Multiphoton Ionization of Molecules: A Comparison between Femtosecond and Nanosecond Laser Pulse Ionization Efficiency

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Multiphoton ionization mass spectra of nonvolatile molecules laser desorbed into a supersonic beam are recorded. It is shown by indirect measurements that the laser desorption of neutrals is not mass limited, but lead to the formation of neutrals with intensities large enough for intense signals. To investigate the efficiency of the multiphoton ionization process with varying laser pulse durations, simultaneous laser pulses of 500 fs and 5 ns or 100 fs and 5 ns have been applied to the neutral beam. The energies of both femtosecond and nanosecond laser pulses are held in a comparable magnitude, and thus produce, in the resulting ion intensity, very large differences up to 4 orders of magnitude. For larger evaporated molecules (> 500 u) the ionization efficiency from nanosecond laser pulses drops significantly in comparison to femtosecond laser pulse excitation. A variety of possible reasons for the different ionization and dissociation behavior in femtosecond and nanosecond laser pulse excitations are discussed in this paper. It is rationalized that even with very short laser pulses and large molecules the "ladder switching model" for ionization and fragmentation is valid. (*J Am Soc Mass Spectrom* 1995, 6, 1059–1068)

The developments of photon-induced ionization methods have opened new pathways in the mass spectral investigation of large molecules with low volatility. Especially the matrix-assisted laser desorption ionization (MALDI) technique has demonstrated the accessibility of the mass range to about 250.000 u and beyond [1]. In addition to this method, other photon-based techniques, such as multiphoton [2] and single-photon [3] ionization have been applied successfully to mass spectrometry of molecules with low volatility. Between these methods fundamental differences exist in terms of the ions formed as well as the mass range. Ions formed in the MALDI process are generally even electron species whereas the impact methods like electron ionization lead to radical cations or odd electron species. Despite intensive research the gas-phase formation of radical cations from larger molecules has not been observed yet. Dependent on the experimental setup and the ionization procedure, the ion intensity of the radical cations decreases with increasing mass, and reaches zero intensity between 3.000 and 5.000 u as shown in this article as well as by other authors [4]. This decrease of ion signals can be attributed to several different reasons such as the decrease of neutral desorption efficiency, transport efficiency between desorption and ionization, or molecule ionization efficiency. As shown in this article, experi-

mental evidence can prove that neither the neutral desorption nor the transport efficiency through the supersonic beam account for this observed behavior.

This experimental result has led to the question whether the formation of radical cations is mass limited. A recent theory [5, 6] has established a picture that shows that energy redistribution processes are essential for the observation of large molecular ions in the gas phase.

Because the energy randomization process in an activated molecule occurs typically through a fast internal redistribution of vibrational energy, it seems that the decreasing ionization efficiency could be the explanation for decreasing ion signals with higher masses. The typical time scale for a C—H stretching mode is around 60 fs. As a result it is possible to redistribute a lot of energy (up to tens of electronvolts) very fast in other vibrational states without ionizing the molecule. This effect should be even more pronounced if activation occurs through a multiphoton absorption process, because energy redistribution should take place already in the intermediate stage, which leads to an even faster decrease in the ion yield than in the electron or single-photon ionization procedures.

Different mechanisms have been discussed for the energy randomization process, because the transfer of energy through coupled vibrations would be too slow to prevent ionization of larger molecules. One of the proposed mechanisms could be the formation of geminate charge pairs through a fast electron transfer pro-

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cess, which would lead to an extremely fast distribution of the activation energy and thus prevent the occurrence of ionization. However, it is interesting to follow the principal ideas of this theory and derive some proposals that can be proven experimentally. One proposal that can be proven easily is a shortening of the activation laser pulse typically from a nanosecond to a femtosecond pulse duration. Although the pulse shortening has some influence in the activation scheme of the multiphoton ionization as will be discussed later, this pulse shortening should dramatically influence the reachable mass range for the formation of radical cations and thus shift the mass limit beyond 10,000 u. In addition to extension of the mass limit, pulse shortening also should lead to a drastic increase in the ionization yield, because shorter laser pulses suppress energy randomization processes in the molecule.

It should be kept in mind that in addition to the pulse duration, two other parameters drive the formation of ions by photon absorption: these are the laser intensity and the pulse energy. In this case we tried to keep the pulse energy and therefore the absolute number of photons per laser pulse constant, which thus allowed a direct comparison of the resulting mass spectra. Changes in the mass spectra could then be attributed directly to the altered activation mechanism. This idea is based on the assumption that the fundamental processes in the laser activation that lead to ionization and fragmentation are changed by using nanosecond or femtosecond laser pulses as indicated in Figure 1. The first step in the investigation of the ionization procedure and that answers the question of a mass limit would therefore be to check whether these generally accepted activation schemes are valid for larger molecules. In this paper we investigate the mass spectral behavior of some smaller molecules up to mass 1881 u by multiphoton absorption from laser pulses with 10-ns, 500-fs, and 100-fs pulse duration.

Experimental Setup

The experiments were performed on a Bruker (Bruker-Franzen, Bremen, Germany) TOF-1 Reflectron

Figure 1. Suggested ionization mechanisms for 1 + 1 multiphoton activation that utilized nano-, pico-, and femtosecond laser pulses.

time-of-flight (ReTOF) mass spectrometer described in greater detail elsewhere [7]. As shown in Figure 2, samples with low volatility are desorbed by a CO₂ laser ($\lambda = 10.6 \mu\text{m}$) in front of the nozzle of a pulsed valve. By correctly timing the triggering of the desorption laser and the opening of the valve, desorbed molecules are entrained into a supersonic jet formed by argon. The solid samples are prepared in matrix of polyethylene and platinum powder as described recently [8]. Polyethylene acts in the desorption step as a matrix that improves and stabilizes the desorption yield of the neutral molecules, and thus leads to a high density of neutrals in the supersonic jet. Signals of polyethylene and platinum are never observed in the mass spectrum. All investigations are made with a fixed relationship of sample molecules to polyethylene and platinum powder. Samples of high volatility are seeded into the argon prior to expansion through the nozzle. This setup ensures that the internal temperatures of the molecules are kept as low as possible [9].

Neutral molecules evaporated by one opening of the jet valve are ionized by both nano- and femtosecond laser pulses. Both laser beams pass through the same optical systems to avoid errors of different lens focus. To minimize fluctuations in the density of neutral molecules, especially in case of laser-desorbed samples and under matrix conditions, both laser beams are focused with a small time delay into the supersonic jet; thus in every desorption cycle two mass spectra,

Figure 2. Experimental setup for comparison of nano- and femtosecond multiphoton ionization mass spectra.

one of the nanosecond and one of the femtosecond ionization, are produced. The delay in time was usually about 5–20 μ s and additionally a small shift in space (about 0.5 mm) was introduced. As a result the flight time spectrum recorded on the transient recorder presents a superposition of two mass spectra from both the nanosecond and the femtosecond activation. It should be noted that the time delay between the two lasers is adjusted to avoid interferences of mass signals. By changing the triggering sequence of the ionizing lasers, for example, nano- before femtosecond laser and vice versa, no differences in the mass spectra could be observed. The neutral density of the desorbed molecules in the desorption chamber can be considered to be constant in a time duration of about 50 μ s [10]. The mass separation is performed with a ReTOF system and the mass signals are detected by a tandem multichannel plate detector. The data are recorded on either a LeCroy 200-MHz transient recorder (LeCroy, Chestnut Ridge, NY) or a Tektronix TDS 540 digital storage oscilloscope (Tektronix, Inc., Beaverton, OR). All mass spectra collected represent at least the sum of 25 laser shots (valve openings).

The nanosecond laser system consists of a Lumonics HY-1200 Nd:YAG laser (Lumonics, Ottawa, Canada) pumping a Lambda Physics FL 3002 dye laser with a pulse duration of approximately 5–8 ns and a maximum output energy up to 500–900 μ J, dependent on the laser dye used. The femtosecond laser system is pumped by 80-mJ pulses from a Lambda Physics EMG 150 XeCl excimer laser ($\lambda = 308$ nm). The main optical components of the first part of the femtosecond laser system (manufactured by Laser Laboratorium, Göttingen) are a quenched cavity dye laser, a short cavity dye laser, amplifiers, a gated saturated absorber, and a distributed feedback dye laser (DFDL) as described recently [11]. The wavelength of the femtosecond laser system can be changed by using different distances between the DFDL and the transmission grid. The duration of the DFDL pulse also is restricted by the maximum length of the interference pattern in the DFDL divided by the speed of light. Using an interference structure in the DFDL of about 1 μ m, a pulse duration of about 500 fs is reached. The second part of the femtosecond laser system consists only of an amplification part and a BBO (barium beta borate) crystal for frequency doubling. The maximum output energy can be adjusted to up to 50 μ J. The 100-fs laser pulses are more difficult to produce. Here a glass fiber to broaden the spectral width and a grid compressor to shorten the pulse duration, an amplifier, and a BBO crystal for frequency doubling are used. The maximum output energy can be adjusted to about 2 μ J. The pulse duration can be arranged between 65 and 120 fs.

Due to this setup the energy for two different laser pulses could be held at the same magnitude. If no ion signal at a given energy from the nanosecond laser pulse was seen, the pulse energy of this laser was increased stepwise until the molecular ion signal was

at a comparable intensity to the femtosecond ion signal at the former energy.

The acquisition of single-photon and electron ionization mass spectra occurred in the same mass spectrometer system as described previously [3a, 12]. The samples were obtained from either Sigma Chemicals (Deisenhofen) or Serva (Heidelberg) and were measured without any further purification. The following amino acids and peptides were used for the investigation: tryptophan, glycyl-tryptophan, tryptophan-leucine, leucyl-leucyl-tryptophan, tryptophan-(leucine)₃, leucine-enkephalin, methionine-enkephalin, SLP (sleep-inducing peptide), angiotensin III, gramicidin S, angiotensin I, gramicidin D, melittin.

Results and Discussion

Desorption Investigations

With the above-mentioned experimental setup the ion intensities and total ion currents (TIC) of different laser-desorbed molecules have been investigated via different ionization methods, which lead to the formation of radical cations. In Figure 3 the molecular ion intensities of a group of tryptophan-containing peptides versus their molecular masses are plotted. In these samples the tryptophan has been chosen as a chromophoric group for the ionization procedure, because this residue has the lowest ionization energy within the peptide chain. An absorption of one or more photons or the interaction with an electron leads therefore to the initial formation of the radical cation in the indole system of this moiety. This is especially true in the case of multiphoton ionization of these samples, where through the recording of intermediate state spectra, the absorption in the indole system even in larger peptides can be proven experimentally [13]. It should be noted that in any change of the aromatic

Figure 3. Data for molecular ion intensities of different peptides measured with electron ionization (EI), single-photon ionization (VUV), and multiphoton ionization (MUPI).

system, for example, tryrosine or even phenylalanine used as a chromophoric group for ionization, the same results for ionization and desorption have been obtained [14].

The results shown in Figure 3 demonstrate clearly that with increasing mass the intensity of the molecular ion decreases for every ionization method that has been used. The only difference between the ionization methods is obviously the termination of the measurable ion abundance at different masses. Although in electron ionization the accessible mass range in our experimental setup yields the lowest mass range, single-photon and multiphoton ionization give still detectable intensities at higher masses. In any case, molecular ions beyond 2500 u cannot be detected. This does not mean that radical cations cannot be formed beyond this mass. It is well known from polymer samples [15] and cluster radical cations [16] that radical cation formation is possible. Köster et al. [17] demonstrated that clusters of gramicidin D can be formed in a supersonic beam to yield up to the dodecamer with a mass of 22572 u. However, it should be kept in mind that the ionization in clusters occurs through the formation of a monomer ion. Furthermore, the energy transfer in large clusters is limited through the low energetic van der Waals modes compared to the ion lifetime demonstrated in several examples [18].

To demonstrate that the desorption process is not responsible for this effect, the desorption yield of neutral molecules is measured also. In Figure 4 these data are set in correspondence to the multiphoton ionization (MUPI) signal of the molecular ion and the total ion current. The desorption yield has been measured by recovering the sample after the desorption, followed by high-performance liquid chromatography (HPLC) separation and quantification [19] according to a procedure given by Williams [20]. Although the absolute number of desorbed molecules drops surely

Figure 4. Data for the molecular ion intensities measured with multiphoton ionization (MUPI) compared to the measured neutral desorption yield (see text) and the total ion current (TIC).

with increasing mass of the molecule, clearly this effect is much smaller than the decrease of the molecular ion yield. Therefore it can be concluded that the desorption process has no major influence on the decreasing ion yield by formation of radical cations. To prove that even larger molecules can be desorbed intact with a CO₂ laser and entrained into the supersonic jet, neutral molecules such as cytochrome *c* are recovered at the skimmer and in the ion source. Here this recovered sample was detected in a quantity sufficient to yield a strong ion signal by any ionization method. It should be noted that the same results have been obtained by Reilly and Reilly [4]. However, application of multiphoton ionization conditions to this neutral beam resulted in no observable ionization signal from the cytochrome *c*. These experimental findings lead to the suggestion that neither the desorption process nor the entrainment of neutrals into the supersonic beam leads to the observed decrease of the ionization efficiency, but that something in the ionization process itself is the cause.

It should be noted that these experimental results also could be explained through a different effect on the ionization procedure. It is a well known rule in mass spectrometry [21] that as a molecule gets larger the absolute number of fragmentations should increase. The reason for this rule stems from the observation that an increase in the mass leads to a decrease of ionization energy. Because the appearance energy of the fragment ions and the excess energy applied in the ionization procedure to the molecular system should stay the same, especially in the photon ionization methods, this should lead immediately to an increased amount of fragmentation and therefore a strong decrease of the molecular ion intensity. The data shown in Figure 4 present clearly the opposite behavior. The total ion current of the different molecules follows exactly the behavior of the molecular ion intensity. It should be noted that this behavior is not limited to these special peptides, but is observed also for a large number of different compounds as well. This clearly must mean that the ionization efficiency of any molecule drops with the increasing mass.

Ionization Investigations

Several models have been discussed as explanations of these experimental results. Especially the idea of the formation of a geminate charge pair, which prevents the formation of large radical cations, found great interest. Although any experimental test of this model is very demanding, some proof can be undertaken easily. As is done in this paper, if one concentrates on the method of multiphoton ionization, the model predicts an increase of the observable mass range by changing from nanosecond to femtosecond activation in the two-photon ionization procedure. As already mentioned, the change in the activation scheme underlies this proposed mechanism. Usually the nanosecond

laser pulse activation is explained through the "ladder switching model" [22] as shown in Figure 1. After formation of the molecular ion through absorption of two or more photons, any fragmentation occurs via an absorption-fragmentation process either in this ion or the formed fragment ions. In contrast, dependent on the width of the laser pulse, shorter laser pulses should lead to an activation explained by the *molecular ion ladder model* [23] or even *high excited autoionizing states* [24]. Furthermore it is generally accepted that femtosecond activation should lead to an increased fragmentation, which thus produces a large number of fragment ions. Therefore a multiphoton ionization mass spectrum should be similar to those obtained by electron ionization.

The following text presents a comparison of the dependence of ionization and dissociation efficiency from femtosecond (100 or 500 fs) and nanosecond (5–8 ns) laser pulse excitation by holding the photon number (pulse energy) in comparable magnitude. As already mentioned in the Experimental section, special care has been taken to avoid any influences of neutral density fluctuation in both the femtosecond- and nanosecond-activated mass spectra. Almost simultaneous acquisition of both the femtosecond and nanosecond mass spectra from the same desorption-jet pulse enables any fluctuations to be neglected, because both lasers are separated in time by only a few microseconds. To avoid optical saturation effects in the absorption of photons, the laser pulse energies have been kept typically below $2 \mu\text{J}$ with femtosecond activation. In nanosecond activation the typical laser energies were approximately 10–30, which yielded soft ionization conditions dependent on the molecular size. Only for hard ionization (focusing conditions of the laser beam) have higher laser energies been used with values of approximately $40 \mu\text{J}$. As demonstrated in preliminary experiments [25] the approach to hold the absolute photon number of both femtosecond and nanosecond laser pulses equal allows a direct comparison of the ionization efficiency and therefore the cross section of the different molecules.

As can be seen from Table 1 and Figure 5, a slight change in the ionization yield for benzene molecular ions is observed when the activation laser pulse is shortened from 5 ns to 500 and 100 fs, respectively. In Table 1 the different laser pulses and the resultant peak areas of the benzene molecular ion signals are

Table 1. Comparison of the molecular ion intensities of benzene activated with different laser pulse durations

Pulse time	Intensity ^a (peak area)	Intensity ratios	
		ns/fs	fs/fs
10 ns	101		
500 fs	7,328	0.013	
100 fs	18,237	0.006	0.402

^a Arbitrary units.

Figure 5. 5-ns, 500-fs, and 100-fs multiphoton ionization mass spectra of benzene.

shown. Also incorporated in this table are the ratios between the different peak areas. With the same absolute number of photons applied to the neutral benzene molecules the 500-fs laser pulse produces nearly 2 orders of magnitude more molecular ions than the nanosecond activation pulse. A change of the laser pulse to even shorter times (approximately 95 fs) only affects an increase of about a factor of 3 compared to the value at 500 fs. Clearly femtosecond activation of molecules lead to an increased formation of ions in the mass spectrum.

This increase can be accounted for by three different processes. Either the absorption cross sections are grossly different for the different time domains or the

laser pulse shortening must change the activation scheme of the neutral molecule as discussed before. As demonstrated in the mass spectra of benzene, in all cases a soft ionization is observed with a nearly exclusive formation of the molecular ion, which is contrary to the predicted behavior. It should be noted that even an increase in the laser intensity in the femtosecond activation did not change the fragmentation processes observed in the benzene molecular ion compared to the nanosecond activation [26]. As shown in subsequent text this behavior is detected in the mass spectra of larger molecules as well. From this we conclude, because the intensity of both the nano- and femtosecond laser beam is adjusted to the same value, that with short laser pulses the autoionization model is not applicable to benzene and larger molecules.

From the data obtained in our experiments we can estimate the absorption cross section for the first absorbed photon at the 500-fs time frame to approximately $5 \times 10^{-17} \text{ cm}^2$. This is nearly in the same range as the values obtained for the nanosecond activation with $3 \times 10^{-17} \text{ cm}^2$. It should be noted that these values are on the same order as those of Boesl et al. [27] have measured for the same sample. However the absorption cross sections for the second photon are slightly different and larger for the femtosecond pulse than for the nanosecond pulse. Clearly this increase cannot be responsible for the higher ion yield. Of course a slight change of the cross section immediately has an effect on the amount of ions formed, but again this should lead also to a higher degree of fragmentation, because the absorption cross section for subsequent photons in the formed ion is much higher than any other process. Clearly this is not observed in the mass spectra of benzene.

One should remember that the absorption cross section for the second photon also includes the radiative deactivation of the intermediate state. This is the third possibility, which can explain the higher amount of ions formed in the femtosecond activation. In the nanosecond multiphoton ionization, molecules in the intermediate state can either absorb the next photon, which leads to ionization, or undergo a singlet-triplet intersystem crossing to a long-lived triplet or the radiative deactivation of the intermediate electronic state may occur, which thus leads back to ground state molecules. Typically this fluorescence deactivation has rates in the nanosecond time domain. In the femtosecond time frame the deactivation process is cut off, because the second photon is delivered to the electronic excited state faster than any deactivation may take place. This sufficiently explains the experimental behavior found in benzene as well as in larger molecules.

Tryptophan. As already mentioned, the aromatic ring system in the amino acid tryptophan ($m = 204 \text{ u}$) introduces high optical transition moments at rela-

Figure 6. 5-ns and 100-fs MUP mass spectra of tryptophan under soft ionization conditions. The spectra were measured at threshold energy conditions with both lasers and a time delay between them of $10 \mu\text{s}$.

tively low excitation energy. The O-O transition for the change from the ground to the first excited electronic state has been measured at 286 nm [28]. Therefore an applied wavelength of $\sim 260 \text{ nm}$ will excite the molecule into high vibrational excited states S_1 or even into S_2 . In any case a high density of states can be expected. As a result a resonant excitation at all wavelengths below this transition can be found.

A comparison of the ionization signals of nanosecond and 500-fs laser pulses for tryptophan as shown in Figure 6 produced no significant observable difference in the fragmentation pattern if both lasers were adjusted to threshold ionization conditions. It should be remembered that in this figure, from one desorption pulse, both lasers induce two mass spectra, which are recorded in a time spacing of $10 \mu\text{s}$. This guarantees a direct comparison of the different ion signals produced by both lasers. Below a pulse energy of about $2 \mu\text{J}$ in the nanosecond multiphoton activation, no ion formation could be observed. This threshold energy has been kept constant and the energy of the femtosecond multiphoton ionization has been changed to meet the threshold conditions for ion production. The part of the time-of-flight mass spectrum due to the femtosecond activation is produced with a laser energy of $0.4 \mu\text{J}$. These energy values correspond to an absolute number of photons applied to the neutral molecule in the case of the nanosecond laser pulse of 2.62×10^{12} and 5.24×10^{11} in the femtosecond ionization. These numbers clearly show that femtosecond activation is more effective to produce ions than the corresponding nanosecond pulse. In the femtosecond time regime the same amount of ions are produced with less photons than in the nanosecond regime.

As demonstrated in Table 2, the pulse energy of the femtosecond activation could be adjusted between 2 and $21 \mu\text{J}$ for both laser pulses on the same value. As a

Table 2. Intensity ratios for the m/z 130 fragment and the molecular ion of tryptophan

ns/fs energy (μ J)	2	7	12	16	21
fs ([130/204])	6.67	6.8	6.15	6.64	6.32
ns ([130/204])	2	1.65	1.73	1.5	1.53

result the mass spectra should reflect any changes in the activation procedure for ionization and fragmentation. Here the question that plays a central role is to decide whether or not an increase of the pulse energy would lead to a change in the fragmentation pattern of the molecular ion. As long as the pulse duration stays in the nanosecond time domain the fragmentation of the molecular ion will occur through the well known ladder-switching model. The central fragment is produced by the breakdown of the tryptophan molecular ion to form dehydroindole at mass 130 as recently demonstrated [29]. As long as the energy of the laser pulse used for ionization and fragmentation of the molecule is not saturated, the intensity ratios between the molecular ion and this fragment ion should be constant. Furthermore the pulse energy applied to the molecular system should be kept as low as possible to avoid the formation of further fragments from the fragment ion under investigation. As given in Table 2 a constant behavior of the intensity ratios clearly can be observed in the case of the nanosecond ionization. The measured values for the intensity ratio between mass 130 and mass 204 do not change with the increase of the laser pulse energy and yield values between 2 and 1.5. The slight decrease of the ratio easily can be explained by the fact that further fragmentation of the dehydroindole fragment ion at higher laser pulse energies already is induced, which leads to smaller fragments.

In the case of the femtosecond ionization, a different activation model, the so-called *autoionization model*, is usually the base for the explanation of the formation of ions. Here the energy necessary for ionization and fragmentation is deposited at once in the neutral molecule through autoionizing states, as shown in Figure 1. As a result of this model the intensity ratios between the first fragment and the molecular ion should depend strongly on the laser energy as long as the energy values are close to the ionization threshold. In the energy range shown in Table 2 this is true, because the only intense fragment formed in the ionization-fragmentation process is the already known dehydroindole ion at mass 130.

As displayed in Table 2, the intensity ratios show no change with increased laser energy. Again a slight decrease of the ratio is observable, but this is again due to the fact that at higher pulse energies, further fragmentation is observed. Clearly the values of the intensity ratio are higher in the femtosecond ionization than in the nanosecond part.

These experimental results lead to the conclusion that at least in tryptophan the autoionization model does not have any influence on the explanation of the fragmentation processes of this molecular ion with short laser pulses: The ladder switching model seems to be appropriate for the fragmentation of this molecular ion.

Larger molecules. Figures 7 and 8 show individual mass spectra obtained with 5-ns and 500-fs pulses from gramicidin S and gramicidin D. Again the soft ionization mass spectra of both pulse durations are taken at threshold energies. In the case of the gramicidin S, the nanosecond pulse energy was 40 μ J, which corresponds to 1.24×10^{14} photons. The mass spectrum displays a measurable molecular ion as shown in the inset of the soft ionization spectrum. In the femtosecond mass spectrum of gramicidin S shown at the bottom of Figure 7, a pulse energy of 24 μ J, which corresponds to 3.14×10^{13} photons, has been applied to the molecule to produce a mass spectrum similar to that produced by the nanosecond ionization. Again this shows the validity of the statement that femtosecond activation leads to more ions than the nanosecond ionization at the same pulse energy, or as shown in this figure, roughly the same amount of molecular ions are produced with less energy in the femtosecond time domain as in the nanosecond.

As demonstrated in Figure 8, the same behavior is observed in the mass spectra produced with individual laser pulses from gramicidin D. Again the laser pulse energy in both cases was adjusted to threshold conditions to yield a necessary pulse energy of 40 μ J for the nanosecond ionization and 24 μ J for the femtosecond activation. As in the case of the gramicidin S, fewer photons produced nearly the same mass spectrum if they were applied to the neutral molecule in a 500-fs pulse. Comparison of the molecular ion regions of both mass spectra, as given in the inset, reveals this behavior clearly.

The mass signals in the lower part of the mass spectrum are unspecific at these threshold energies and are not due to any fragmentation of the molecular ion from either the gramicidin S or gramicidin D; they stem from the by-products in the sample and perhaps the mass spectrometer system. The foregoing statement can be proved easily by increasing the energy and therefore the number of photons delivered to the neutral molecules. These conditions are met by decreasing the focus size of the laser beam, which leads to hard ionization conditions with a high degree of fragmentation. These mass spectra also are shown in both figures. It should be noted that in these spectra the ion signals in the lower part are now due to real fragmentation of the sample molecules and are no longer caused by the background. As already shown in the literature [9a], this is also observed for the nanosecond mass spectra. Again it should be emphasized that

Figure 7. Multiphoton ionization mass spectra of gramicidin S. Upper spectrum taken with 5-ns pulse duration and soft ionization conditions; middle spectrum taken with 500-fs pulse duration and hard ionization conditions; lower spectrum taken with 500-fs pulse duration and soft ionization conditions. The insets show the molecular ion region.

a careful inspection of the data reveals immediately the validity of the ladder-switching model for multiphoton ionization for larger molecules also.

Conclusion

It has been shown in this article that the desorption of neutrals by the impact of laser radiation is not limited with respect to the mass of the analyte molecules. Therefore the apparent mass limit observed for the formation of radical cations is a result of the ionization process. By using laser pulses with different time profiles, it can be shown that none of the proposed mechanisms—especially not the geminate charge pair formation—can be proven experimentally. Although several

predictions of the model are experimentally verified, such as the increased ion yields by changing from nano- to femtosecond laser pulses, it should be noted that these experimental findings are explained sufficiently by the ladder switching mechanism, contrary to results published in the literature. Even at relatively high laser intensities ($\sim 10^{10}$ W/cm², 24 μ J) no indications for autoionization of larger molecules could be found in the mass spectra.

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Figure 8. Multiphoton ionization mass spectra of gramicidin D. Upper spectrum taken with 5-ns pulse duration and soft ionization conditions; middle spectrum taken with 500-fs pulse duration and hard ionization conditions; lower spectrum taken with 500-fs pulse duration and soft ionization conditions. The insets show the molecular ion region.

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