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Mechanism for the Degradation of Ribostamycin by the Laser Spray

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A laser spray interface for use in liquid chromatography/mass spectrometry (LC/MS) has been investigated with respect to the degradation of a thermally labile compound, ribostamycin. It was confirmed that few fragment ions were formed when the laser beam was focused to the center of the stainless steel capillary. When the laser beam was slightly off-centered, the sample ions suffered from the thermal degradation by the heated wall of the stainless steel capillary. A feasible observation of fragment ions for the thermally labile compounds by the wall heating would be useful for the structural elucidation of the sample molecules.

1. Introduction

In our recent work,^{1–5)} mechanism for the formation of gaseous ions from the infrared-laser irradiated liquid sample solution was investigated in detail. It was found that the laser spray gave stronger ion signals than electrospray, especially in the negative-ion mode operation.⁵⁾ This is mainly due to the enrichment of the sample concentration by the selective evaporation of solvent by the laser irradiation, the efficient separation of positive and negative ions by the strong electric field exerted on the tip of the stainless steel capillary, and the formation of finer charged liquid droplets by the laser spray than by electrospray.⁴⁾ It was also found that the higher voltage can be applied to the stainless steel capillary for the laser spray than for electrospray, probably because the occurrence of the gas phase breakdown is suppressed by the dense gas flow effusing out of the stainless steel capillary (Paschen's law).

In our previous paper,⁵⁾ it was suggested that thermal degradation takes place in the laser spray due to the superheating⁶⁾ of the aqueous solution. In the present work, a detailed investigation was performed whether a thermally labile compound suffers from the thermal degradation by the superheating caused by the laser irradiation. It was concluded that the thermal degradation due to the superheating of the laser-beam irradiated liquid was minor if there is any. Besides, the observation of fragment ions for the thermally labile compound was possible by adjusting the laser beam slightly off-centered from the center of the stainless steel capillary.

2. Experimental Method

The general experimental procedures were similar to those described previously.^{1–5)} Briefly, a stainless steel capillary (0.13 mm i.d., 0.3 mm o.d.) supplied sample

solution by means of a computer-regulated pump (Shimadzu LC-10Ai, Kyoto, Japan). For the laser spray, a Synrad (Bothel, WA, USA) model 48-5, Duo-Lase 50-W infrared (IR) laser (10.6 μm) was used. The terminal tip of the stainless steel capillary was irradiated from the opposite side of the capillary by a laser beam focused to ≈ 0.1 mm. The most intense ion signals were obtained with a laser power of 3–5 W ($3\text{--}5 \times 10^4 \text{ J cm}^{-2} \text{ s}^{-1}$), approx. +3 kV applied to the stainless steel capillary, and a sample flow rate of about $100 \mu\text{L min}^{-1}$.

In the absence of bubble-forming nuclei, liquids may be heated to temperature far above their boiling points (spinodal). There is, of course, an absolute limit of superheating, determined by the limit of mechanical stability of fluids. The limit of superheating is about 10% below the critical temperature (T_c) of many substances.⁶⁾ Although water/methanol solvent generally gives somewhat better sensitivities than water solvent for the laser spray, water was adopted as the solvent in the present experiment. This is because liquid water has higher T_c (374.1°C) than alcohols (e.g., T_c of methanol is 218.3°C). Apparently water is the best solvent to check the thermal degradation of thermally labile compounds by the superheating.

3. Results and Discussion

In the matrix-assisted laser desorption ionization (MALDI), fragment ions for the thermally labile compounds can be readily formed with increase of the laser power that is useful for the structural elucidation of the sample molecules.⁷⁾ Because the present method, the laser spray, can be regarded as the electric field-assisted MALDI using the solvent as a matrix, occurrence of the sample degradation by the laser irradiation may be conceivable. In this study, a detailed study was made whether the thermal degradation takes place in the laser spray ionization using ribostamycin as a test sample which is known to be thermally labile to give fragment ions a–e in Fig. 1.

Figure 2 shows the intensities of the most intense fragment ion b and the molecular-related doubly-

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Fig. 1. Structure of ribostamycin.

Fig. 2. The ion intensities of $(M+2H)^{2+}$, the fragment ion b, and the ratio of ion intensities $[I(b)]/I[(M+2H)^{2+}]$ as a function of laser power for aqueous solution of 10^{-5} M ribostamycin. The center of the focused laser beam was adjusted to that of the stainless steel capillary. Sample flow rate, $100 \mu\text{L min}^{-1}$; voltage applied to the stainless steel capillary, +3 kV; voltage applied to the ion sampling orifice, +120 V.

charged ion $(M+2H)^{2+}$, and the ratio of the ion intensities $[I(b)]/I[(M+2H)^{2+}]$ as a functions of laser power for 10^{-5} M aqueous solution of ribostamycin. The intensities of fragment ions a, c, d, and e are weaker than that of b by factors less than 1/5 and only the fragment ion b was considered here. In this experiment, the spot of the focused laser beam was set just the center of the stainless steel capillary. The heating of the wall of the stainless steel capillary could be suppressed minimal in such an arrangement. With the laser power in the range of 1–3 W, the laser-sprayed liquid droplets were coarse and no cone-shaped spray was formed. The coarse liquid droplets sprayed were attracted toward the interface plate in front of the ion sampling orifice. The sprayed charged liquid droplets were vaporized on the surface of the heated interface plate. The strong appearances of $(M+2H)^{2+}$ and the fragment ion b are notable. It seems likely that the coarse charged liquid droplets form abundant gaseous ions when they hit the heated interface plate. The formed gaseous ions were sampled into the ion sampling orifice by the electric field applied between the interface plate (+300 V) and the ion sampling orifice (+50~+150 V) to give fairly strong ion signals as

shown in Fig. 2. With the laser power of ≥ 3.5 W, a stable trumpet-shaped laser spray composed of much finer liquid droplets was established. Concomitantly, the ion intensities of $(M+2H)^{2+}$ and other fragment ions decreased as shown in Fig. 2. The decrease of the ion signals with increase of the laser power 3→3.5 W must be due to the very wide trumpet-shaped dispersion (almost 180° wide) of the charged liquid droplets resulting in the decrease of the gaseous ions sampled into the ion sampling orifice. It is interesting to note that the appearance of the laser spray observed by the naked eye did not change with increase of the laser power from 3.5 to ~ 9 W and the extra laser power larger than 3.5 W did not lead to the increase of the intensities of the sample ions as shown in Fig. 2. The explosive vaporization of the liquid sample leading to the formation of the gaseous ions seems to be already established with ~ 3.5 W. At present, it is not well understood why the increase of the laser power does not result in the increase of the laser-sprayed ion intensities.

In this experiment, the ions generated by the laser spray in the atmospheric pressure were sampled through the ion sampling orifice and accelerated by the field between the ion sampling orifice and the skimmer electrode which was held at the ground potential. The distance between the ion sampling orifice and the skimmer electrode is about 2 cm, thus the approximate electric field applied between the ion sampling orifice and the skimmer electrode is given by $V_0/2$ where V_0 represents the voltage applied to the sampling orifice. In the mass spectrum of ribostamycin, all of fragment ions a–e (see Fig. 1) were observed. These fragment ions may be formed either by the laser irradiation of the sample solution and/or by the collision-induced dissociation (CID) of the molecular-related ions in the drift region between the ion sampling orifice and the skimmer electrode. It was found that the fragment ions a–e disappeared completely with decrease of the voltage (V_0) applied to the ion sampling orifice. Figure 3 shows the relationship between the orifice voltage V_0

Fig. 3. Relationship between the voltage applied to the ion sampling orifice and the ratio of the intensities $[I(b)]/I[(M+2H)^{2+}]$ for aqueous solution of 10^{-5} M ribostamycin. The center of the focused laser beam was adjusted to that of the stainless steel capillary. Sample flow rate, $100 \mu\text{L min}^{-1}$; voltage applied to the capillary, +3 kV; laser power, 5 W.

and the ratio of the intensities of the fragment ion b and the molecular-related ion $(M+2H)^{2+}$, $[I(b)/I(M+2H)^{2+}]$. The fragment ion b (and all other fragment ions also) disappears with V_0 of about +50 V and lower. Exactly the same results were obtained with an increase of the laser power up to 9 W. This clearly indicates that the thermal degradation of the sample does not take place by the laser irradiation in the range of 1–9 W. It is apparent that the fragment ion b observed with the laser power in the range of 1–9 W in Fig. 2 were formed by CID in the ion drift region. That is, the thermal degradation due to the superheating⁶⁾ is minor in the present laser spray. However, when the laser power was increased to ≥ 9.5 W it was found that fragment ions persisted to appear with the orifice voltage lower than 50 V. It is apparent that the sample ion suffers from the degradation by the laser irradiation. With the laser power of ≥ 9.5 W, the terminal tip of the stainless steel capillary started to become red hot due to the wall heating. An increase of the ion intensities of the fragment ion b, a decrease of the molecular-related ion $(M+2H)^{2+}$, and a steep increase of the ratio $[I(b)/I(M+2H)^{2+}]$ in Fig. 2 with the laser power of ≥ 9.5 W may be due either to the direct fragmentation of the molecular-related ion $(M+2H)^{2+}$ in solution by the laser irradiation (superheating⁶⁾) or to the fragmentation of the molecular-related ion $(M+2H)^{2+}$ by the wall heating of the stainless steel capillary. Because the tip of the stainless steel capillary became red hot with the laser power of ≥ 9.5 W, we think that the observed fragmentation may be mainly caused by the wall heating with the laser power of ≥ 9.5 W. If the superheating plays an important role for the sample degradation, the relative intensities of fragment ions should increase with the increase of the laser power in the range of 3–9 W in Fig. 2, as in the case of MALDI.⁷⁾ The nearly laser-power independent ratio $[I(b)/I(M+2H)^{2+}]$ in Fig. 2 in the range of 1–9 W suggests that the superheating leading to the sample degradation is minor under the present experimental conditions.

Fig. 4. The ion intensities of $(M+2H)^{2+}$, the fragment ion b, and the ratio of ion intensities $[I(b)]/[I(M+2H)^{2+}]$ as a function of laser power for aqueous solution of 10^{-5} M ribostamycin. The center of the focused laser beam was set slightly off-center from the center of the stainless steel capillary. Sample flow rate, $100 \mu\text{L min}^{-1}$; voltage applied to the stainless steel capillary, +3 kV; voltage applied to the ion sampling orifice, +100 V.

Figure 4 shows the intensities of the molecular-related ion $(M+2H)^{2+}$, the fragment ion b, and the ratio of the ion intensities $[I(b)]/[I(M+2H)^{2+}]$ as a function of the laser power. In this experiment, the laser spot was intentionally set off-center slightly, from the center of the terminal tip of the stainless steel capillary. In such an arrangement, the wall of the stainless steel capillary became red hot with laser power of ≥ 5 W and it became possible to induce the thermal degradation of the molecular ions by the wall heating. With the laser power of ≥ 6 W, the tip of the stainless steel melted away. The steep increase of the ratio $[I(b)]/[I(M+2H)^{2+}]$ above 5 W is due to the thermal decomposition of the sample by the wall heating.

In Fig. 4, the relative ion intensity of fragment ion b to that of the molecular-related ion $(M+2H)^{2+}$ is smaller than that in Fig. 2 although the laser spot was set off-centered in Fig. 4. This is due to the lower V_0 (+100 V) in Fig. 4 than that (+120 V) in Fig. 2, *i.e.*, milder CID for $(M+2H)^{2+}$ took place in Fig. 4 than in Fig. 2.

4. Concluding Remarks

In our previous paper,⁵⁾ it was found that the laser spray has the better sensitivity than the conventional electrospray for almost all samples dealt with in our laboratory. Especially, the laser spray gives orders of magnitude better sensitivities than electrospray in the negative-mode operation for aqueous solutions of glutamic acid and di-2-ethylhexylsulfosuccinate sodium salt.⁵⁾ The present laser spray may be considered as the hybrid of three techniques, an energy sudden (laser explosion), a high electric field, and the nebulizer. The focused laser beam irradiation onto the liquid surface induces the explosive liquid vaporization, *i.e.*, the energy sudden. The evaporated solvent acts as the nebulizer because the jet flow would lead to the further atomization of the liquid droplets entrained in the jet stream. The positive and negative ions formed in the gas phase are separated efficiently by the strong electric field exerted on the tip of the stainless steel capillary. We found that the angle of the laser spray cone was much wider than that of the electrospray, indicating the better separation of positive and negative ions by the laser spray than by electrospray.

In this work, it is tentatively concluded that the thermal degradation due to the superheating of the liquid is minor if there is any. This is in contrast to MALDI in which the solid matrix is commonly used. It is likely that the temperature rise by the IR laser irradiation is not enough to cause the thermal degradation of the thermally labile compounds. Instead, the fragment ions for thermally labile compounds can be readily observed by the wall heating by adjusting the laser spot set off-center, from the center of the tip of the stainless steel capillary. Thus, the present laser spray may be applicable for the observation of ions extracted intact from the solution without suffering from the denaturing and also for the observation of the fragment ions caused by the wall heating. The observation of fragment ions for thermally stable compounds by the wall heating is now in progress in our laboratory.

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