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**Dynamics of Initial Ionization Events in Biological Molecules:
Formation and Fate of Free Radicals**

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I. Introduction

Study of early time events following the absorption of electromagnetic radiation in biological systems has potentially significant impact on several areas of importance. In this context, the studies being conducted under this program provided insight into the conformational changes as well as the reactions leading to a variety of transformations that culminate from hydrogen atom and proton transfer events. These studies enabled an investigation of molecular details of structure-function relationships.

In a second aspect of the program, investigations were conducted to provide basic underpinning research that contributed to a quantification of the behavior of radionuclides and pollutants associated with advanced energy activities after these materials emanate from their source and become transferred through the environment to the biota and human receptor. The studies on the interaction of ionizing radiation with biological matter provided valuable data in the area of radiation biology, including the generation of free radicals upon the absorption of radiation. They also find use in assessing photoinduced transformation of pollutants.

Our approach to elucidating factors governing the difference between reactions in the gas and condensed phase was to study the initiating steps at progressively higher degrees of cluster aggregation. We employed ultrafast laser techniques, in combination with selected molecules, carefully prepared in tailored compositions, to investigate the primary mechanisms involved in various molecular functional groups following the absorption of electromagnetic radiation. During the last period of the program, we studied various molecules representing chromophores in such biologically important molecules as tyrosine and amines. Determining the influence of solvation on the OH group of phenol-like molecules has been, for example, very instructive in elucidating the mechanisms of energy dissipation as well as proton and hydrogen atom transfer.

This work was originally sponsored through a grant entitled "Clusters: Elucidating the Dynamics of Ionization Events and Ensuing Reactions in the Condensed Phase, Contract No. DE-FG02-88ER-60648 and a final report dealing with that work through 2/28/94 has been submitted. The present report covers the additional period 5/1/94 through 12/31/95. Through the publication of nine new papers on this subject, our work has provided additional critical information on problems in radiation biology.

II. Publications Arising from Grant No. DE-FGO2-93ER61602: Dynamics of Initial Ionization in Biological Molecules: Formation and Fate of Free Radicals

385. "Formation and Metastable Decomposition of Unprotonated Ammonia Cluster Ions Upon Femto-second Ionization," S. A. Buzza, S. Wei, J. Purnell, and A. W. Castleman, Jr., *J. Chem. Phys.* **102**, 4832-4841 (1995).
386. "Growth Pattern of Zirconium-Carbon Clusters: Face-Centered Cubic or Multicage Structures?", S. Wei and A. W. Castleman, Jr., *Chem. Phys. Lett.* **227**, 305-311 (1994).
406. "Ultrafast Laser Induced Coulomb Explosion of Clusters with High Charge States," J. Purnell, E. M. Snyder, S. Wei, and A. W. Castleman, Jr., *Chem. Phys. Lett.* **229**, 333-339 (1994).
418. "Dynamics of Cluster Reactions and Ionization: From Semiconductor to Hydrogen-Bonded/van der Waals Systems," A. W. Castleman, Jr., B. D. May, S. F. Cartier, K. P. Kerns, H. T. Deng, E. M. Snyder, and S. A. Buzza, *NATO ASI Series on Large Clusters of Atoms and Molecules*, Kluwer Academic, Dordrecht, pp 371-404 (1996).
420. "Probing the Structure and Dynamics of Cage-Like Clusters: From Water to Met-Cars," A. W. Castleman, Jr., in *Atomic and Nuclear Clusters* (G. S. Anagnostatos and W. von Oertzen, Eds.) Springer-Verlag, Berlin, pp 160-166 (1995).
422. "Real-Time Dynamics of Ammonia Clusters Excited Through the $\tilde{A}$ State: Formation of the Protonated Cluster Ions," E. M. Snyder, J. Purnell, S. Wei, S. A. Buzza and A. W. Castleman, Jr., *Chem. Phys.* **207**, 355-366 (1996).
424. "Femtosecond Laser-Induced Coulomb Explosion of Ammonia Clusters," E. M. Snyder, S. Wei, J. Purnell, S. A. Buzza, and A. W. Castleman, Jr., *Chem. Phys. Lett.* **248**, 1-7 (1996).
428. "Intracuster Excited State and Ion-Molecule Reaction Dynamics of Weakly Bound Clusters," S. A. Buzza, E. M. Snyder, D. A. Card, D. E. Folmer and A. W. Castleman, Jr., in *Femtochemistry: Ultrafast Chemical and Physical Processes in Molecular Systems* (M. Chergui, Ed.) World Scientific, Singapore, pp 149-156 (1996).
431. "Further Direct Evidence for Stepwise Dissociation of Acetone and Acetone Clusters," S. A. Buzza, E. M. Snyder and A. W. Castleman, Jr., *J. Chem. Phys.*, **104**, 5040-5047 (1996).

III. Overview of Recent Findings (See Publications Listed in Section II for Details.)

The absorption of ionizing radiation initiates both physical and chemical interactions which culminate in transformations that alter the structure and function of biological molecules, and ultimately lead to the damage of biological systems. The influence of ionization on the ensuing chemical reactions which take place following the initial physical event, depends on subsequent mechanisms of energy transfer and dissipation away from the primary site of absorption. The primary molecular groups adjacent to the site of energy absorption ("chromophore"), as well as neighboring solvent molecules or bound ligands, can influence the energy transfer processes through collisional deactivation (removal of energy), via effects in which dissociating molecules are kept in relatively close proximity due to the presence of the solvent (cage effects), and in other ways

where the solvent influences the energetics of the reaction coordinate (i.e., the opening of reaction channels through effects on potential energy barriers to reaction).

A prime objective of our program has been unraveling the reactions and structural changes which occur in various functional groups that are the counterparts of those present in biological systems. Attention has been focused on ones which can be isolated, thereby enabling study of the microscopic details of transformations that occur following the absorption of ionizing radiation. The studies utilized ultrafast laser techniques to provide critical information on mechanisms following the early time events. An inherent nature of biological systems is their extensive interaction with neighboring molecules through ligand-ligand bonding, and especially solvation. Studies of isolated gas phase molecules, while providing some useful data, are of somewhat limited value since they do not involve any effects due to solvation on the reaction energetics or dynamics. On the other hand, condensed phase studies are sometimes difficult to implement and interpret. Studying cluster systems, in which the composition has been carefully tailored, provides a unique way of investigating the detailed mechanisms involving structural rearrangements and their influence on the course of subsequent reactions.

We have published more than 29 papers in the open scientific literature dealing with the results of our findings in this area; those arising from the current period are listed in Section II. Our approach to elucidating factors governing the difference between reactions in the gas and condensed phase has been to study the initiating steps at progressively higher degrees of aggregation. The main thrust of the research has been directed to unraveling fundamental processes on the femtosecond time scale involving: 1) early time events and processes responsible for dissipating the energy away from the site of initial deposition of ionizing radiation, 2) reactions of free radical and other transient intermediates formed as a result of processes occurring during the initial ionizing event, and 3) the selective ionization of biological molecules to study their bonding, ensuing reactions, and contribute to new methods for use in sequencing and analysis.

1. Dynamics of Ionization and Early Time Events

Various molecules representing chromophores in such biologically important molecules as tyrosine and amines have been investigated. Determining the influence of solvation on the OH

group of phenol-like molecules has been very instructive in elucidating the mechanisms of energy dissipation as well as proton and hydrogen atom transfer.

a. Reaction Mechanisms of NH_x and Related Proton and Hydrogen Atom Transfer Reactions

Each of the amino acids, and various base pairs (i.e., adenine, cytosine and guanine) also contain NH_x groups whose reaction following the absorption of ionizing radiation, influences their structure-function relationships. Hence, some of our attention has continued to be focused on unraveling the chemistry associated with the amino groups. In this context, investigation of the reactions of ammonia under varying degrees of solvation provide particular insight into the role of this molecular functional group in influencing the ensuing chemistry of biological molecules.

Recent experiments in our laboratory have examined the role of hydrogen transfer upon the ionization dynamics of ammonia clusters. These experiments have focused on the detection of the protonated ammonia cluster series, $(\text{NH}_3)_{n-1}\text{H}^+$. Two mechanisms have been proposed to account for the formation of protonated ammonia clusters under multiphoton resonant ionization conditions. They are absorption-ionization-dissociation (AID) and absorption-dissociation-ionization (ADI). The ADI mechanism was initially proposed based on theoretical calculations, and supported by findings that hydrogenated ammonia clusters can have lifetimes of a few microseconds following neutralization of protonated cluster cations. The fact that protonated ammonia clusters are formed under electron impact and single photon ionization conditions, provides evidence that the AID mechanism must be operative, at least in some situations. The femtosecond pump-probe studies performed by our group provide a detailed and complete picture for the formation of various protonated ammonia cations produced by ionization of clusters through the C' and A states. Two distinct pathways are considered:

The absorption-ionization-dissociation or AID mechanism (1) involves the direct ionization of the molecule after excitation to an intermediate state followed by proton transfer and fragmenta-

tion, while the absorption-dissociation-ionization or ADI mechanism predicts that after excitation to an intermediate state, hydrogen transfer occurs, followed by ionization of this excited fragment. In either case, the major observed ion series corresponds to protonated clusters. Previously, we have shown that the operative mechanism depends upon the intermediate state accessed by the initial excitation; the ADI mechanism is operative when ionizing through the $\tilde{A}$ Rydberg state of the ammonia monomer at time delays greater than the experimental pulsewidth, while the AID mechanism is operative when ionizing through states corresponding to the $\tilde{B}$ or $\tilde{C}$ Rydberg states. An assumption of the AID mechanism is that the unprotonated cluster is ionized, a process which is then followed by rapid proton transfer (within the ionization region resulting in the detection of the protonated series). Although the major series in the time-of-flight spectra corresponds to protonated clusters, the unprotonated series, $(\text{NH}_3)_n^+$, is also observed. Since these species exist before the proton has been transferred, investigating their behavior after ionization yields insight into the proton transfer process.

The time response features measured in our studies for the $\tilde{A}$ state are in accord with the following mechanism:

It should be noted that the rapid intensity drop observed for all protonated cluster ions when $n \geq 2$ is attributed to step 2, where NH_2 or H containing species cannot be readily ionized. It is proposed that this species is on the peak of the barrier to predissociation, in which it can return to step 1 or proceed to steps 3 and 4. Step 3 leads to formation of long-lived radicals in accordance with the findings of non-zero ion intensity values at long pump-probe delays observed in the data for the $\tilde{A}$ state.

A mathematical analysis of the data was carried out to quantitatively model the reaction dynamics involved in the ionization processes. It is worth noting that several other mechanisms were also considered, but none of the resulting curve fits agreed with our experimental findings. The lifetimes ($1/k$) are obtained from the study. Depending on cluster size, the lifetime of steps 1 and 2 are approximately 0.5-1 ps. The lifetime of step 3 is 20-50 ps, and 1-5 ps for step 4. See Figure 1. Details of the findings are found in paper # 422.

Another study was conducted during the report period to investigate the proton transfer process; a 624nm, 350 fs laser was used to ionize ammonia cluster through states corresponding to the $\tilde{B}$ or $\tilde{C}$ states of the ammonia monomer and the metastable (long time $\sim$ microsecond) decay patterns of the unprotonated series were investigated. The decay channels were studied through the use of reflecting potentials (reflectron) that determine the kinetic energy of both parent and their metastable daughter fragment ions. Once these energies are ascertained, the daughter mass may be determined. The unprotonated cluster series, $(NH_3)_n^+$ where $n = 2$ through 5, were examined using this method, and it was found that the unprotonated series loses an NH_2 unit. This is very significant in elucidating the time evolution of the NH_2 free radical and indicates that the long time behavior involves first a proton transfer followed by fragmentation:

Therefore, studying the metastable decay behavior of the $(NH_3)_n^+$ series under various conditions also contributes to an understanding of some of the driving forces involved in the proton transfer.

Since collision free conditions are not totally attainable in the experiments, this leaves open the question of the spontaneity of the observed proton transfer. By combining the rate equations for both spontaneous and collision driven proton transfer reactions, the role of initial conditions and collisional partners can be examined. The rate equations for the two processes are as follows:

$$-d[(NH_3)_n^+]/dt = k_{\text{spontaneous}} \quad (4)$$

and

$$-d[(NH_3)_n^+]/dt = [(NH_3)_n^+][\text{Collision partner}] \bullet k_{\text{collision}} \quad (5)$$

Combining these two equations, varying the number of collisions, and investigating the metastable decay fraction over a constant time interval, the spontaneous and collisional components may be

Figure 1(a). Plot of the values of the rate constants of the 208 nm pump wavelength ($v=2$) experiments, from the dynamical scheme shown in Figure 1(b), versus cluster size.

Figure 1(b)

determined. The effect of cluster formation conditions upon the proton transfer reaction was also studied. Additional findings of the work can be found in publications 385 and 418.

b. Ultrafast Laser Induced Coulomb Explosion of Clusters with Charge States

Study of the interaction of atoms and molecules with large laser fields can also lend insight into the mechanisms following the absorption of intense electromagnetic fields. Using a femtosecond laser coupled with a reflectron time-of-flight mass spectrometer, we recently undertook a study of the ionization of hydrogen iodide clusters, as well as co-clusters of HI with argon, in intense laser fields. The hydrogen atom system provides insight into the ionization of molecules having this light atom present, while the iodine is a prototypical heavy atom; the argon work can be used to model systems which can undergo simple electron transfer without the additional complication of a chemical reaction. The results are also of value in unraveling the influence of heavy charged particles on energy transfer, ensuing ionization and charge transfer reactions. Comparing the results of HI/Ar_n clusters with those of (HI)_n is instructive in studying the influence of solvation on the charge transfer dynamics, too. Also, we are hopeful that the new methods of studying coulomb explosion may prove to be useful in imaging the structure of biological molecules, an aspect of the problem which we intend to pursue in the future.

Multicharged iodine atoms as highly charged as I⁺¹⁷, and multicharged argon atoms as highly charged as Ar⁺⁸ were found following the absorption of intense radiation. The I⁺ⁿ are identified to be formed through the coulomb explosion of HI and its clusters, while the Ar⁺ⁿ are produced via the coulomb explosion of HIAr_m clusters. To the best of our knowledge, the present study provides the first observation of such highly charged iodine and argon atoms arising from the coulomb explosion of clusters. Significantly, kinetic energy releases of several hundred eV were determined in the resulting process.

The loss of hydrogens due to photofragmentation occurs on a long timescale such that the detected distribution has a composition of H_mI_p⁺, where 0 ≤ m ≤ p+1. Under the present conditions, clusters containing up to 6 iodine atoms are observed. As shown in Figure 2, prominent peaks are

Figure 2. Time-of-flight mass spectrum of multicharged iodine atoms resulting from the coulomb explosion of HI monomer and HI clusters. The voltage across the first two TOF lenses is 500 V.

observed which correspond to I^{+n} fragments. Charged species with $n=17$ are clearly discernible in the figure. In any coulomb explosion of HI monomer and its clusters which takes place in the TOF lens assembly, some I^{+n} fragments will be ejected toward, while others will be ejected away from, the detector. This gives rise to a broad peak for the multicharged fragments having an early arrival time, followed by a sharp peak arising from space focusing for those species having a later time of arrival. The delay in the time of arrival results from the time necessary for the ions to be turned toward the detector by the accelerating field applied between the TOF lenses.

Figure 3 displays a time-of-flight mass spectrum of I^{+n} , with the field gradient lowered to 260 V. Using the peak splittings from this spectrum, the kinetic energies (KE in units of eV) are calculated using the following equation:

$$KE = \frac{(\Delta t)^2}{m} (U_1 - U_2)^2 (0.1204) q^2 \quad (6)$$

where Δt is the peak splitting in microseconds, m is the mass in amu, $U_1 - U_2$ is the voltage gradient, q is the charge, and 0.1204 is a constant which accounts for the units. The values calculated from the above equation, along with those determined from the voltage cut-offs, are given in Table I. The kinetic energies determined from the two independent methods are seen to be in good agreement.

The kinetic energy release values which we have measured cannot arise from the coulomb explosion of HI monomer because they would involve unallowably small internuclear distances between H^+ and I^{+n} . Also, note that most of the total energy in the process would be partitioned into the hydrogen ion fragment. A plausible explanation for the large kinetic energies observed in the present study is that they result from the coulomb explosion of HI clusters. Since the clusters contain more than one iodine atom, it is possible that repulsion of two or more highly charged iodine ions occurs, which would result in a large kinetic energy release. This is further supported by our observation of multiple peaks for each multicharged species, suggesting that they are originating from more than one source. With the current experimental set-up it is not possible to determine the exact parentage of the multicharged ions; but, such large values of kinetic energy release must result from coulomb explosion of the clusters. The apparatus has been modified to enable the desired cluster-size specific experiments.

Figure 3. Time-of-flight mass spectrum of multicharged iodine atoms resulting from the coulomb explosion of HI monomer and HI clusters. The voltage across the first two TOF lenses is 260 V.

Table I
Measured and Calculated Kinetic Energies[‡]

	I^{+2}	I^{+3}	I^{+4}	I^{+5}	I^{+6}	I^{+7}	I^{+8}
Measured	74 ± 14^a	204 ± 21	320 ± 32	405 ± 45	450 ± 42	539 ± 56	696 ± 64
Calculated*	56 ± 11^b	153 ± 28	373 ± 57	563 ± 88	395 ± 88	537 ± 120	886 ± 175
	Ar^{+2}	Ar^{+3}	Ar^{+4}	Ar^{+5}	Ar^{+6}	Ar^{+7}	
Measured	106 ± 10	204 ± 12	288 ± 28	380 ± 25	474 ± 30	546 ± 42	
Calculated*	103 ± 35	257 ± 76	506 ± 114	668 ± 203	916 ± 286	1055 ± 323	

*deduced from peak splittings

‡all values in eV

^aThe uncertainty arises from the inability to precisely measure the energy distribution due to signal fluctuation. Therefore, the uncertainty was determined from the highest and lowest signal intensity.

^bThe uncertainty arises from the inability to precisely choose the maximum point of the broad peak (early arrival time). Therefore, the uncertainty was determined by choosing a point on each side of the expected maximum point.

Similar investigations were conducted on clusters of HI bound to argon clusters. Figure 4 displays a time-of-flight mass spectrum of the resulting Ar^{+n} , which in the present work were observed to extend to $n=8$. Note that each of these ions contains a broad peak at arrival times earlier than the sharp peaks. This observation is similar to that of I^{+n} which was shown to result from the coulomb explosion of HI clusters. Since argon is an atom, multicharged ions can not display peak splittings or significant kinetic energy releases unless the resulting progeny arise from a charged entity comprised of more than one atom. This establishes that some of the multicharged argon is originating from argon bonded to other species, as would be present in a cluster. A well defined distribution of HIAr_m clusters ($m \leq 10$) was observed in the mass spectrum (although not displayed in Figure 4). No clusters with more than one HI were seen. Also, Ar_m clusters with $m \leq 6$ were observed. It should be noted that multicharged argon atoms are observed only when HI-argon clusters are present, suggesting that the multicharged argon atoms are not produced from the coulomb explosion of neat argon clusters, but from the coulomb explosion of co-clusters of HI and Ar.

The kinetic energies measured from the cut-off potentials and the values calculated from the peak splittings are both given in Table I. The kinetic energies for Ar^{+n} determined by the two different methods do not agree as well as those of I^{+n} . As previously stated, multicharged argon atoms are only observed when co-clusters of HI and Ar are present. A likely reason is that the iodine atom is the species being multiply ionized by the laser pulse and the charging of the argon atom(s) occurs due to a charge-transfer reaction with the highly charged iodine. In such a situation, only one or a few argon atoms will undergo a charge-transfer reaction. As the coulomb explosion occurs, the remaining neutral argon atoms will undergo evaporation from the charged cluster with which they are initially bound. Depending on the evaporation rate, the kinetic energies obtained from the two methods may differ. If the evaporation is very prompt, the two methods should give similar kinetic energies, but if the evaporation is delayed, the kinetic energies will differ because of the different time windows of the two methods. (Note, resident times in the first TOF region are typically on the order of hundreds of nanoseconds, whereas the species turned by the reflectron are on the order of tens of microseconds.) Hence, in the case of a delayed evaporation, the mass of the

Figure 4. Time-of-flight mass spectrum of multicharged argon atoms resulting from the coulomb explosion of $HIAr_m$ clusters. The voltage across the first two TOF lenses is 310 V.

exploding fragment is actually greater than detected. This would result in the kinetic energies of the multicharged argon atoms calculated from the splittings to be greater than the energies determined from the voltage cut-offs.

It is abundantly evident that we have detected multicharged species of iodine and argon which have very high values of kinetic energy release. Two different techniques have been utilized to deduce these values, namely peak splittings and direct cut-offs using a reflectron as an energy analyzer. The values obtained for I^{+n} by the two methods are in good agreement, establishing the fact that we have indeed observed species with such large kinetic energy releases.

Rhodes and coworkers¹ previously observed multicharged argon ranging from Ar^+ to Ar^{+8} , however, their work did not lead to observed peak splitting nor large kinetic energy release values, as did our work. Clearly this establishes the fact that the multicharged argon atoms in our study arise from clusters. As seen in Figure 4, two intense sharp peaks are observed for each multicharged ion up to Ar^{+4} . Based on the kinetic energy release measurements, these peaks appear to originate from two different sources. We considered the possibility that the lower energy ions were the result of multiple electron ejection from an argon atom; however, they too have significant kinetic energy, showing that their origin is also from clusters. As with the multiple peaks of I^{+n} , it is not possible to determine the exact parentage of the multicharged argon with the current experimental set-up.

Codling and coworkers² determined total kinetic energy release values for HI, ranging only up to about 20 eV, far below those which we have measured in the present study. Also note that our high values only represent the kinetic energy of one partner in the coulomb explosion. Hence the total values are even larger than those presented for the individual species in Table I. If one neglects internal energy of the repelling fragments, a simple relationship pertains for the energy arising from the coulomb repulsion between two charges of the same sign.

$$KE = \frac{14.40}{r} q_1 q_2, \quad (7)$$

where the kinetic energy is given in values of electron volts, r represents the separation distance in angstroms and q_1 and q_2 the magnitudes of the charge on the individual repelling species. Based on this simple relationship, it would be impossible to account for the magnitudes of the kinetic energy

release in the iodine system by considering a coulomb repulsion between H^+ and an ejected I^{+n} . Indeed, the magnitudes observed in our work indicate that repulsion must be occurring between (at least) two species which have relatively high charge states and very short internuclear separation distances, i.e. a bound collection such as would be present within a cluster. The findings reported herein are further testimony to the wide ranging processes which can be explored within the domain of femtochemistry.³

In conclusion, we have demonstrated that clusters exposed to intense laser fields can coulomb explode giving rise to particles with high charged states and surprisingly large values of kinetic energy release. Currently we are rebuilding our time-of-flight mass spectrometer system that will enable us to determine the exact parentage of these species which give rise to this interesting and surprising phenomenon. Details of the findings can be found in papers 406 and 424.

2. Reactions of Free Radicals and Transient Intermediates Involving Functional Groups of Biological Molecules

a. Reaction Mechanisms of Carbonyl Groups

Various other molecules having similar functional groups as those of direct biological interest, were studied. In particular we investigated acetone to elucidate the chemistry of molecules with $C=O$ groups to elucidate the behavior of this functional group in thymine, cytosine, uracil and guanine.

Extensive investigations have been performed on the dissociation dynamics of acetone, the simplest ketone. Excitation from the ground state of acetone to the first excited singlet state (S_1) occurs via a $\pi^* \leftarrow n$ transition, resulting in dissociation to acetyl and methyl fragments:

Based upon translational energy of the fragments, this process has been predicted to occur on the time scale of less than 1 ps. Fragmentation channels in the ion state have also been investigated for both acetone and acetone clusters. Ionization of acetone clusters results in the observation of the following species:

Cleavage of a carbon - carbon bond in the acetone skeleton is depicted by reactions (10) and (11), which results in the loss of a methyl or acetyl moiety and observation of the associated acetyl or methyl unit bound with an acetone cluster. A possible proton transfer reaction is depicted by (12), which accounts for the detection of the protonated acetone cluster series.

Since Reactions (10) and (11) also happen in the S_1 state, Reaction (8), the origin of these observed ions is an interesting question: do the acetone clusters ionize first and then fragment to free radical cations, or do the clusters fragment first, followed by the ionization of the acetone associated fragments? Since the potential energy surfaces have been elucidated recently, the time dynamics of the intermediate singlet state (S_1) may be investigated and the aforementioned question resolved.

In the current study, we have employed a 350 fs laser at several different harmonics (energy/photon) to investigate the dissociation dynamics. The singlet state is accessed by either 1 photon of either 312, or 208 nm or 2 or 3 photons of 624 nm from the ultrafast laser. Once this state has been excited, ionization is achieved by further absorption. Refer to Figure 5 for potential energy surfaces and points of access. The laser beam is separated into two beams; these beams can be delayed in time with respect to each other, enabling the intermediate state to be accessed at different pump-probe delay times. The beam arriving first (pump) prepares the intermediate state, while the time delayed beam (probe) ionizes the molecule.

The ionization response as a function of time (pump-probe delay) for the acetyl fragment, acetone monomer, dimer, and trimer species is shown in Figure 6. At negative time delays the 208nm photon accesses high vibrational levels within the singlet state, while subsequent absorption of one 312nm photon exceeds the ionization potential. At positive time delays, the 312nm photon accesses the S_1 state close to the barrier leading to the methyl and acetyl fragment channels. Refer to Figure 5. The time profiles reveal that ionization of the acetone monomer, dimer and trimer follow a direct mechanism. That is, unless the ionizing pulse (probe) arrives within ~500 fs of the pulse which excited the S_1 state (pump), ionization is not accomplished. Examining the time profiles of the fragment ion, CH_3CO^+ reveals that the fragmentation is occurring after ionization of

Figure 5. Potential Energy Surface for the Acetone System.

208/312nm Pump-Probe Experiment Acetone and Acetone Clusters

* NOTE: All responses have been normalized to the point where acetone monomer saturation was observed.

Figure 6

the molecule and not within the intermediate state. Another aspect of the experimental data that points to this conclusion is the enhanced ionization that is observed. Enhancement is defined as the difference between ionization by pump beam only (probe is blocked) compared to ionization when both beams are unblocked and overlapped in time. For example, the acetone monomer is enhanced by a factor of twenty greater than the acetyl fragment.

Considering the potential energy surfaces involved, the probe beam, in either case, (208nm or 312 nm depending on time delay) may not have sufficient energy to ionize the fragment generated in the intermediate singlet state. To overcome this restriction, we have repeated the experiments using resonant multiphoton ionization (REMPI) with 624nm photons. The laser beam is split into two equal parts and delayed in time with respect to each other in a Michelson interferometric configuration. The photon densities for these experiments are several orders of magnitude greater than in the aforementioned 208/312nm experiments. This allows for absorption of multiple photons which would exceed the energy deficiency in the probe pulse, thereby exceeding the ionization potential for fragment ions. The time plots for the acetone dimer, trimer and their acetyl fragments (refer to reaction (10)) are shown in Figure 7. Not shown in Figure 7 are the time profiles for the protonated cluster species, which also show similar responses. As of yet, it has not been resolved whether the ionization is a 2 or 3 photon resonant process; however, in either case the pump would assume the role of either the state prepared by one-208nm photon or one-312nm photon. Examination of the time profiles depicted in Figure 7 reveal that there is not any significant ionization when the pump and probe beams are delayed greater than 500 fs time, leading to the conclusion that the fragmentation is occurring on the ion state surface rather than in the intermediate state.

These studies were conducted to elucidate the ionization mechanisms of the acetone monomer and clusters along with their fragment species. We have found that the fragmentation is occurring in the ion state rather than in the intermediate, S_1 , state. Examination of the time profiles reveal that the acetone species, fragments included, live for approximately 500fs. This is the first experimental determination of the predissociative lifetime of the S_1 state and is in agreement with previously estimated values. See papers 428 and 431.

624nm Pump-Probe Experiment Acetone and Acetone Clusters

Figure 7

3. Selective Ionization of Biological Molecules

a. Efficient, Selective, and Intact Ionization of Biomolecules Using Femtosecond Laser Pulses

Much of the presently available information about the nature of molecular systems and the structures and dynamics in particular comes from experimental studies in the gas phase. Among all the gas phase techniques, mass spectrometry (MS) is one of the most powerful and versatile for probing molecular properties of biomolecules. The traditional MS method, like all other techniques, does have its limitations, such as intact introduction of non-volatile chemicals into the gas phase and ionization of these fragile molecules without severe fragmentation. Achieving efficient, selective, and intact ionization of biomolecules has been one of our recent primary research areas, through the use of femtosecond laser techniques. The unique femtosecond laser pulses offer many fascinating advantages due to the exceptional high field strength and short pulse duration. We have demonstrated that the femtosecond laser gives the most efficient ionization as compared to the traditional ionization methods in the nanosecond time scale, for a number of molecular systems of biological importance; for example, we have successfully employed this for water, pyrimidine, benzene, tryptamine, and similar molecules. The key finding is that the femtosecond pulses result in minimal fragmentation because of the short interaction time between the laser pulse and the molecules of interest.

Using a reflectron time-of-flight mass spectrometer coupled with both femtosecond and nanosecond lasers, recently we have conducted several extensive studies on pyrimidine. In particular, we have compared the dependence of fragmentation on laser wavelength and pulse width, and also we have investigated the effects of solvating ligands such as ammonia and water.

The apparatus used in these studies is a reflectron time-of-flight (TOF) mass spectrometer coupled with both femtosecond and nanosecond laser systems. The 310 nm nanosecond pulses are generated by frequency doubling the output of a pulsed dye amplifier. The amplifier contains DCM dye and is pumped by the second harmonic of a 10 Hz, Nd:YAG laser. The output energy is ~2 mJ/ and the pulse duration is ~10 ns. The femtosecond laser pulses are generated by a colliding pulse mode-locked (CPM) dye laser. The output wavelength, energy, and pulse width are ~620 nm, ~200 pJ, and ~150 fs, respectively.

(i) Fragmentation of pyrimidine

Figure 8 displays time-of-flight mass spectra of pyrimidine ionized with a femtosecond laser at wavelengths of 310 nm and 620 nm. Several differences are immediately obvious. At 310 nm, the prominent form of pyrimidine is the intact or unprotonated species, whereas the protonated species is more prominent at 620 nm. Also, a greater degree of fragmentation is observed at 620 nm, as seen by the increase in intensity of the two prominent fragments of pyrimidine, CN^+ and $\text{C}_3\text{H}_3\text{N}^+$. These observations can be explained by the ladder climbing mechanism. The laser fluence is an order of magnitude greater at 620 nm, which gives a larger degree of fragmentation as a result of climbing higher up the ladder.

We also conducted experiments to ascertain the origin of the protonated pyrimidine species. We wanted to determine whether they originated from pyrimidine clusters or from solvated pyrimidine. For this determination, we performed experiments in which we ionized ND_3 -solvated pyrimidine with a femtosecond laser at wavelengths of 310 nm and 620 nm. At 620 nm the prominent pyrimidine species is PD^+ (where P = pyrimidine), rather than PH^+ . This observation indicates that the protonated species originates from fragmentation of pyrimidine-solvated ammonia clusters rather than from pure pyrimidine clusters. Ammonia was not used as the backing gas for the spectra shown in Figure 8, but a trace amount was present.

Figure 9 displays a time-of-flight mass spectrum of pyrimidine which is ionized with a nanosecond laser at 310 nm. Unlike the femtosecond ionization at 310 nm (shown in Figure 8a) which results in predominantly P^+ species, the nanosecond ionization results in predominantly PH^+ species. This result is also observed upon nanosecond ionization at wavelengths of 280 nm and 355 nm. This large degree of fragmentation can be explained by the well known ladder switching mechanism. Due to the broad nanosecond pulse width, the ion has time to rearrange and absorb additional photons which results in fragmentation.

(ii) Solvation of Pyrimidine

Figure 10 displays a time-of-flight mass spectrum of water-solvated pyrimidine clusters, which are ionized with femtosecond laser pulses at 620 nm. It is observed that pyrimidine can be solvated with a large number of water molecules. A distribution of water-solvated pyrimidine

Figure 8

NANOSECOND IONIZATION OF PYRIMIDINE AT 310 NM

Figure 9

Femtosecond Ionization of Pyrimidine/Water at 620 nm

Figure 10

clusters is also observed. Again, the retarding effect due to solvation, on fragmentation, is readily observed. These findings are very interesting and significant for studying the structure/function of biological molecules, and we had hoped to pursue further investigations of this type for larger biological molecules.

IV. BIBLIOGRAPHY

1. U. Johann, T. S. Luk, I. A. McIntyre, A. McPherson, A. P. Schwarzenbach, K. Boyer, and C. K. Rhodes, "Multiphoton Ionization in Intense Ultraviolet Laser Fields," in *Proceedings of the Topical Meeting on Short Wavelength Coherent Generation*, D. T. Attwood and J. Bokor, Eds. (AIP Conference Proceedings No. 147, AIP, New York, 1986), pp 157-168.
2. K. Codling, L. J. Frasinski, and P. Hatherly, and J. R. M. Barr, *J. Phys. B.* **20**, L525 (1987).
3. A. H. Zewail, *J. Phys. Chem.* **97**, 12427 (1993).
4. E. W. Schlag, J. Grotemeyer, and R. D. Levine, *Chem. Phys. Lett.* **190**, 521 (1992).