Infrared Desorption of Neutral Molecules Embedded in Transparent Matrices

R. C. Beavis, J. Lindner, J. Grotemeyer, and E. W. Schlag

Institut für Physikalische und Theoretische Chemie, Technische Universität München

Z. Naturforsch. **43a**, 1083–1090 (1988); received July 25, 1988

The desorption of intact molecules by pulsed IR irradiation is shown to depend on the IR absorption of the matrix. IR transparent matrices, such as NaCl or NH_4NO_3, increase the total yield and the shot-to-shot stability of the sample. IR absorbing matrices decrease the yield of intact molecules and increase the amount of pyrolysis products.

Using semi-transparent sugar matrices with dipeptide samples, the most intense fragment, i.e. the parent molecule having lost 18 u, is suppressed. This fragment is formed by pyrolysis during IR desorption. Three other prominent fragments are caused by the UV photoionization step.

The experimental results suggest that a model involving bulk stresses and strain may be necessary to explain the observations.

Introduction

A method for the mass spectrometric observation of intact, thermally labile molecules was developed at this Institute, employing a low power, pulsed CO_2 laser to desorb the neutrals [1, 2]. The 10.6 μm radiation was focussed onto a solid sample, and the molecules desorbed from the surface were entrained and cooled by a supersonic jet. After passing through a skimmer, the molecules were ionized by resonance enhanced multiphoton ionization (MUPI) [3–5].

The use of an infrared (IR) laser for the production of ions from surfaces has been employed for some time [6–8]. A CW CO_2 laser, if focussed onto a sample for a sufficient time, produces ions, but the delay time between the start of laser irradiation and the emission of ions is on the order of hundreds of milliseconds [9]. High-powered pulsed CO_2 [10] and Nd-YAG (1.06 μm) [11–13] lasers have also been used to produce ions from organic surfaces. The production of ions by IR radiation was shown to be caused by adduct ion formation. Ions, such as Na^+, leave the surface and combine in the gas phase with desorbed neutral molecules via ion-dipole interactions [9, 14].

Several interesting studies have also been done on IR and thermal desorption of neutrals using post-ionization techniques. Allison et al. [15] and Röllgen et al. [16] showed that the emitted neutrals could be ionized in the gas phase by using an intersecting alkali metal ion beam, producing sodium adduct ions from the desorbed neutrals. Cotter et al. [17], using electron impact post-ionization, demonstrated that ions and neutrals were produced by two different processes. Prompt ions were detected during the first few microseconds after the laser pulse, but the production of neutral molecule continued for approximately one hundred microseconds.

On the basis of a theoretical analysis [18, 19], the desorption of neutral molecules by an IR laser has been attributed to purely thermal effects. More subtle quantum mechanical effects may also be involved in this process [20–23]. The experimental conditions involved in these models are difficult to achieve in the laboratory, however, so their applicability to mass spectrometry experiments has not yet been established.

In the present work, the mechanism of IR desorption and subsequent fragmentation was studied by altering the physical characteristics of the sample. To alter the sample, without changing the actual molecular species present, it was mixed with compounds having well known IR absorption characteristics. The role of surface chemistry in the production of pyrolysis products of the dipeptides was examined by the use of organic matrices. A method for the suppression of these reactions was developed from these experiments.

Experimental

1. Apparatus

The desorbed molecules were detected using a reflectron time-of-flight mass spectrometer (Bruker

Reprint requests to Dr. J. Grotemeyer, Institut für Physikalische und Theoretische Chemie, Technische Universität München, Lichtenbergstraße 4, D-8046 Garching, BRD.

0932-0784 / 88 / 1200-1083 $ 01.30/0. – Please order a reprint rather than making your own copy.

TOF I), coupled with a pulsed supersonic jet. The apparatus has been described previously [24], but is briefly discussed below.

The sample holder was mounted just below the nozzle of the supersonic jet. The infrared laser (LP 30, Pulse System Inc., low pressure, pulsed, multimode CO_2 laser, 10.6 μm) was fired and the pulsed jet valve opened. The desorbed molecules then traverse the 1 mm between the probe surface and the jet and are entrained. The jet swept the molecules out of the desorption chamber, through a differentially pumped skimmer and into the ionization laser beam (Lambda Physics FL 2001 tunable dye laser, pumped by a Lambda Physics EMG 102 excimer laser), tuned to ionize the molecules being studied by resonance enhanced MUPI. The distance from the jet nozzle to the ionizing laser was 80 mm, the diameter of the skimmer was 1 mm, and the diameter of the ionizing laser beam was 0.1 mm. The ions produced by the laser were accelerated and mass analyzed by a reflectron time-of-flight instrument.

2. *Sample Preparation*

The organic molecules examined were treated in as simple a manner as possible. One milligram of the sample was mixed with 50 μl of water containing the matrix material. Twenty microliters of the mixture were spread over a stainless steel probe tip, with an area of 40 mm^2. The mixture was air dried. If the sample did not dissolve in water, the sample was slurried by thorough agitation.

Results

The dipeptides used in these experiments were semi-transparent at the laser IR frequency, i.e. there was no absorption band in the IR spectrum of the molecules either centred on, or with a significant tail at 10.6 μm. The absorption coefficient of the sample molecules used was on the order of 100 cm^{-1}. The laser wavelength 10.6 μm is important because it is poorly absorbed by most organic molecules. Only ring systems with some strain or the addition of hetero-atom (e.g. P or S) into an organic molecule result in an absorption band in this region. The only nearby strong band is caused by the C–O stretch mode in hydroxyl groups near 1000 cm^{-1}. Therefore, the majority of biological molecules that have been examined by this technique to date [25] have been at best semi-transparent, and not strong IR absorbers.

1. *Inorganic Matrices*

The first class of matrices used were IR absorbers: Na_3PO_4, Na_2CO_3, Na_2HPO_4 and sodium acetate. The phosphates absorb very strongly, owing to a resonance absorption of the P–O bond, while the other two matrices were medium strength absorbers, owing to tails of absorption peaks centred in the 1000 cm^{-1} region. Because these materials were easily soluble in water, relatively large quantities of matrix could be mixed with a dissolved peptide sample.

Mixing IR absorbers with peptides generally resulted in a decrease in the peptide yield. In most cases, the addition of the matrix completely suppressed the yield of peptide in the MS. This result can be explained in terms of the temperature reached by the matrix/sample mixture during the IR irradiation. Calculations based on a model for the temperature of a semi-transparent material irradiated by an intense source [26] showed that under our irradiation conditions, the temperature would reach at least 1300 K by the end of the laser pulse. Such a temperature is sufficient to pyrolyze the peptide molecules, either on the surface or during their flight to the supersonic jet. Presumably, once the molecule has entered the jet, the vibrational cooling provided by the jet would be sufficient to rapidly suppress any pyrolysis. However, the molecule is in flight for approximately ten microseconds before entrainment, sufficient time for unimolecular decay of vibrationally excited species. Because of the limited dynamic range of TOF instruments, a decrease in overall yield of intact sample molecules by two orders of magnitude would be sufficient to render the signal undetectable.

The second class of matrices were IR transparent: NaCl, KBr and NH_4NO_3. These materials are almost completely transparent at 10.6 μm and water soluble. The general result of adding these matrices was to *increase* the yield of intact neutral molecules detected by MUPI. Figure 1 a shows the typical increase in intact neutral molecule yield as a function of the matrix-to-sample ratio for dipeptides with non-polar sidechains. Dipeptides with polar sidechains, such as lysine, did not show an improvement in yield (Figure 1 b). However, in both cases the addition of the inorganic matrix increased the number of IR shots possible on one spot of the probe. The increase in the

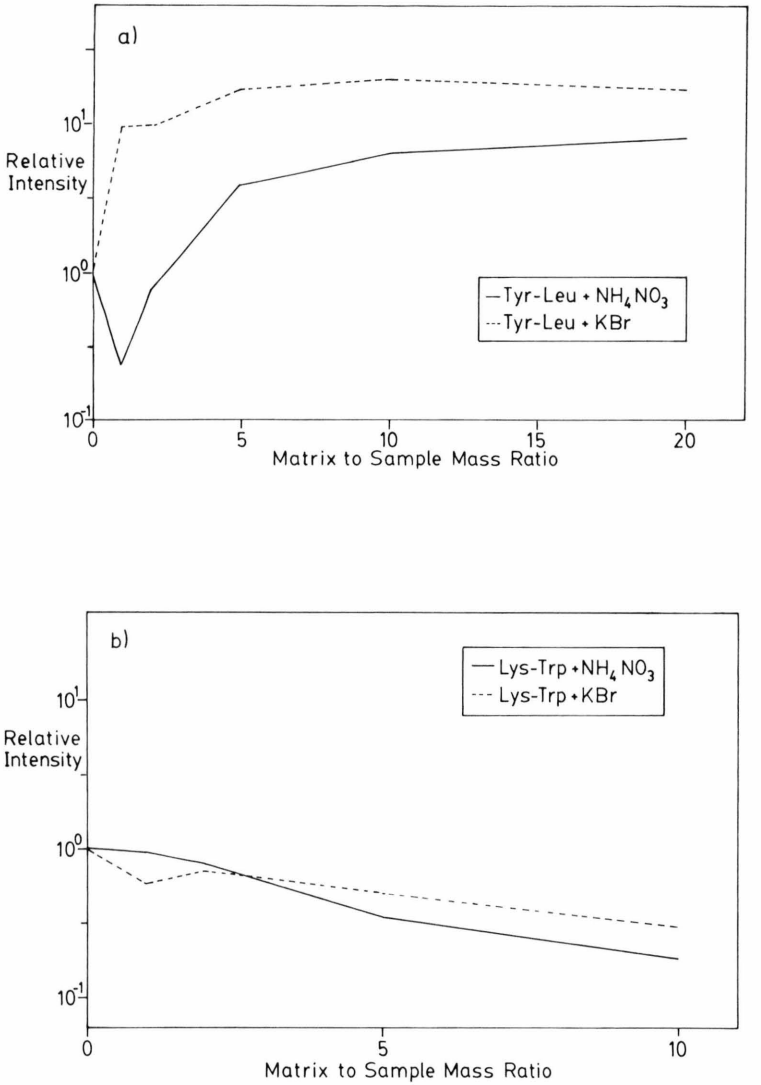

Fig. 1. The dependence of the molecular ion signal intensity on the presence of an IR non-absorbing matrix. 100 IR shots accumulated. (a) Non-polar dipeptide: Tyrosine-Leucine (MUPI wavelength = 280 nm). (b) Polar dipeptide: Lysine-Tryptophan (MUPI wavelength = 285 nm).

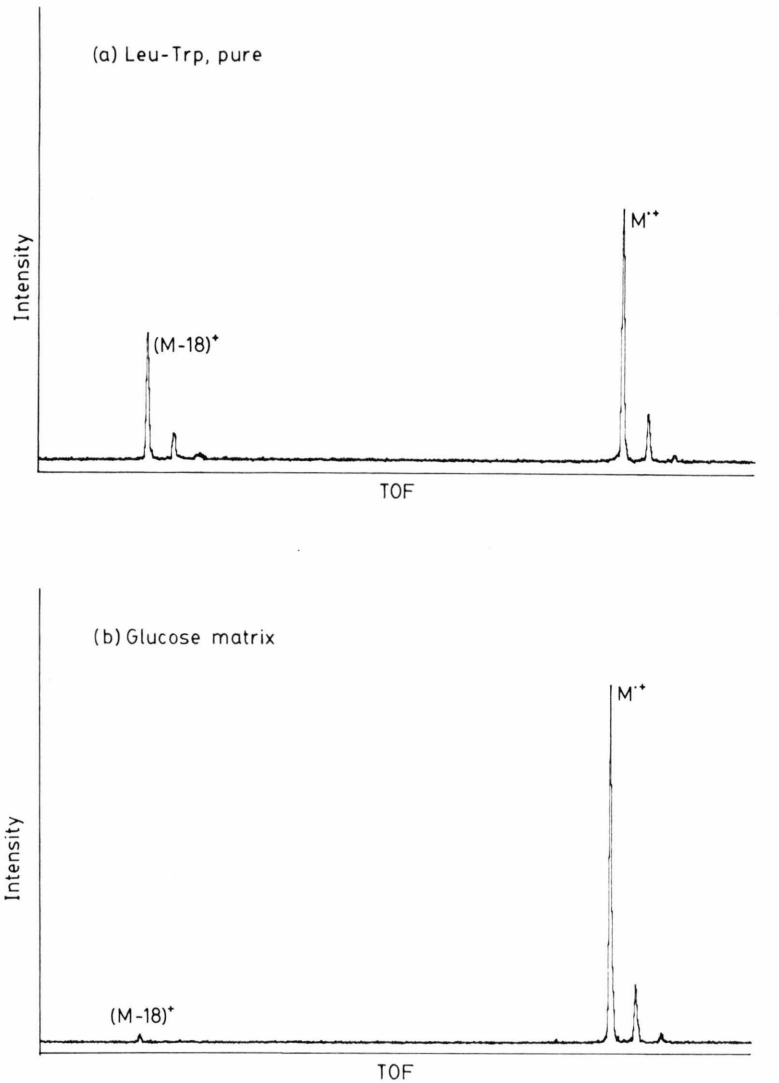

Fig. 2. The suppression of the $(M-18)^+$ species by addition of a saccharide matrix to a dipeptide sample. 100 IR shots accumulated, MUPI wavelength = 285 nm. (a) Pure Leucine-Tryptophan. (b) Leucine-Tryptophan plus a glucose matrix.

number of shots possible per spot (i.e. shots producing intact neutral molecules) was typically a factor of 100 greater than without a matrix.

2. *Organic Matrices*

The type of organic matrices used were mono- and disaccharides. These molecules show a range of IR absorption characteristics, owing to shifts in the C–O vibrational mode near 1000 cm^{-1}. These materials also show rather similar solubility in water and other physical properties. None of the sugars tested was transparent at 10.6 μm, but several were semi-transparent. The sugar followed the same general trend as the inorganic salts: the more transparent the matrix, the better the yield of neutral molecules. For sugars with a resonant vibrational band at 940 cm^{-1}, such as ribose, the yield of neutral molecules completely vanished.

An interesting observation with sugar matrices was the suppression of one type of pyrolytic reaction commonly found when desorbing peptides with an IR laser. Spectra taken by MUPI of peptides frequently show an ionic species at $(M\text{-}18)^+$. This species has been ascribed to UV photofragmentation in the MUPI step, although the mass of the signal has been at times reported as M-17 u [27]. In our studies, $(M\text{-}17)^+$ was not found to be the most intense signal [28]. Adding a semi-transparent sugar to a peptide suppressed the $(M\text{-}18)^+$ species (Figs. 2 and 3). This suppression strongly suggests that the $(M\text{-}18)^+$ ion is not formed from an M^+ ion during MUPI, but that it is a neutral species in the jet. It is, therefore, a pyrolysis product, which is suppressed by the presence of an excess of sugar. Derivation studies showed that only the C-termal end of the molecule participates in the pyrolysis, suggesting a loss of water to form a C-terminal ketene [29]. The formation of a stable ketene would not be possible in the sugar matrix, because at temperatures high enough to cause pyrolysis of the peptide molecule, the sugar would also pyrolyze. The resulting production of water would drive the ketene formation reaction back towards the initial peptide.

The suppression of the M-18 u pyrolysis species makes possible the examination of two other species formed by the MUPI process. Figure 4 shows the region of the TOF spectrum around M-18 u for a Trp-Phe sample with and without a sugar matrix. The two additional species, $(M\text{-}16)^+$ and $(M\text{-}17)^+$, are typical of spectra produced by dipeptides having an N-terminal aromatic group. The peak shapes in the spectrum taken with a sugar matrix suggest that the $(M\text{-}16)^+$ species is more stable than $(M\text{-}17)^+$, i.e. a significant fraction of the $(M\text{-}17)^+$ ions undergo a metastable decay in the ion acceleration region of the MS. The addition of the matrix removes the signals of the $(M\text{-}18)^+$ ^{13}C satellites from the $(M\text{-}17)^+$ and $(M\text{-}16)^+$ signals.

Figure 5a shows the evolution of signal strength during a sequence of 80 laser shots on one spot. Each point is the accumulated intensity for twenty shots within this sequence. The species followed in Fig. 5a are: i) the intact molecular ion (M^+); ii) UV photofragments of M^+, i.e. $(M\text{-}17)^+$ and $(M\text{-}16)^+$; iii) the pyrolysis product $(M\text{-}18)^+$; and iv) the quinolinium ion $(130)^+$, which could be produced by UV photofrag-

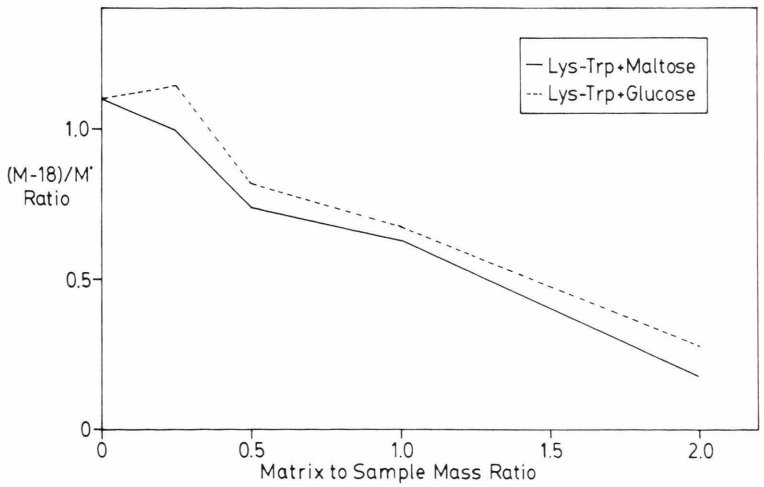

Fig. 3. The ratio of the intensity of the $(M\text{-}18)^+$ signal to the $M^{\cdot+}$ signal as a function of the concentration of matrix in the sample for two different sugars. 100 IR shots accumulated, MUPI wavelength = 280 nm.

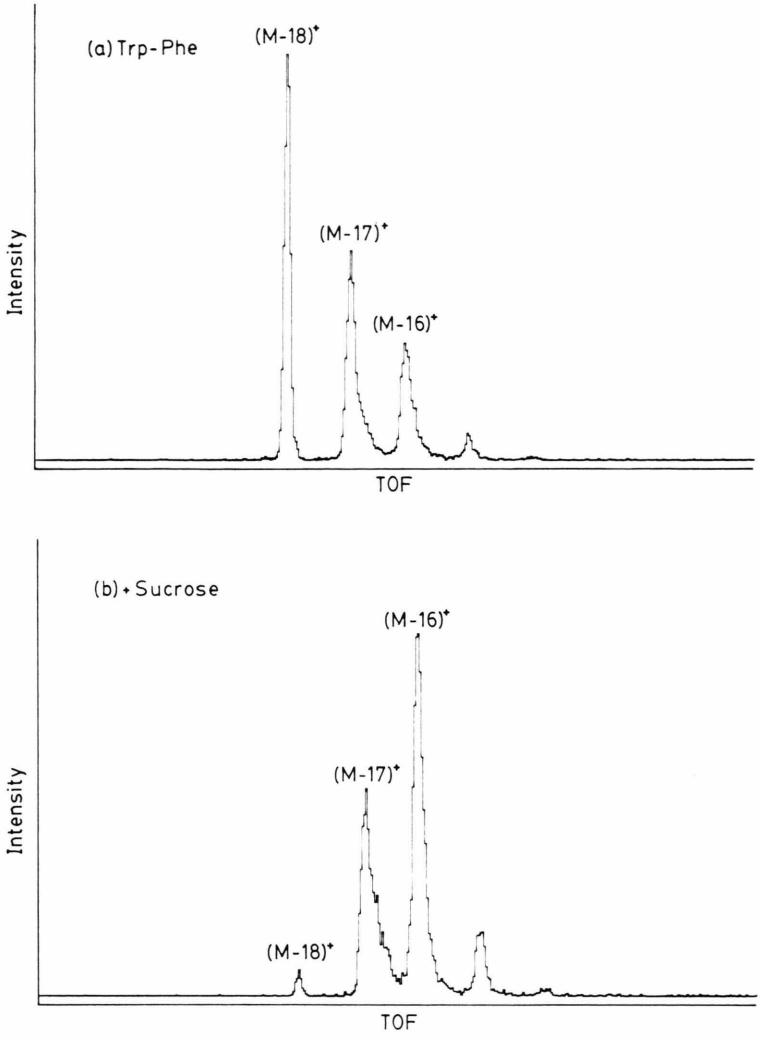

Fig. 4. Mass spectra around the $(M-18)^+$ peak for Tryptophan-Phenylalanine (mw = 352.2 u). 100 IR shots accumulated, MUPI wavelength = 285 nm.
(a) Pure Tryptophan-Phenylalanine.
(b) Tryptophan-Phenylalanine with a sucrose matrix.

mentation of any of the preceding species. Figure 5b shows the same data, normalized to the $(M-18)^+$ ion intensity. This graph clearly demonstrates that the $(M-18)^+$ species cannot be a UV photofragment of the intact molecule. The true UV photofragment should follow the same trend as the M^+ ion, e.g. $(M-17)^+$ and $(M-16)^+$. The more complicated behavior of the quinolinium ion is caused by its dependence on the intensities of all of the more massive species.

Discussion

The somewhat surprising observation that IR transparent matrices enhance neutral molecule emission during IR laser irradiation has some precedent for ions. It has been shown that IR desorption of sodium adduct quasimolecular ions is enhanced by using a sodium chloride or ammonium chloride matrix [30]. In that case, however, the enhancement in signal was for quasimolecular ions, and the increase in the total number molecules present in the gas phase was not measured.

Owing to the semi-transparency of organic molecules at 10.6 microns, multiphoton processes at low laser powers are unlikely. Also, this low absorptivity means that when dealing with a relatively thick layer of organic material, the temperature distribution at the surface and in the bulk will be important. The absorption coefficient, therefore, affects the rate of

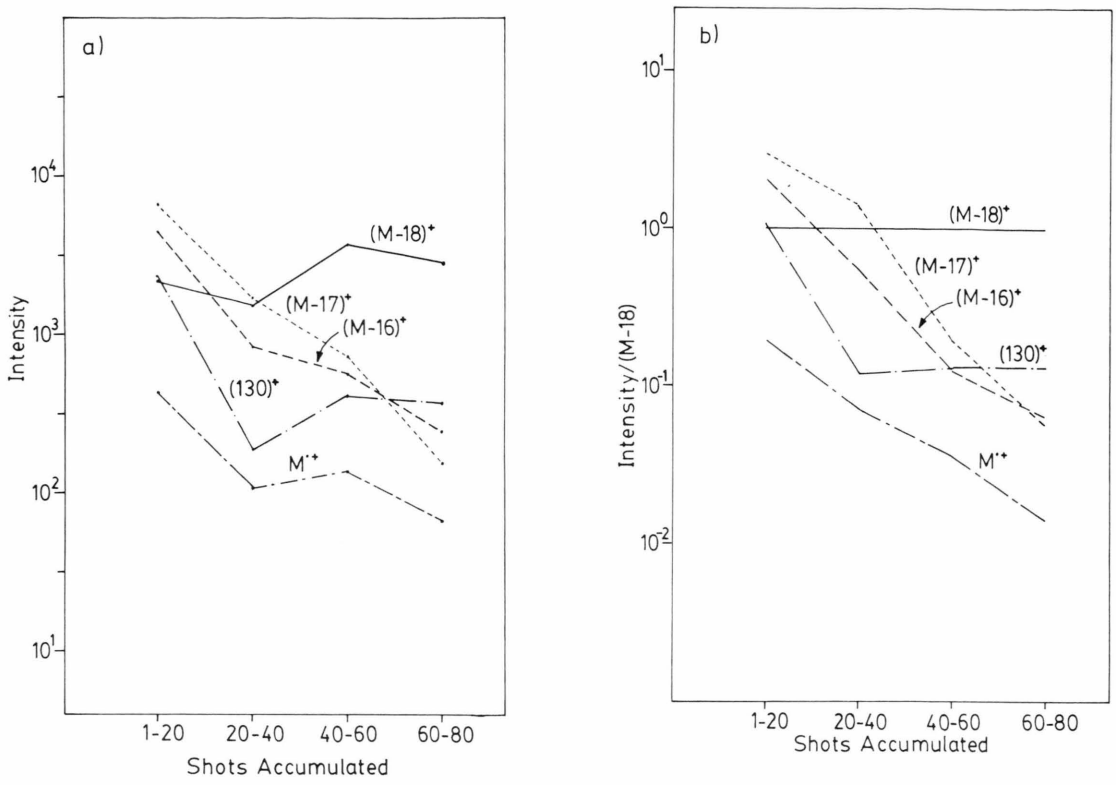

Fig. 5. The signal intensities of various important ions in mass spectra taken at intervals during IR irradiation of a Tryptophan-Phenylalanine sample. (a) Absolute intensities. (b) Intensities normalized to the $(M-18)^+$ ion intensity.

pyrolytic damage to the surface during irradiation. Early studies on the evaporation of thermolabile molecules from rapidly heated wires suggested that normally thermolabile molecules could be desorbed if heated rapidly enough [31]. This effect could be explained in terms of the activation energies of the evaporation and pyrolysis reactions. The explanation given clearly predicts that for large molecules (larger than 400 u) pyrolysis should dominate evaporation. Further work with CW CO_2 lasers confirmed this result, although the question of decreased ionization efficiency for larger molecules could not be discounted.

Results with pulsed CO_2 laser have been different. High molecular weight molecules (e.g. bradykinin) have been desorbed with little pyrolytic decomposition. Even with supersonic jet cooling, there should be some molecules that are not entrained (cooled) for 10–20 μs after desorption [32] Therefore, if a large molecule was heated sufficiently to evaporate by means of excitation of the molecules vibrational degrees of freedom, a significant fraction of the molecules evaporated should undergo unimolecular decay entrainment (based on lifetimes for excited large quasi-molecular ions obtained from TOF studies [33, 34]. These decays do not occur.

Thermal evaporation is not the only possible process that can be responsible for the desorption of intact molecules. Several authors have suggested that desorption phenomena produced by various primary particles are caused by purely mechanical forces. Shock wave induced surface "unloading" has been suggested [35–37] for both pulsed UV laser and high energy particle impact desorption. While a shock wave probably does not occur in our experimental situation, transient high pressures can be produced by two other mechanisms: i) warming of a semi-transparent material by a laser on a time scale short with respect to the time necessary for the resulting expansion to relax with the unirradiated surroundings [38]; and ii) strong localized absorption in an otherwise transparent material at a defect or impurity [39]. Both of these mechanisms produce localized high stress

fields, which relax by producing strain fields. The strain field can relax slowly by surface "unloading" during the formation of fractures, and the unloading will continue much longer than the original laser pulse. Neither mechanism requires high temperatures on the surface or in the bulk of the material. They only require that the change in temperature be rapid; a condition met in a pulsed laser experiment.

The existence of bulk motion caused by IR radiation induced strains is evident in our apparatus. Solid, clear films of peptides on the sample probe show marring on the surface caused by fracture formation. Less homogeneous samples eject macroscopic pieces, resulting in a deposit of powder on the pulsed valve nozzle tip and the bottom of the desorption chamber. The deliberate seeding of a transparent pressed KBr pellet with small IR absorbing particles, e.g. graphite, produces very pronounced damage to the crystal surface and results in the ejection of neutral KBr cluster molecules. Experiments based on this principle will be reported in a future publication [40].

A desorption mechanism based on the relatively slow relaxation of a strain field induced in a relatively transparent sample by a low power IR laser pulse (see also [41] and [42]) explains many of our experimental results. This mechanism does not discount the role played by thermal evaporation in the desorption of molecules as small as dipeptides. It makes possible, however, a significant yield of large molecules that cannot reach high enough internal temperatures to evaporate without decomposition. It also explains the increase in yield for a sample embedded in a transparent matrix, creating a semi-transparent composite material. The slowly relaxing strain field implies that the production of molecules is not limited to the duration of the laser pulse. In fact, intact molecules are emitted from the surface for at least 80 μs, after taking account of spreading before and after entrainment [27]. This period is much longer than would be expected if the desorption was purely thermal, as the temperature of the sample drops rapidly after the laser pulse.

Conclusions

The IR desorption yield of neutral organic molecules can be increased by the addition of an IR transparent or semi-transparent matrix. The desorption yield is decreased by the addition of IR absorbing matrices, presumably by increased thermal decomposition of the sample caused by the increased sample temperature. Decomposing semi-transparent matrices, such as sucrose and maltose hydrate, can suppress pyrolysis reactions induced by the IR laser. The suppression of the dehydration pyrolytic decomposition of peptide molecules was used to study the UV photofragmentation produced by MUPI. It was used as a means of distinguishing between fragments produced by MUPI and species produced by stable pyrolysis products in the supersonic jet.

Acknowledgements

This work was supported by grants from Deutsche Forschungsgemeinschaft (GR 917 1/1) and Bruker-Franzen Analytik, Bremen. Dr. Beavis received support as a NATO Postdoctoral Fellow.

[1] W. E. Henke, H. von Weyssenhoff, H. L. Selzle, and E. W. Schlag, Verh. Deutsch. Physik. Ges. **3,** 139 (1983).
[2] H. L. Selzle, H. von Weyssenhoff, and E. W. Schlag, Z. Naturforsch. **40 a,** 674 (1985).
[3] U. Boesl, H. J. Neusser, R. Weinkauf, and E. W. Schlag, J. Chem. Phys. **86,** 4857 (1982).
[4] J. Grotemeyer, U. Boesl, K. Walter, and E. W. Schlag, Org. Mass Spectrom. **21,** 595; 695 (1986).
[5] L. Lin and D. M. Lubman, Rev. Sci. Instrum. **59,** 557 (1988).
[6] M. A. Posthumus, P. G. Kistemaker, H. L. C. Meuzelaar, and M. C. Ten Noever de Brauw, Anal. Chem. **50,** 985 (1978).
[7] R. J. Cotter, Anal. Chem. **53,** 719 (1981).
[8] R. Stoll and F. W. Röllgen, Org. Mass Spectrom. **16,** 441 (1981).
[9] R. Stoll and F. W. Röllgen, Z. Naturforsch. **37a,** 9 (1982).
[10] G. J. Q. van der Peyl, J. Haverkamp, and P. G. Kistemaker, Int. J. Mass Spectrom. Ion. Phys. **42,** 125 (1982).
[11] B. Schueler, P. Fiegel, F. R. Krueger, and F. Hillenkamp, Org. Mass Spectrom. **16,** 502 (1981).
[12] D. Zakett, A. E. Schoen, and R. G. Cooks, J. Amer. Soc. **103,** 1295 (1981).
[13] M. P. Chiavelli and M. L. Gross, Int. J. Mass Spectrom. Ion Phys. **78,** 37 (1987).
[14] P. G. Kistemaker, G. J. Q. van der Peyl, K. Isa, and J. Haverkamp, in: Ion Formation from Organic Solids (A. Benninghoven, ed). Springer Series in Chemical Physics, 1982.
[15] D. Bombick, J. D. Pinkston, and J. Allison, Anal. Chem. **56,** 396 (1984).
[16] G. Schmelzeisen-Redeker, U. Giessmann, and F. W. Röllgen, Org. Mass Spectrom. **20,** 305 (1985).
[17] R. B. van Breeman, M. Snow, and R. J. Cotter, Int. J. Mass Spectrom. Ion Phys. **49,** 35 (1983).

[18] G. J. Q. van der Peyl, J. Haverkamp, and P. G. Kistemaker, Int. J. Mass Spectrom. Ion Phys. **42,** 125 (1982).
[19] F. P. Novak, K. Balasamugan, K. Viswandadham, C. D. Parker, Z. A. Wilk, D. Mattern, and D. M. Hercules, Int. J. Mass Spectrom. Ion Phys. **53,** 135 (1983).
[20] T. J. Chuang, Surf. Sci. Rept. **3,** 1105 (1983).
[21] B. Fain, Chem. Phys. Lett. **118,** 283 (1985).
[22] Z. W. Gortel, H. J. Keuzer, P. Piercy, and R. Teshima, Phys. Rev. **B 28,** 2119 (1983).
[23] B. Fain, V. Fleurov, and S. H. Lin, Chem. Phys. **122,** 17 (1988).
[24] U. Boesl, J. Grotemeyer, K. Walter, and E. W. Schlag, Anal. Instrum. **16,** 151 (1987).
[25] J. Grotemeyer and E. W. Schlag, Angew. Chem. **100,** 461 (1988).
[26] H. S. Carslaw and J. C. Jaeger, Conduction of Heat in Solids, 2nd ed., Chapt. 2, Oxford University Press 1959.
[27] R. Tembreull and D. M. Lubman, Anal. Chem. **59,** 1003 (1987).
[28] R. C. Beavis, J. Lindner, J. Grotemeyer, and E. W. Schlag, Chem. Phys. Lett. **146,** 310 (1988).
[29] J. March, Advanced Organic Chemistry, 2nd ed., McGraw-Hill, London 1983.
[30] D. V. Davis, R. G. Cooks, B. K. Meyer, and J. L. McLaughlin, Anal. Chem. **55,** 1303 (1983).
[31] R. J. Beuhler, E. Flanigen, L. J. Greene, and L. Friedman, J. Amer. Chem. Soc. **96,** 3990 (1973).
[32] P. Arrowsmith, M. S. de Vries, H. E. Hunziker, and H. R. Wendt, Appl. Phys. **B 46,** 165 (1988).
[33] K. G. Standing, R. Beavis, G. Bolbach, W. Ens, D. E. Main, and B. Schueler, Secondary Ion Mass Spectrometry, SIMS V (A. Benninghoven et al., eds.). Springer Series in Chemical Physics, 1986, p. 57; p. 185; p. 479.
[34] K. G. Standing, R. Beavis, G. Bolbach, W. Ens, F. Lafortune, D. Martin, B. Schuler, X. Tang, and J. B. Westmore, Anal. Instrum. **16,** 173 (1987).
[35] G. Carter, Radiat. Eff. Lett. **43,** 193 (1979).
[36] Y. Kitazoe and Y. Yamamura, Radiat. Eff. Lett. **50,** 39 (1980).
[37] I. S. Bitensky and E. S. Parilis, Nucl. Instrum. Methods **B 21,** 26 (1987).
[38] R. Bullough and J. J. Gilman, J. Appl. Phys. **37,** 2283 (1966).
[39] G. H. Connors and R. Y. Thompson, J. Appl. Phys. **37,** 3434 (1966).
[40] R. C. Beavis, J. Lindner, J. Grotemeyer, I. M. Atkinson, F. R. Keene, and A. E. W. Knight, J. Amer. Chem. Soc. **110,** 7534 (1988).
[41] J. H. Wasilik, IEEE Trans. Nucl. Sci. **NS 27,** 1632 (1980).
[42] Handbook of Laser Science and Technology, Vol. VI, Optical Materials: Part 2 (M. J. Weber, ed.). CRC Press 1986.