Influence of the Wavelength in High-Irradiance Ultraviolet Laser Desorption Mass Spectrometry of Organic Molecules

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The influence of the wavelength on laser desorption of ions from organic solids is exemplified for various amino acids and dipeptides: some absorbing and some nonabsorbing at 266 nm and all nonabsorbing at 355 nm. The presence of classical absorption lowers the threshold irradiance for the detection of sample-specific ions and increases the ratio of molecular-to-fragment ions. These observations promise to be of considerable value for future practical application of laser desorption mass spectrometry of organics. The enhanced ion yield of nonabsorbing molecules in an absorbing matrix is presented as an example. Two models for the wavelength influence are discussed, one assuming a predominantly resonant one-photon energy transfer for highly absorbing samples and the other postulating an increased ion yield via excited states.

Processes resulting in (quasi-molecular) ion formation from organic solids in laser desorption mass spectrometry (LDMS) as well as the influence of the pertinent experimental parameters still need further clarification (*1*). In particular, the extremely wide range of laser parameters (continuous wave (CW) to picosecond pulses, irradiances from 10^0 to 10^{12} W/cm^2, far-UV to IR wavelengths) as well as the varying size of the laser spot and different ion source geometries complicates the interpretation of the available data with regard to the general desorption processes. Whereas at least parts of the results of laser desorption (LD) initiated by IR lasers can qualitatively be described by applying thermal models (*2–6*), only general theoretical approaches exist for LD with pulses of 10 ns or less of visible or UV radiation (*7*) as used in a laser micro mass analyzer (LAMMA).

With regard to the influence of the laser wavelength on the desorption process, the problems described above are particularly obvious. The relevance of the laser wavelength for the energy deposition in the IR has been shown by Hess (*8*) for condensed layers; Röllgen (*9*) assumed analogous resonant energy deposition occurred for CW IR LD of sugars. For these techniques ion kinetic energy measurements (*10*) have proved thermalization prior to ion desorption. For LD with nanosecond pulses in the visible and UV regions the laser wavelength so far has been thought to be of minor influence (*11–13*). No systematic investigations have however been reported. Antonov et al. (*14*) initially discussed their results on the LD of adenine and anthracene (λ = 294 and 347 nm, respectively) in terms of a wavelength-dependent resonance absorption. The interpretation of their results must however remain somewhat uncertain because of a limited technical performance of their instrument, which did not provide the necessary mass resolution to decide whether radical or protonated molecular ions were detected. These authors now favor gas-phase ionization resulting in radical ions as the dominating process in their experiments (*15*).

To address these questions a series of LD experiments with aliphatic and aromatic amino acids (AAs) as well as a number of dipeptides were run on a LAMMA-1000 instrument at wavelengths of 266 or 355 nm, respectively. Amino acids and dipeptides were chosen because they exhibit strongly differing absorption characteristics (strong to medium classical absorption by the aromatic AAs, no absorption by aliphatic AAs at 266 nm, and no considerable absorption by any of the AAs at 355 nm). Significant differences in threshold irradiance for ion detection as well as in the characteristic features of the LD mass spectra are reported in the following, thus proving the important role of the wavelength as an experimental parameter.

EXPERIMENTAL SECTION

The main features of the LAMMA-1000 instrument used are described elsewhere (*16*). Samples were prepared from about 10 mmol/L aqueous solutions and dried onto bulk aluminum substrate forming layers of ~5–10 μm thickness. The layers were irradiated with pulses of 10-ns half-width of a frequency-quadrupled (266 nm) or -tripled (355 nm) Nd-YAG laser. The laser was focused to a spot area of about 3 μm in diameter. The irradiance at the sample could be varied from 1×10^7 to 5×10^{10} W/cm^2 with absorption filters. A linear time-of-flight system equipped with a double microchannel plate detector was used for mass separation and ion detection. All mass spectra were taken in the positive ion mode. For all LD experiments it proved to be of great advantage that the sample could be observed through the optical microscope while irradiated. In this way additional information about the sample state (microscopic sample structure, irradiated area, and macroscopically visible changes induced by the irradiation) is available.

RESULTS

Threshold Irradiances. In this first section the results of the threshold irradiance for the detection of sample-specific ions are presented. The concept of a threshold irradiance is appropriate because at this value a decrease in irradiance by ~20% only causes the intensities of sample-specific ions to reproducibly and sharply decline by a factor of 10–50 to noise level. All threshold irradiances are given as normalized quantities, i.e., as a multiple of those for tryptophan (Trp) E_0^{Trp}) at 266 nm, which was the lowest for all samples under all conditions. The corresponding absolute value has been determined to be $E_0^{Trp} = 2 \times 10^7$ W/cm^2 (10-μm^2 spot area, 10-ns pulse width, 20 nJ at the sample), which approximately coincides with the threshold for the observation of Al^+ from the pure Al substrate.

At 266 nm the threshold irradiances of tyrosine (Tyr) and phenylalanine (Phe) were factors of 2 and 5, respectively, above that for Trp, which is in qualitative agreement with the order of their decreasing classical absorption. For all aliphatic AAs analyzed (alanine, leucine, serine, threonine, proline, histidine), the threshold irradiance was about a factor of 12 above that of Trp. Experiments on dipeptides comprising different absorbing and nonabsorbing amino acids gave corresponding results. At 355 nm all AAs and dipeptides needed an equal and considerably higher irradiance for the generation of specific ions. In the mean, the irradiance needed was about a factor of 50 above that necessary for Trp at 266 nm.

At 266 nm significant differences between aromatic and aliphatic AAs were also manifest for irradiances above

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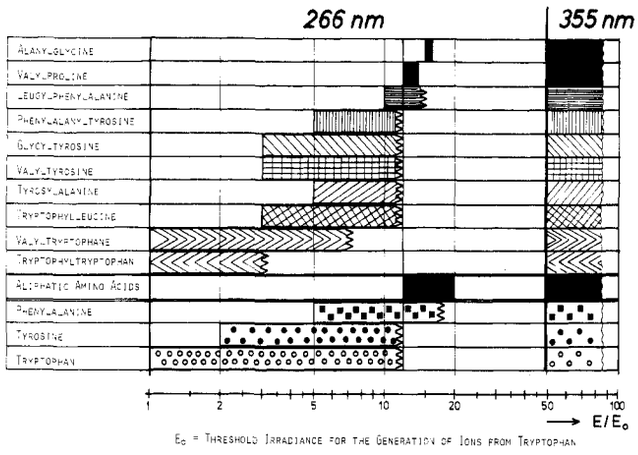


Figure 1. Threshold irradiances and working range for UV LD of amino acids and dipeptides.

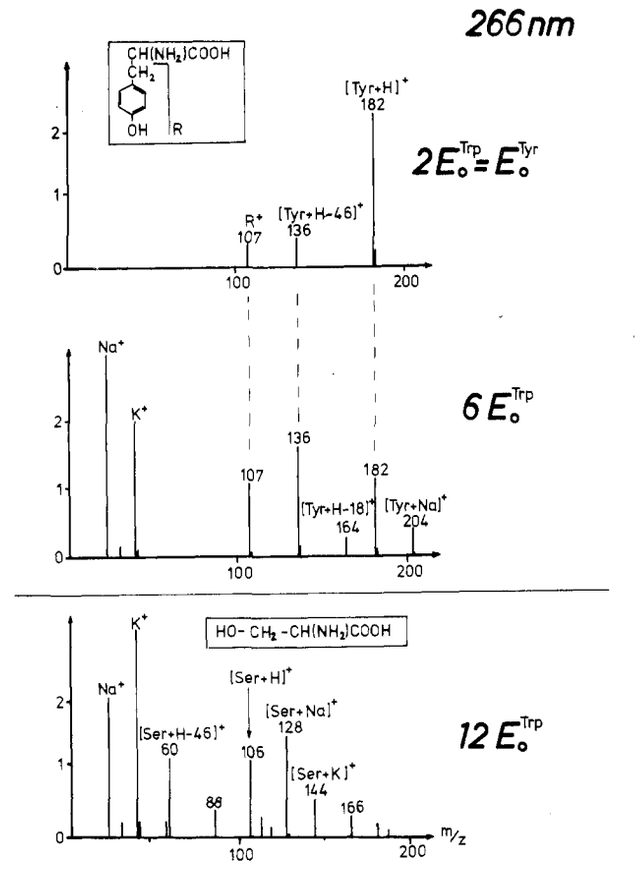


Figure 2. LD mass spectra of tyrosine at threshold (a) and three times above threshold (b) and of serine at its threshold (=12 × E_0^{Trp}) (c), λ = 266 nm.

threshold. Whereas aromatic AAs and dipeptides exhibited a relatively large working range (the irradiance range where sample-specific ions are reproducibly detected), LD mass spectra of aliphatic AAs and dipeptides could only be taken at near-threshold irradiances, because an increase by a factor of 2 already caused saturation by alkaline ions and nonspecific fragment ions. At 355 nm the working range was similarly narrow with no difference between aliphatic and aromatic samples. These results are summarized in Figure 1.

Characteristics of LD Mass Spectra. At their threshold irradiance, using 266 nm, all aromatic AAs, as well as the dipeptides containing at least one aromatic AA, reproducibly yield mass spectra showing intense $(M + H)^+$ ions. No alkaline or substrate ions are observed at this level. In the Trp spectrum the fragment ion due to the aromatic residue, R^+,

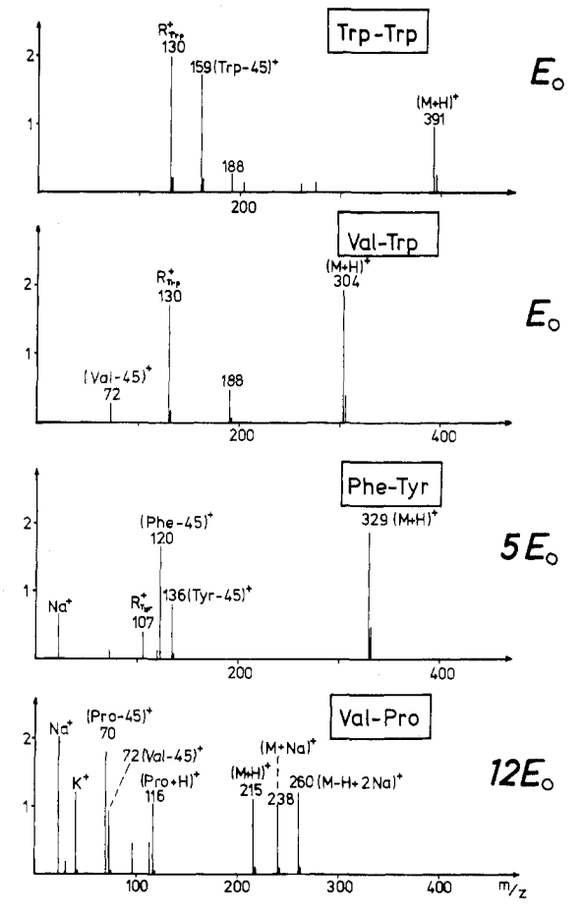


Figure 3. LD mass spectra of dipeptides at their threshold irradiance, λ = 266 nm.

is the most intense mass signal already at threshold. With increasing irradiance the $(M + H)^+$ signal decreases and fragment ions due to decarboxylation $(M + H - 46)^+$ or the aromatic residue (R^+) become the base peaks among the sample-specific ions. Above $5 \times E_0^{Trp}$ alkaline ions appear in the mass spectra and cationized molecules are observed. For all aliphatic AAs analyzed LD mass spectra at 266 nm and threshold irradiances show equal characteristic features: protonated and cationized molecules of comparable intensities and abundant $(M + H - 46)^+$ ions. With increasing irradiance the $(M + H - 46)^+$ signals increase at the expense of the quasi-molecular ions. At present the available data for LD-MS does not actually allow one to decide whether the $(M - 45)^+$ ion originates from a $M^{+\cdot}$ precursor or is generated as $(M + H - 46)^+$ by loss of formic acid from the protonated species. We have adopted the latter nomenclature, in analogy to MS/MS results obtained for CI-MS and because $M^{+\cdot}$ have never been observed in any of the AA spectra. Day et al. (*17*) present a more detailed discussion of this problem.

A typical series of LD mass spectra taken at a wavelength of 266 nm is shown in Figure 2. Figure 3 shows a representative series of mass spectra of dipeptides. For all dipeptides measured, a general trend of ion formation and fragmentation can be derived. At low irradiance, necessary for dipeptides containing at least one Trp, $(M + H)^+$, R^+, and a fragment ion (AA (N-terminal) $- 45)^+$ are observed. If Trp is the C-terminal AA, an additional peak at $(Trp - 17)^+$ due to the cleavage of the C-terminal C–N bond appears; $(M + H - 46)^+$ ions are never observed. At higher irradiances, as necessary for Phe–Tyr, an additional peak due to (AA (C-terminal) $- 45)^+$ appears, indicating a reformation of the C-terminal amino acid. A fragmentation from a protonated molecule or a (neutral) degradation, as proposed for thermal

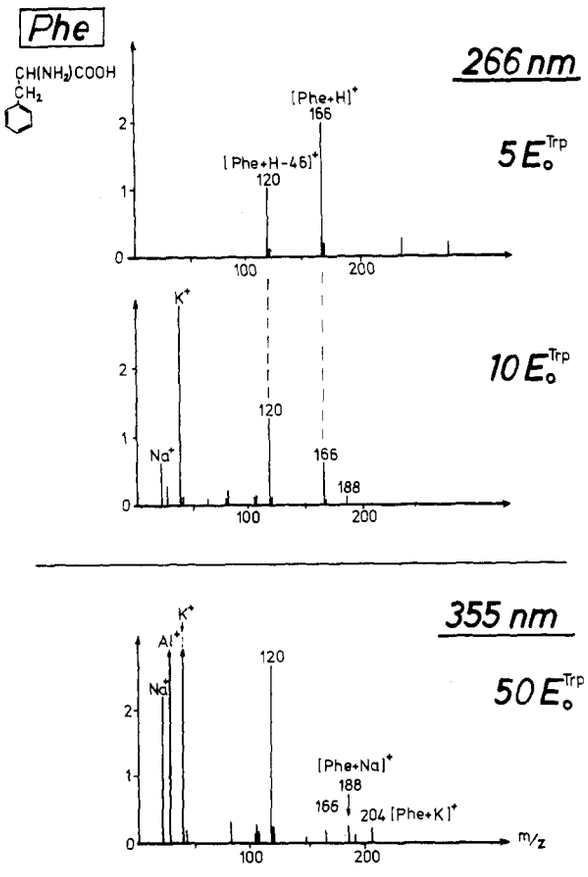


Figure 4. LD mass spectra of phenylalanine for threshold irradiance at 266 nm (a) and two times threshold irradiance at 266 nm (b) and for threshold irradiance at 355 nm (c).

decay by Bombick et al. (*18*) as the first step followed by protonation, could both reasonably explain the formation of such ions. Cleavage of the peptide bond producing acylium ions is only marginal.

Mass spectra of aliphatic dipeptides, using 266 nm, can be explained by applying the above considerations. In the case of C-terminal proline, additional mass peaks at 114 and 96 occurred indicating the cleavage of the peptide bond and subsequent water elimination.

At 355 nm fragment ions are generally the most intense specific ions. In the low mass range nonspecific ions and abundant alkaline and aluminum ions are also observed. Figure 4 shows the changes in the LD mass spectra of Phe going from threshold irradiance at 266 nm (a) to twice that (b) and to threshold irradiance at 355 nm (c). For dipeptides LD at 355 nm often failed to produce molecular ions. If molecular ions do occur, cationization is much more intense than protonation. The systematic differences in the mass spectra as seen at 266 nm have completely disappeared, in agreement with the uniform threshold irradiances at 355 nm. The remaining differences in the spectra of different samples taken at a wavelength of 355 nm presumably reflect the dependence of LD on the molecular structure and/or the varying microscopic sample morphology. Some additional observations are worth reporting.

Reproducibility. In general, mass spectra at 266 nm are by far more reproducible as compared to 355 nm. At 355 nm the ratio of successful laser shots, i.e., the ratio of shots yielding specific ions to those producing only nonspecific alkaline and low mass fragment ions, increase from about 0.05 for aliphatic AAs and dipeptides to a value of 0.5 for Trp. At 266 nm aliphatic AAs and dipeptides yield a factor of 0.5; aromatic AAs and dipeptides give specific mass spectra for nearly each laser shot. At 266 nm LD to a large extent is independent

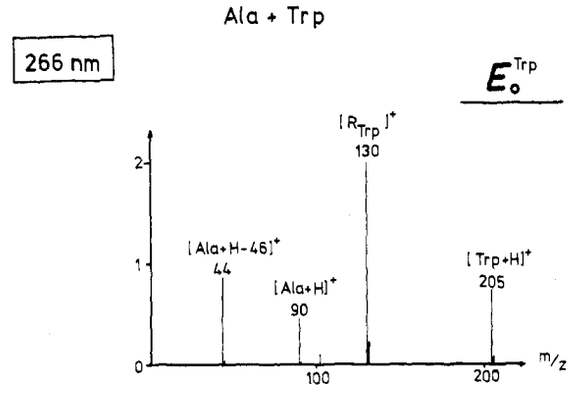


Figure 5. LD mass spectrum of a 1:1 mixture of alanine and tryptophan at 266 nm and threshold irradiance for tryptophan.

of sample thickness and micromorphological structure. Aromatic samples yield equal LD spectra from samples, prepared as described above, and from the sample powder, supplied commercially dusted onto the substrate.

Alkaline Ion Attachment. For ion formation by alkaline attachment an optimum yield is found at or near the threshold irradiance for the appearance of alkaline ions themselves. Under these conditions alkaline ions and cationized molecules have comparable intensities. At higher irradiances, alkaline ions are desorbed with much higher intensities, but the signals of the alkaline-ion-attached molecules do not increase accordingly, and often they even decrease. This is particularly pronounced at the wavelength of 355 nm. This observation would be hard to reconcile with a model of a gas-phase ion/molecule reaction as proposed for CW IR LD by Röllgen (*5*) and Kistemaker (*2*) and for pulsed-IR LD by Kistemaker (*3, 4*), unless the decreasing intensity of alkaline-ion-attached molecules would result from the increasing initial energy of these ions with increasing irradiance. The latter assumption is however somewhat unlikely, because in the range of irradiances used, initial ion energy generated from nonmetallic samples does not change appreciably.

Sample Damage. At threshold irradiance for ion detection and 266 nm samples of aliphatic AAs show a microscopically just-visible sample damage of ~3–4 μm in diameter, whereas damage to a sample of aromatic AAs remained below the resolution limit of the microscope, ~1 μm.

"Matrix-Assisted" Laser Desorption. The mass spectrum of a mixture of alanine (Ala) and Trp taken at Trp threshold irradiance is shown in Figure 5. A strong signal of the Ala quasi-molecular ion was observed in addition to that of Trp. It is important to note that its desorption took place at an irradiance of about a tenth of that necessary for obtaining spectra of alanine alone.

Tryptophan thus must be regarded as an absorbing matrix resulting in molecular ion formation of the nonabsorbing alanine. This kind of "matrix-assisted LD" has also successfully been applied to reproducibly desorb other nonvolatiles, e.g., stachyose (*19*).

DISCUSSION

The striking similarity of the main features of all LD mass spectra, irrespective of the wavelength used, has led investigators to believe that in this technique the wavelength is at least a parameter of minor importance (*11–13*). The correlation between the wavelength-dependent laser threshold irradiances for ion generation and the classical extinction coefficient due to vibrational absorption bands, as reported for the IR (*8*), still support this general notion, because the desorption process stipulated for these results is a thermal one and no qualitative differences of spectra taken at different wavelengths near 10.6 μm were seen. For wavelengths in the

visible and UV many, if not most, of the substances investigated exhibit only negligible absorption at the wavelengths used, and where present, spectra taken somewhat above threshold irradiance were qualitatively very similar for both groups of substances. It is generally assumed that in this wavelength range energy transfer between the laser field and the sample takes place via a nonlinear multiphoton process. The threshold irradiances of 10^7 W/cm^2 and above found experimentally and the general, i.e., sample nonspecific trend of decreasing threshold irradiance with decreasing wavelength (leading to a decrease in the number of photons to be absorbed simultaneously in a single excitation process), certainly support this notion.

The data presented in this paper however prove that at least for nanosecond pulses in the UV and in the preferential working range near threshold irradiance, a match between a molecular absorption band and the laser wavelength leads to qualitatively different spectra as evident from Figure 2 and outlined in the Results section. These features, particularly the high yield of parent molecular ions and the low degree of fragmentation, may moreover be desirable in practical applications. To account for the observations two mechanisms are suggested and will be discussed: the first involves a partly or fully linear energy transfer; the second involves ion formation via excited state molecules. Though both processes will in reality not be independent of each other, they are separated here for the sake of clarity in the discussion.

Assuming classical, i.e., linear absorption, the total energy absorbed per chromophore (e_a) of the top molecular layer at a given laser irradiance (E) during the laser pulse duration (τ) can be derived from Beer's law to be

$$e_a = \frac{2.3\epsilon_n E\tau}{N_A} \quad (1)$$

where ϵ_n is the molar extinction coefficient and N_A is Avogadro's number. For tryptophan in aqueous solution $\epsilon_n = 4.5 \times 10^3$ L mol^{-1} cm^{-1} at 266 nm. With $\tau = 10^{-8}$ s and $E_0^{Trp} = 2 \times 10^7$ W/cm^2 the energy deposited per molecule is about 20 eV or 4–5 photons. This energy would certainly suffice for the desorption, cleavage of about one covalent bond per molecule (the R fragment is the base peak), and the observed initial energy of a few electronvolts at threshold. Assuming a singlet excited-state lifetime of $<10^{-9}$ s and only negligible intersystem crossing as reported in the literature and taking into account the well demonstrated photostability of tryptophan (*20*), the absorption of 4–5 photons in a 10-ns pulse is just feasible. In the mass spectrometer substances are in the polycrystalline, solid state, rather than in aqueous solution, which could lead to very substantial differences in the absorption characteristics. Preliminary results (*21*) obtained for 0.5–2 μm thick layers of tryptophan, evaporated by the molecular beam technique onto quartz substrates, however indicated that the solid-state absorption spectrum is almost identical with that obtained for dilute solutions. For tryptophan classical absorption may be the only, or at least the dominating, energy-transfer process. The above assumptions are also supported by results obtained for tyrosine at 266 nm with single pulses of 30 ps in duration, as shown in Figure 6 (taken from ref 22). Whereas the spectrum obtained with 10-ns pulses shows abundant molecule-specific ions, the one taken with a 30-ps pulse contains unspecific fragments only, although the total incident (threshold) energy was nearly the same in both cases. In contrast to the predominantly linear sequential absorption of several photons during the 10-ns pulse, excited-state lifetime will allow only one classical electronic excitation of the molecule during the 30-ps pulse. The laser intensity, up by a factor of 300 for the 30-ps pulses as compared to the 10-ns pulses, will however lead to a strong

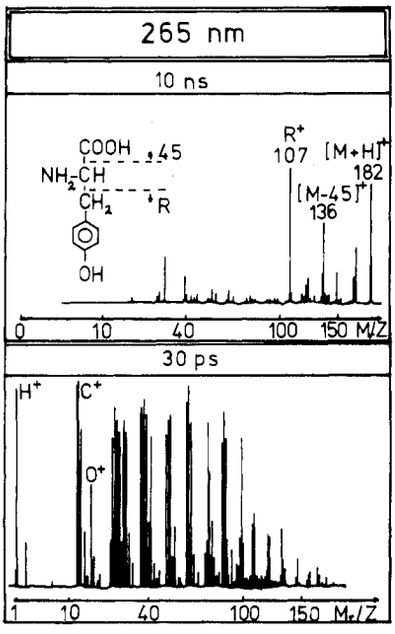


Figure 6. LD mass spectra of tyrosine at 266 nm near threshold irradiance with 10-ns (above) and 30-ps (below) pulse widths.

excited-state absorption and most likely even to nonlinear multiphoton absorption of fragment molecules and ions, resulting in a very strong decomposition of the molecule.

The threshold irradiances of 4×10^7 W/cm^2 and 10^8 W/cm^2 for tyrosine and phenylalanine are factors of 2 and 5, respectively, above that of tryptophan and therefore do not quite reflect their decrease of molar extinction by factors of 4 and 20, respectively. This on the one hand points toward the onset of a partial contribution of nonlinear absorption if equal energy deposition per molecule at desorption threshold is assumed. The spectra of these molecules are on the other hand very similar to those of tryptophan. In particular for tyrosine at threshold the $(M + H)^+$ ion forms the base peak in the spectrum. This observation could also be taken to indicate that resonance excitation of sample molecules has additional effects besides enhancing linear energy transfer.

Within the models developed for resonant excitation of chromophores in the solid state, excited states form excitons, which in the case of organic crystals should be localized (Frenkel excitons). It is assumed that most of their energy is transferred to the lattice by exciton–phonon coupling. The desorption of neutrals and ions would then result from a short, strong perturbation of the lattice. The limited degree of fragmentation in conjunction with the initial ion energy of several electronvolts is taken as an indication that this collective process of lattice disintegration leading to desorption takes place far away from thermodynamic equilibrium. The results found for the matrix-assisted desorption of nonabsorbing molecules (Figure 5) seem to support this model of desorption as a collective process.

At 355 nm one observes not only a higher threshold irradiance as compared to 266 nm but also a more pronounced threshold behavior and smaller working range (Figure 1). This is to be expected, because energy transfer takes place via nonlinear absorption, which requires an irradiance of about 4 times that at 266 nm for samples nonabsorbing at either wavelength. This will necessarily result in an increased energy transfer to the sample, even at threshold, for equal pulse lengths as used in these experiments. At the wavelength of 355 nm the mass spectra always show signals of the aluminum substrate in addition to those of the sample. This could indicate that at this wavelength part of the energy transfer could be mediated by the substrate. This question needs

further clarification and is currently under investigation.

In the following a second mechanism is discussed that, in addition to linear energy transfer, could result in enhanced parent ion formation. It must be assumed that upon lattice disintegration during the laser pulse some of the molecules or ions remain in the singlet excited state or get excited by photon absorption after lattice disintegration. Excited amino acid molecules could then act as proton donators for ground-state molecules because of their increased acidity. At least for the aromatic amino acids in solution, a shift of the pK value toward lower values upon excitation is well-established (*23*). In this way absorbing amino acids should show a higher ion-to-neutral ratio than nonabsorbing ones. Two observations support this assumption. At threshold the amount of material removed per laser shot, as estimated from the area which under microscopic observation appears affected, is higher for the aliphatic amino acids by at least a factor of 10, as compared to the aromatic ones, yet the total ion current is about the same in both cases. Also Hardin and Vestal (*24*, *25*) have reported that at a nonresonant wavelength of 483 nm the alkali-attached ions greatly dominate over the protonated ones, a trend which is also visible in the spectra reported here of the aliphatic AAs taken at 266 nm and of all the AAs taken at 355 nm.

In the model discussed here, it is assumed that ion formation by proton transfer, chemical rearrangements if present, and most of the fragmentation take place in a time shorter than or at most equal to about the laser pulse time of 10 ns. During this time interval the density of the sampled material will decrease from solid-state density by only 4 orders of magnitude due to the expansion velocity of the material of $\sim 10^5$ cm/s as judged from the initial ion energy. Transient phenomena taking place in this transition region are difficult to assess theoretically and the ongoing discussion as to whether or not to apply gas-phase or condensed-phase chemistry to these processes may be somewhat futile. The model is also supported by the observation that increased signals of alkali ions appearing in the spectra with increasing irradiances are not paralleled by an increase of the signals of alkaline-ion-attached parent molecules, in contrast to the IR-desorption experiments by Kistemaker and Röllgen in which locally separate desorption of neutral molecules and alkali ions followed by gas-phase attachment was shown to be the dominating process.

Fragmentation of molecules is often taken as a measure of the "thermal load", i.e., the temperature to which the desorbed material has been exposed. Besides the fact that thermodynamic equilibrium, required for such an interpretation, is most probably not reached in the process of laser desorption as discussed here, there is in this case also the possibility of direct photofragmentation as a consequence of the electronic singlet-state excitation of the molecules. The preferential formation of the residue ion in tryptophan, which carries the chromophore, may indicate such a photofragmentation or photoenhanced fragmentation. In order to avoid complication due to differing molecular chemistry, this paper concentrated on the discussion of the wavelength influence on the desorption of amino acids and dipeptides by 10-ns UV laser pulses because of their strong chemical similarity. All other aromatic compounds measured so far (e.g., carboxylic acids, alcohols) have however followed the same trend.

The data and the interpretation presented in this paper promise to be of considerable value for future practical applications of laser desorption mass spectrometry. The choice of an appropriate laser wavelength could be used to induce selective, or at least enhanced, desorption of absorbing organic molecules if suspected to be contained in a mixture. On the other hand, the use of a strongly absorbing matrix at a fixed laser wavelength offers a more controllable energy deposition and thus "soft" ionization and enhanced ion yield for organic samples independent of their individual absorption characteristics.

Registry No. Alanylglycine, 687-69-4; valylproline, 20488-27-1; leucylphenylalanine, 3063-05-6; phenylalanyltyrosine, 17355-18-9; glycyltyrosine, 658-79-7; valyltyrosine, 3061-91-4; tyrosylalanine, 730-08-5; tryptophylleucine, 13123-35-8; valyltryptophane, 24587-37-9; tryptophyltryptophan, 20696-60-0; phenylalanine, 63-91-2; tyrosine, 60-18-4; tryptophan, 73-22-3.

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