist (T. A. Baldwin and T. Hayes) on protein folding. Photodissociation of partially reduced bovine insulin could greatly accelerate our efforts in this area. That is, MALDI is excellent for following the kinetics of the disulfide reduction reaction (described in 2) listed below), but fragment ions are not formed and it is impossible to determine (by MS) which disulfide bonds are reduced. If we could induce dissociation of the partially reduced peptide, and fragmentation occurred near the sulfur containing residues, we could determine which disulfide bonds are reduced first.

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Photodissociation Studies of MALDI formed ion by FTICR

Fourier-transform ion cyclotron resonance (FTICR) mass spectrometry is a trapped ion method, consequently ions can be irradiated for extended periods of time to improve the conversion to photofragment ions. On the other hand, ICR photodissociation methods involve long irradiation times and it possible to perform photodissociation on photofragment ions (sequential processes) and ion-molecule collisions can occur and new product ions may be formed and also undergo photodissociation. In addition, because the timeframe for ICR is much longer than that for beam instruments (ms-s as compared to 1-100 us) the types of photofragment ions observed may differ. The shorter timescale sampled by beam experiments could favor simple-cleavage fragment ions, whereas the longer timescale sampled by ICR experiments could favor ions formed by rearrangement reactions. Thus, comparison between the two experiments are beneficial for both analytical and fundamental reasons.

We recently completed a study on a series of compounds that serve as model systems for ICR photodissociation. The studies demonstrate the utility of ICR photodissociation for characterizing large molecules. Abundant photofragment ions are observed for all compounds examined and extensive photofragmentation of vitamin B_{12} is observed at relatively low laser power. In addition, we show that UV absorbing peptides, e.g., a dinitrophenyl derivatized peptide, can be photodissociated to yield structurally significant ions. Follow-up studies to this work are underway and we plan to examine these same systems using the TOF/TOF apparatus. These studies are also important for both TOF and FTICR MALDI experiments (see below).

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1) "How Do Mixed Matrices Help Ion Detection in FTICR Mass Spectrometry Experiments?" T. Solouki; D. H. Russell PitCon 93

II. MATRIX-ASSISTED LASER DESORPTION IONIZATION STUDIES

The MALDI projects encompass numerous areas concerning analytical laser desorption ionization (LDI) and fundamental studies on the mechanism of ion formation. The fundamental studies differ from conventional studies on the ion formation mechanism. That is, most studies on LDI ion formation focus on the
physics of the process (e.g., energy deposition in the solid or liquid phase), whereas our studies focus on the chemical processes that yield the ionic species. In particular, we are focussing our attention on the proton transfer reactions leading to formation of the [M+H]+ ion.

General Studies on MALDI Developing conditions that produce a high abundance of intact ions by MALDI is extremely important to many areas of our research, especially photodissociation. Consequently, we have put a large amount of time into understanding experimental parameters that influence the ion yield.

Development of new matrices In an effort to understand the ion formation process and to optimize the conditions for ion formation we have examined a large number of potentially useful MALDI matrices. The thoughts guiding our selection of potential matrices are the well-known excited state acidities/basicities of substituted aromatic compounds. These concepts are adequately laid out in the last proposal.

The papers listed below all contain sections dealing with comparing ion yields obtained using different matrices and/or reports on new matrix compounds. One feature of particular note is a result in paper #2. For instance, 4-nitroaniline and 4-nitrophenol are excellent matrices for taxol and taxanes, but the conventional matrices (sinapic acid and alpha-cyano-4-hydroxy-cinnamic acid) do not work at all for this class of compound. Also, 4-nitroaniline yields almost exclusively the [M+Na]+ ion, whereas 4-nitrophenol yields predominantly the [M+H]+ ion. The ions yields are not the result of excess Na in the 4-nitroaniline matrix, thus the ion yields must be related to the intrinsic acidity of the 4-nitrophenol matrix relative to that for 4-nitroaniline. More detail studies on this are underway.

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5) “Analysis of High Mass Proteins using 4-Nitroaniline in Matrix-Assisted


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Photodissociation of Ions formed by MALDI The general area of photodissociation of MALDI formed ions is discussed above, but one particular aspect that is important to both the mechanism of MALDI and photodissociation are the neutrals (see Figure 1 above). The neutral background signal serves to interfere with detection of photofragment ions (in the beam experiments), thus we have examined the neutral products as a function of matrix and other experimental parameters.

Mechanistic Studies of MALDI using the E-TOF The E-TOF instrument built a couple of years ago is now being used to measure the kinetic energies of ions formed by MALDI. These measurements clearly show that ions are formed with two distinct kinetic energies. The dominant fraction of MALDI ions show negative kinetic energy shifts, energies less than the ion source accelerating voltage. We interpret these data as evidence of ion formation in the gas phase, e.g., away from the probe surface by 20-100 um. The amount of kinetic energy shift is matrix dependent and laser power dependent. Several papers (at least 4) on this work in in preparation.

MALDI of Aerosols The MALDI-aerosol experiment (described in last renewal application) has rapidly progressed since December 1992. We now understand the key parameters in this experiment. One of the most important parameters is to dry (by gas phase collisions) the sovent/matrix/analyte droplets. Another important parameter is the size distribution of the droplets, small droplets (less than 10 um
diameter) ionize the best.

We are now using the aerosol-MALDI experiment to probe fundamentals of H+-transfer reactions, and we are pursuing both on-line reaction monitoring of solution phase reactions and LC of biomolecules. Both aspect of the experiment are working beautifully!!

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Continued progress in this area is limited by hardware. The experiment needs a high rep rate laser (500 hz. minimum) and faster TOF data acquisition. The area of fast data acquisition for TOF is rapidly advancing and we are working with two companies involved in this area. I have written a new proposal to NSF Biology Division for funds to develop the LC-MS portion of this apparatus, and a proposal for development of aerosol-MALDI/FTICR will be submitted to NSF in September 1993. Additional funds are also needed for the fundamental work covered under the DOE/OBES program. This is the reason for the supplemental request that I submitted to you several months ago.

III. FUNDAMENTAL ION CHEMISTRY

The current emphasis of the ion chemistry research is on long-lived excited states of transition metal ions. Several metal systems have been studied, e.g., Cr+, Co+, and Mn+, but the most emphasis has been placed on Fe+. In part, the emphasis on Fe+ is due to the complexity of this system and the diverse range of reactivities observed for this ion. We have developed ICR based methods to measure the distribution of electronic states formed (paper #1), and a two-section ICR cell experiment (paper #2) for studying the collisional relaxation reactions of the metal ion excited states. The method used in paper #2 was also used to isolate a specific electronic state of Fe+, viz. 6S (3d^5s^2). The state selected ion-chemistry paper is truly novel and has considerable impact on the entire area of metal ion chemistry.

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2) "Ion-Molecule Reaction Chemistry of Fe+ with NO: Excited Versus Ground State Reactions," J. V. B. Oriedo; D. H. Russell J. Am. Chem. Soc. in press
