The Matrix Suppression Effect and Ionization Mechanisms in MALDI

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Abstract

At appropriate matrix : analyte mixing ratios, small to moderate sized analyte ions (1000-20000 Da) can fully suppress positive matrix ions in MALDI mass spectra. This is true for all matrix species, including radical cations and adducts with protons or alkali metal ions. Full matrix suppression is also observed regardless of the preferred analyte ion form, be it protonated or an alkali adduct. These facts lead us to propose a mechanism for prompt, primary (not secondary gas-phase) MALDI ionization in which excited matrix is the key species. At least two such excited molecules are believed necessary for free ion generation. This model is found to be consistent with the available data, as well as making several predictions which are confirmed by new observations. The model also predicts that the matrix suppression effect will not be observable with heavy analytes because their large excluded volume precludes desorption at the necessary mixing ratios.

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Introduction

Although it is not widely discussed in the literature, MALDI practitioners have often noted a remarkable phenomenon: when analytes of moderate size (1000-20000 Da) are mixed with matrix in relatively high mole ratio (10:1 to about 2000:1 matrix to analyte ratio), the appearance of positive matrix ions in the mass spectrum can be completely suppressed (1,2). Along with the fact that analyte fragmentation is typically weak in MALDI, this leads to nearly ideal mass spectra: strong analyte parent ions but no other signals of any kind. Since the effect has been observed with a number of matrices (1,2) including nicotinic acid, α-cyano-4-hydroxycinnamic acid (CCA) and 2,5 dihydroxybenzoic acid (DHB), it seems to be a general phenomenon in MALDI. It would clearly be advantageous to understand the effect, so as to extend it to the widest possible range of analytes. At the same time we can also expect to learn something about the molecular level mechanisms of the MALDI method.

In their paper describing CCA as a MALDI matrix, Beavis et al. (3) presented a spectrum of substance P (1347 Da) in which the matrix peaks were nearly absent. They did not specifically note this partial matrix suppression effect, nor did they study the matrix-to-analyte mixing ratio dependence of the matrix signals, but they did propose a disproportionation ionization mechanism involving nonionized matrix as an intermediate. They also noted the possibility of proton generation from the matrix as a first step, followed by efficient transfer to the analyte, but did not go on to verify these hypotheses.

Matrix ion suppression was explicitly studied by Chan et al., (1) using nicotinic acid as the matrix, and insulin (5807 Da), lysozyme (~14300 Da) and myoglobin (~18000 Da) as the analytes. They investigated matrix-to-analyte ratios of about 600 to 6000, and found that larger analytes required larger ratios for good suppression. They tentatively proposed a suppression model based on the competition between matrix and analyte for free protons. According to this model, if the analyte has a greater proton affinity than the matrix species present, it will not only collect free protons but also abstract them from protonated matrix.

When analyte is sufficiently concentrated that every protonated matrix molecule will interact with at least one analyte during desorption, only analyte signal will remain after desorption. While a step forward in understanding the effect, this model does not explain the proton generation process, nor consider the fate of radical cations and alkali metal adducts. It should also be noted that while Phillips et al. (4) did find evidence for proton competition between two analytes, they found no correlation between analyte proton affinity and signal strength if only one analyte was present. This suggests that proton competition between matrix and analyte is less important than assumed by Chan et al.

Juhasz, Wang and Biemann (2) also reported matrix suppression using DHB and CCA as matrices. The analytes covered the mass range from 688 Da (a small peptide) to 8565 Da (ubiquitin). They found that lighter analytes could suppress the matrix signals over a wider mixing ratio than heavier analytes. In the case of angiotensinogen (1646 Da) in DHB, suppression was observed over mixing ratios of 100 to 3500. Infrared desorption was found to lead to strong matrix suppression, but for both UV and IR desorption no suppression was observed for negative ions. These authors also suggested that a nonionic matrix precursor species could be the reagent which ionizes the analyte. Both vibrationally and electronically excited matrix were proposed as candidates, but no conclusion was drawn, nor a detailed mechanism presented.

More recently, Bökelmann, Spengler and Kaufmann have studied ion abundances of DHB and protonated substance P at one microsecond after the desorption laser shot (5). They observed a strong reduction in the matrix signal when the DHB/P ratio was <200. They also attributed the effect to depletion of active matrix molecules in a manner similar to that of Chan et al.. However, they also observed that the maximum in P signal does not occur under conditions of strong matrix suppression, but rather at ratios where the matrix signals are still quite strong. This seems inconsistent with a straightforward competition model in which analyte is the limiting reagent at high dilution, and primary matrix ions are limiting at low mixing ratio. In such a scheme analyte signal should reach its maximum value when analyte is in stoichiometric ratio to the active matrix species, leading to the fullest possible reaction

extent. The matrix should thus be fully or nearly fully used up at the mixing ratio for maximum analyte signal, which is not the case.

We report here observations suggesting the models proposed to date are inadequate to fully explain the matrix ion suppression effect. We propose instead a new analyte ionization mechanism in which a common species is the precursor for both protonated and cationized analyte ions. Consistent with some of the above suggestions, we believe that this species is not the often invoked photoionized matrix, but rather singly excited matrix neutrals.

This model is found to be consistent with available data, and its predictions are confirmed by new results. It leads to considerations for optimization of analyte signal as a function of molecular weight, and puts limits on the observability of the matrix suppression effect.

Experimental

The MALDI experiments were performed in a conventional 2 m linear time-of-flight instrument which was built in our laboratory. The total 2-stage static acceleration voltage was ±25 kV. Desorption was peformed with a Laser Science Inc. (74 Chapel St., Newton, MA 02158-1010 USA) VSL-337ND-T nitrogen laser. The laser was attenuated by glass plates and an adjustable iris. The energy after attenuation was measured with a pyroelectric detector inserted into the beam. Using a 47 cm focal length lens, the spot size on the sample was estimated to be an ellipse of approximate dimension 0.1 mm X 0.2 mm, leading to power densities on the order of 5 X 10^6 W/cm^2 and fluences of 15 mJ/cm^2 for strong MALDI signal.

The samples were prepared with 2,5 dihydroxybenzoic acid (DHB) that had been purified by sublimation. All other reagents were used as received: substance P, Sigma, 98%; valinomycin, Fluka, >98%; and cesium iodide, Fluka, >99.5%. The solvent was in all cases distilled water.

Solutions were mixed immediately prior to use, 1-2 μl was deposited on the holder, and dried under vacuum. The operating pressure during MALDI measurements was typically 1 X 10^{-6} mbar or lower. In all the spectra presented here 100 laser shots were summed to improve the signal-to-noise ratio.

Results and Discussion

Because many analytes are known or believed to be observed in MALDI as protonated species, the matrix suppression models noted in the introduction have focussed on the means by which analyte could take up protons in the desorption plume. The most detailed model to date, that of Chan et al. (1), specifically considers only competition for protons under conditions where every primary proton carrier will interact with at least one analyte. None of the models takes into account the fact that different analytes may be ionized in different ways. While many appear as the protonated molecule, many others are primarily observed as adducts with alkali metal ions from the preparation solution (cationized).

According the the competition model of Chan et al., and by analogy to the analyte-analyte competition observed by Phillips et al. (4), we expect that an analyte with high proton affinitiy in high concentration may suppress all other protonated ion signals. However, it is not clear why there should simultaneously be any suppression of cationized species or of radical cation signals. Nevertheless, full non-analyte signal suppression can be observed, as is shown in Fig. 1. Associated with matrix suppression is some loss in absolute sensitivity: the P signals in the two spectra are comparable, even though there is 10 times more peptide in the more concentrated sample. Substance P is detected here almost exclusively as the $(M+H)^+$ ion, yet there are no cationized signals of any kind in the lower spectrum of Fig. 1. In particular, the normally substantial m/z=154 DHB^+ and m/z=177 $DHB{\bullet}Na^+$ signals are absent.

The converse is also true: if an analyte "prefers" to be detected as the sodium adduct, there is no obvious reason to expect suppression of protonated or radical cation matrix ions. Nevertheless, full matrix suppression is also readily observed in such cases, as is shown in Fig. 2. The analyte valinomycin is detected nearly exclusively as the Na^+ adduct, yet all matrix ions have been suppressed, including typically strong m/z=154 DHB^+, and m/z=155 $DHB{\bullet}H^+$.

This suggests that both types of analyte signal, protonated or cationized, are created by chemical ionization pathways involving a common precursor. Such a model is in contrast to most schemes presented for MALDI ionization mechanisms (6,7) wherein the two types of signal are treated more or less independently.

It is difficult to rationalize the above results if matrix photoions are assumed to be the primary common starting species. The means by which matrix ions would influence alkali metal adduct ion formation is particularly problematic. In fact, most theories of cationized analyte formation invoke either a pick-up type gas-phase capture mechanism, in which the matrix plays no active role, or preformed analyte•Na^+ ions, which are simply liberated by desorption (8).

Furthermore, there are data which seem inconsistent with efficient direct multiphoton ionization of matrix as a dominant process in MALDI. Most important is that the MALDI signal depends on the laser pulse energy rather than the pulse peak power (9), the opposite of what is expected for multiphoton absorption. Excited state lifetimes are also short in many MALDI matrices making sequential two-photon absorption unfavorable (10). These considerations do not rule out some contribution of direct photoionization to MALDI signals, but suggest that this contribution is not dominant.

If, on the other hand, it is not the ionized matrix which is most important but rather the electronically excited molecule, then new mechanisms come into consideration. Such a scheme is presented below (mH= matrix, m*H=electronically excited matrix, A=analyte, X=counterion in sodium salt):

$$mH + h\nu \rightarrow m^*H$$ **Scheme 1**

<u>protonation</u>

$$m^*H + A \xrightarrow[m^*H]{} m^- + AH^+$$

<u>cationization</u>

$$m^*H + NaX \rightarrow m^{*-}Na^+ + HX \quad \text{or} \quad m^-Na^+ + h\nu \rightarrow m^{*-}Na^+$$

$$m^{*-}Na^+ + A \xrightarrow[m^*H]{} m^- + ANa^+$$

The first key feature we wish to focus on is the central role of the excited matrix species as a precursor for both protonated and cationized analyte ions. On both pathways we assume that the analyte ion affinity (either for protons or for Na^+) is sufficient to abstract the relevant ion from the matrix carrier at every opportunity. In this aspect, it resembles the competition model. Two possibilities are presented for the first step of the cationization pathway. The second alternative is included because some matrix sodium salt is typically present. Small quantities of such salts have been shown to be efficient in promoting cationization of some analytes (11).

The second key feature is the presence of a second excited matrix molecule in close proximity on both pathways. Note that this differentiates the present protonation model from simple single-step excited state proton transfer. Also, on both pathways two excited molecules pool their energy, but an ionized matrix intermediate is not required or postulated. This is in contrast to one of the mechanisms proposed in Ref. 6.

There are two reasons for including a second excited molecule. First, the 3.7 eV from a single 337 nm nitrogen laser photon is insufficient for efficient charge separation, since the Coulomb energy is 4-6 eV. Second, it is known (10) that desorption begins at laser fluences where matrix fluorescence quenching becomes significant, indicating that the excitation density is high enough for two excited matrix molecules to meet with substantial frequency. As will be shown below, the requirement for (at least) two excited matrix molecules also leads to predictions that are experimentally verifiable.

If processes like Scheme 1 are necessary for creation of MALDI ions, there should also be a similar pathway to matrix ions such as mH^+ (6), when analyte is not in the vicinity:

$$m^*H \xrightarrow[m^*H]{} mH^+ + e^- \text{ or } m^- + (mH)H^+ \text{ or } m + H \text{ , etc.} \qquad \textbf{Scheme 2}$$

This provides an efficient mechanism for production of primary matrix ions and radicals without direct two-photon ionization. Such products are observed for DHB, including neutral H atoms (12). These primary ions may continue to react with analyte in the gas phase, leading to further secondary analyte signal, as is suggested by observations of an optimum delay for post-desorption extraction (8).

It is important to emphasize that these mechanisms are intended to explain only prompt ions which are created very early in the desorption event. In particular they are not relevant for preformed ions or ions that are created by secondary gas-phase reactions in the plume (8). Related mechanisms can, however, be constructed for these other cases. Preformed complex salts may easily be carried into the gas phase by desorption, but substantial further energy is required to separate the cation complex from the counterion. This energy may sometimes be supplied by excited matrix in a manner similar to that of Scheme 1:

$$ANa^+X^- \xrightarrow[2(m^*H)]{} ANa^+ + X^- \qquad \textbf{Scheme 3}$$

An analagous reaction replacing ANaX in Scheme 3 with NaX may also liberate the cations which attach to analyte in later stages of desorption (8).

Reactions such as in Scheme 3 may play a role in MALDI, but they are not the focus of this paper. Other mechanisms not requiring matrix are very probably also active. Cations of simple salts such as NaCl are, for example, easily produced from the pure salt, at fluences similar to those used with matrix. Such direct generation mechanisms may also work for preformed analyte salts, though matrix salts themselves often function poorly in this way (11).

The model in Scheme 1 can be tested in numerous ways. One of the predictions it makes is the production on both pathways of matrix anion (m^-) in at least equal concentration to the ionized analyte. In Fig. 3, we show the negative ion mass spectra of the same samples in Figs. 1 and 2, where full matrix suppression was observed in positive ion mode. Only the polarity of the acceleration voltages was changed. In both cases there is no suppression, and instead very strong matrix anion signals appear. These results are consistent with the observations of Juhasz, Wang and Biemann (2). Other ions also are found, presumably due to secondary ion chemistry not included in Scheme 1.

The matrix ions observed are similar in both spectra, even though substance P is preferentially protonated and valinomycin preferentially cationized in positive ion mode. Note also that the lack of matrix anion products in Scheme 3 suggests that the majority of the observed valinomycin•Na^+ signal in Fig. 2 is not due to preformed sodium adducts or gas-phase reactions.

The mechanism of Scheme 1 assumes that the ion transfer step of H^+ or Na^+ from matrix to the analyte is energetically favorable. In other words, matrix ion suppression will always be observed when coalescence of two excited matrix molecules occurs in proximity to an analyte molecule, so that Scheme 2 has no opportunity to occur. This sets limits on the concentration ranges where matrix suppression can potentially be observed, since this requires all matrix molecules be near at least one analyte. From this one might expect that suppression is strong when there is on average one layer of matrix between neighboring analyte molecules. This is in fact the approximate upper end of the concentration range for matrix suppression with substance P and valinomycin. Comparison of their molecular dimensions with those of the matrix molecules suggests that approximately 24 matrix molecules are needed to fully surround one analyte. This matrix layer is shared equally with nearest analyte neighbors, the matrix to analyte molar ratio is then 24/2=12, similar to that used here for valinomycin to induce matrix suppression.

Conversely, if analyte and matrix are somehow separated, so that Scheme 2 can again take place independently, a sample for which matrix ions are normally suppressed should once again show matrix signals. Such an effect is shown in Fig. 4. The solution of substance P in DHB that was used in Figs. 1 and 3 was dropped onto a porous graphite sample holder, rather than onto a metal one. The solvent distributes both matrix and P over the large surface of the graphite, increasing their physical separation. As expected from the model, DHB signals are once again strongly observed.

A similar effect is predicted if the density of excited matrix molecules is increased. If many such molecules are created, the chance is increased that excited pairs will be created in the few remaining regions far enough from analyte that Scheme 2 becomes possible. This was tested by increasing the laser fluence, as shown in Fig. 5. At fluences not far above threshold, matrix suppression is observed (although weak alkali signals still appear). As the fluence is increased on the same sample, matrix peaks begin to appear, as expected.

The poor resolution in the upper spectra is clearly due only to the high fluence, since similar results are obtained with DHB alone, as seen in the top spectrum. The fragmentation and loss of resolution at high fluence appear to be a result of the large excitation density necessary for appreciable matrix ionization.

With the nanosecond lasers typically used, MALDI depends on the laser pulse energy (fluence) than the rate at which the energy is delivered, or peak power (9). This is fully consistent with Scheme 1, where only the excitation density is predicted to be important, not the rate at which excitations are created. This suggests a modified MALDI experiment in which the necessary excitation density is created by two temporally separated low-intensity pulses. The results of such an exmperiment are shown in Fig. 6. By splitting the nitrogen laser output and using mirrors to delay one of the beams, two pulses were brought onto the sample with 5.5 ns time difference. The measured pulse width was 3.2 ns, so there was no significant temporal overlap. Clearly both pulses are individually near-threshold, but together yield a good MALDI spectrum. In a complementary experiment, the excitation pulse was

used in addition to a normal desorption pulse. In this case, the signal typically increased by a factor of 2-4, with no loss in mass resolution.

While at least two excited matrix molecules are invoked in Schemes 1 and 2, the true minimum number could be greater. For example, the MALDI signal of four biomolecules was recently experimentally found to have a seventh power fluence dependence (13). This suggests that as many as seven matrix excitations could be necessary for the combined process of desorption and ionization of a single analyte. Nevertheless we believe that excited matrix pairs are the most likely ionizing agents for several reasons. Consider first the range over which excited matrix pairs may strongly interact. If the excitations are localized on single matrix molecules, energy transfer to other molecules will fall off quickly with distance, e.g. r^{-6} for a dipole-dipole mechanism. This suggests that consideration of only nearest neighbors will be a good approximation.

This is also supported by the excitation density necessary for neutral desorption, which has been found to occur when about 1 in 17 DHB molecules is photoexcited (10). A cubic volume containing 17 molecules will be $17^{1/3} = 2.57$ molecular diameters on a side. At the desorption threshold excited matrix molecules are then separated by 2.5 diameters, or by one non-excited molecule, on average. This must be an upper limit for the interaction range. The true range must be smaller since excited matrix pairs with this separation will become frequent at sub-threshold pulse energies. Active pairs must then be separated by the next smaller interval, or be nearest neighbors.

That pairs (or other groupings) of excited matrix molecules are needed for ionization is also consistent with the increase in threshold fluence observed at high concentrations of various analytes (14). If enough analyte is present that only very thin regions of matrix exist, then the average number of matrix neighbors for each matrix molecule begins to drop. This reduces the probability that the necessary number of neighboring excitations (2 or more) can be created by the laser pulse.

We have tested this aspect of the model by means of a dilution experiment. We have found that CsI can be co-crystallized with DHB in relatively large mixing ratios. The linear dimensions of CsI are similar to those of DHB, so assuming uniform mixing, each CsI should reduce by about one the number of possible active neighbors for each DHB. From the pair probability arguments above this should lead to reduction of signal as the mole fraction of CsI is increased. As seen in Fig. 7, at about 40 % mole ratio CsI in DHB, the matrix peaks drop dramatically. This drop is somewhat steeper than expected, but may also be a result of changes in crystal habit and microscopic mixing. On the other hand, the drop occurs at nearly 50% CsI content, again suggesting that it is excited matrix pairs which are important, not larger clusters of excitations.

At present, no cases are known to us where molecules of more than 20000 Da have been found to induce matrix suppression. While separation of heavy ion signals from matrix interferences is generally not a problem, it could still be of interest to extend the effect to larger masses. The proposed model predicts this will not generally be possible.

Successful matrix suppression requires a low mixing ratio to maximize Scheme 1 and minimize Scheme 2. If the ratio is chosen such that there is just enough matrix to surround the analyte, then the amount of matrix available for lifting the analyte off the surface will increase as the surface area of the analyte, while the energy required for lifting will go as the mass, or roughly the molecular volume. At some molecular size, the typical analyte will simply be too massive to be desorbed, given the small amount of matrix available. This is consistent with the observation that larger analytes require larger mixing ratios to be both efficiently desorbed and still induce matrix suppression (1,2). This is also consistent with the observations of Medina et al. (14) where it was found that the threshold laser fluence at a given mixing ratio is larger for larger analytes, especially at the low mixing ratios (<5000) relevant for matrix suppression.

One possible objection to the proposed model is that it requires the matrix to have at least one labile proton. As the majority of widely used matrices are carboxylic acids, this is

generally not a problem, but there are also basic matrices (15) and matrices with no carboxyl groups. However, the model requires not that the ground state be acidic, but rather the longest-lived excited state. The possible role of excited state proton transfer (ESPT) in MALDI has long been discussed (16). The potential of typical MALDI matrices for ESPT is not generally known, especially under dense conditions of the early expansion. Nevertheless, many of the "basic" matrices are aromatic amines, which are often much more acidic in the excited state (17). Therefore the existence of basic (ground state) matrices does not invalidate the model. It is also possible that not all matrices function in the manner of Scheme 1, as could be indicated by the ability to induce matrix suppression or not.

An alternative explanation for matrix suppression concerns the physics of the MALDI plume. MALDI desorption events are known to create about 10^4 ions per laser shot, in an area of a few hundred square micrometers. This charge density should lead to some divergence of the ion cloud due to Coulomb repulsion. Large ions will feel the same forces as small ions, but this leads to smaller accelerations due to the mass differences. One can therefore imagine that small ions will be rapidly radially ejected from the cloud leaving behind predominantly large ions on the spectrometer axis, which then are preferentially detected. To test this we have carried out simulations of the ion cloud (18). Although the large ions do diverge from the acceleration axis much slower than the light ions, the light and heavy ion packages are also rapidly separated by the accelerating field. After a very short time, the light ions are too far from the heavy ions to be significantly affected. As a result, they are actually less divergent, since the total charge density in the light ion cloud is reduced compared to an expansion with no heavy ions.

Finally, it should be mentioned that apparent suppression of matrix signals has been observed in pulsed extraction experiments, but this is not a result of the same effect discussed here. Light matrix ions can, for example, be neutralized on the sample holder if a reverse bias voltage is applied for a limited time before the extraction pulse (19). Pulsed extraction also has the effect of time-focusing ion signals of a limited m/z range at the detector, but simultaneously defocusing other m/z signals (20). As a result, optimization at m/z=2224 in

one study (21) strongly defocused matrix peaks below m/z=600, so that they appeared as a broad hump.

Conclusions

The matrix ion suppression effect in MALDI has been reexamined. Comparing the effect for protonated and cationized analyte species leads us to reject the ion competition model. We also find photoionization models for primary ion generation unlikely, and propose a new general model for prompt MALDI ion creation processes. In this model excited, but not ionized, matrix molecules are the common precursor for all subsequent ion products (excepting possible pre-formed ions liberated by desorption). Simultaneous neighboring presence of two such excitations is required for ionization, so the primary events occur in the first nanoseconds of the desorption where both neutral and excited molecule densities are high.

Numerous aspects of the model were found to be consistent with existing and new data:

- As observed, the model predicts that large quantities of negative matrix ions are produced, regardless of analyte cation form (protonated or sodiated).
- The observed mixing ratios for matrix suppression are in agreement with those predicted.
- The matrix-analyte proximity requirement for matrix suppression was tested by physical separation on graphite, and also by increasing the excitation density. In both cases matrix signal reappeared as predicted.
- The time-delayed two pulse MALDI experiment functions as predicted by the model. With both pulses individually below threshold there is no signal at all, but with both pulses present a normal spectrum is obtained.
- The dependence of MALDI signal on excited matrix aggregates was found consistent with a matrix dilution experiment, and suggests that excited matrix pairs are the active species.

It is predicted that heavy analyte molecules will seldom, if ever, be able to suppress the matrix ions in MALDI spectra, because of their physical size. If heavy analyte is concentrated enough to capture all primary charges, it lowers the matrix concentration to levels insufficient for lifting the molecule off the surface. At the same time, any analyte of

moderate size should be able to induce matrix suppression, so long as it can be well mixed with the matrix at the appropriate ratio.

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Figures

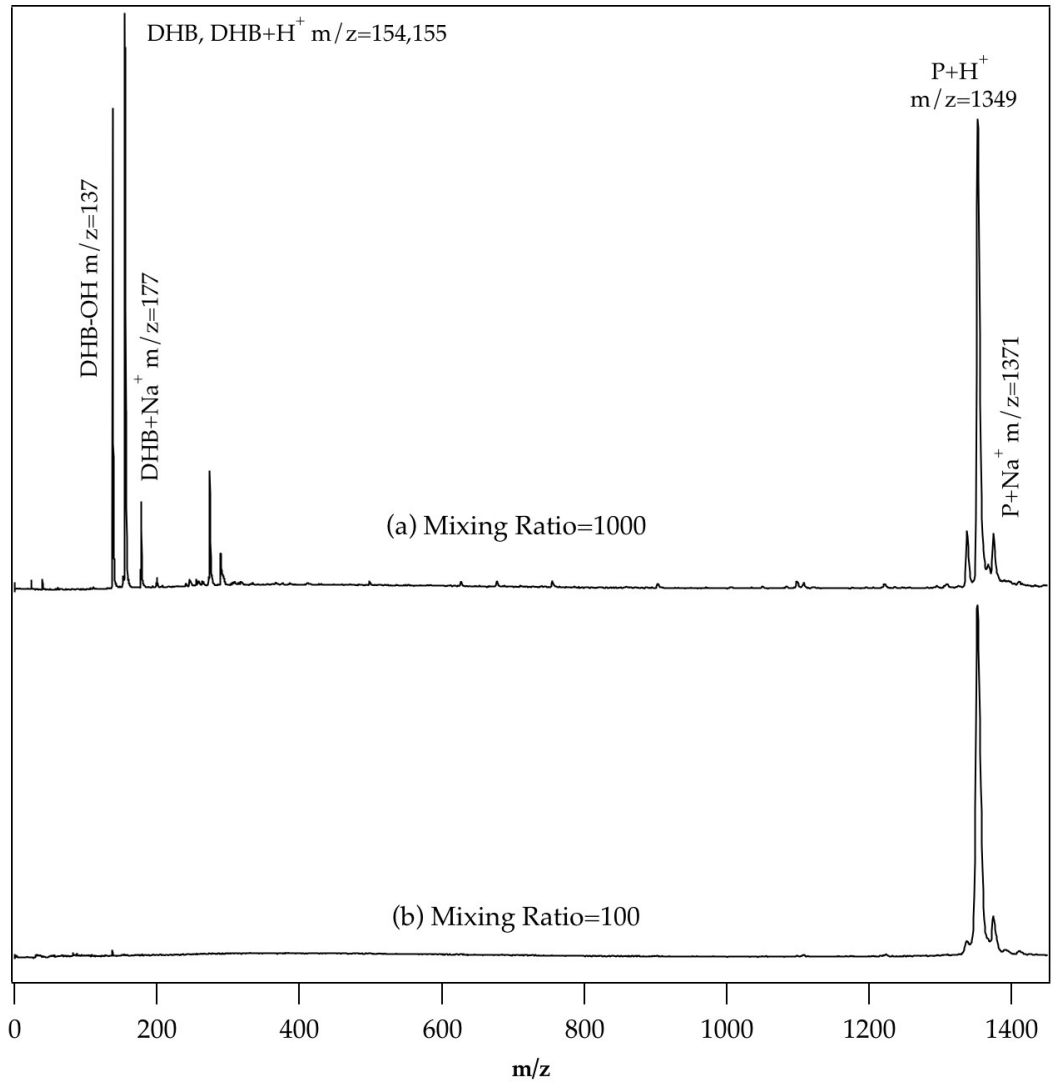

Figure 1. Positive ion MALDI mass spectra of substance P in DHB matrix. In the upper spectrum the matrix-to-analyte mole ratio was 1000. The spectrum shows strong matrix peaks, as is typical of the method. In the lower spectrum, the mixing ratio was decreased to 100. Substance P is primarily detected as the proton adduct, yet all matrix peaks are suppressed in the lower spectrum, including sodiated species and radical cations.

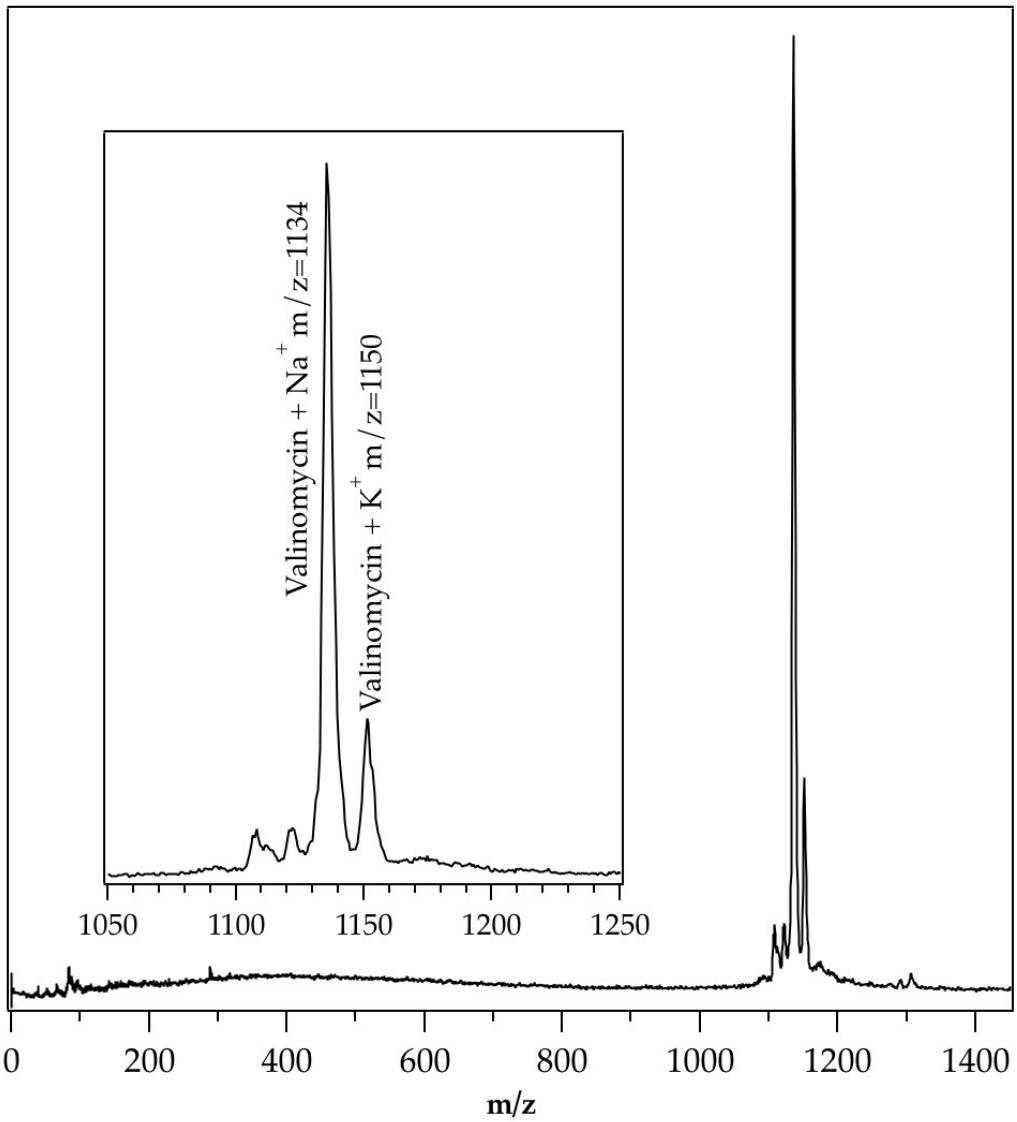

Figure 2. Positive ion MALDI mass spectra of valinomycin in DHB at a matrix-to-analyte mole ratio of 10. Valinomycin is primarily detected as the sodium adduct, yet all matrix peaks are suppressed, including protonated species and radical cations. The insert is an expanded view of the molecular ion region. The weak broad hump peaking at m/z=400 is an instrumental artifact unrelated to any ion signals.

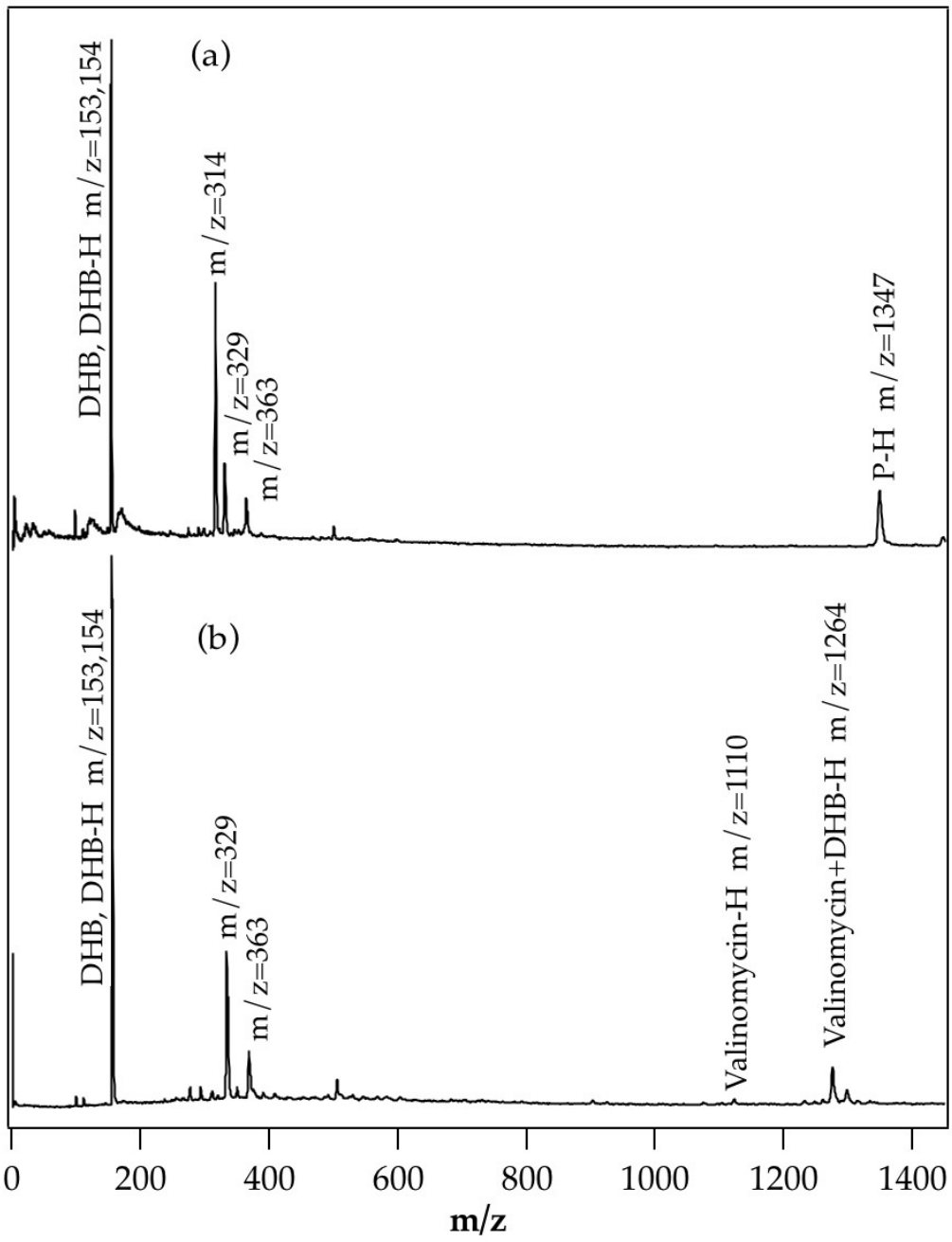

Figure 3. Negative ion MALDI spectra of substance P and valinomycin in DHB matrix. The samples were the same as used in Figures 1 and 2, for which complete positive matrix ion suppression was observed. The only change was reversal of the acceleration voltage polarity. As predicted by the model, large quantities of negative matrix ions are observed in both cases.

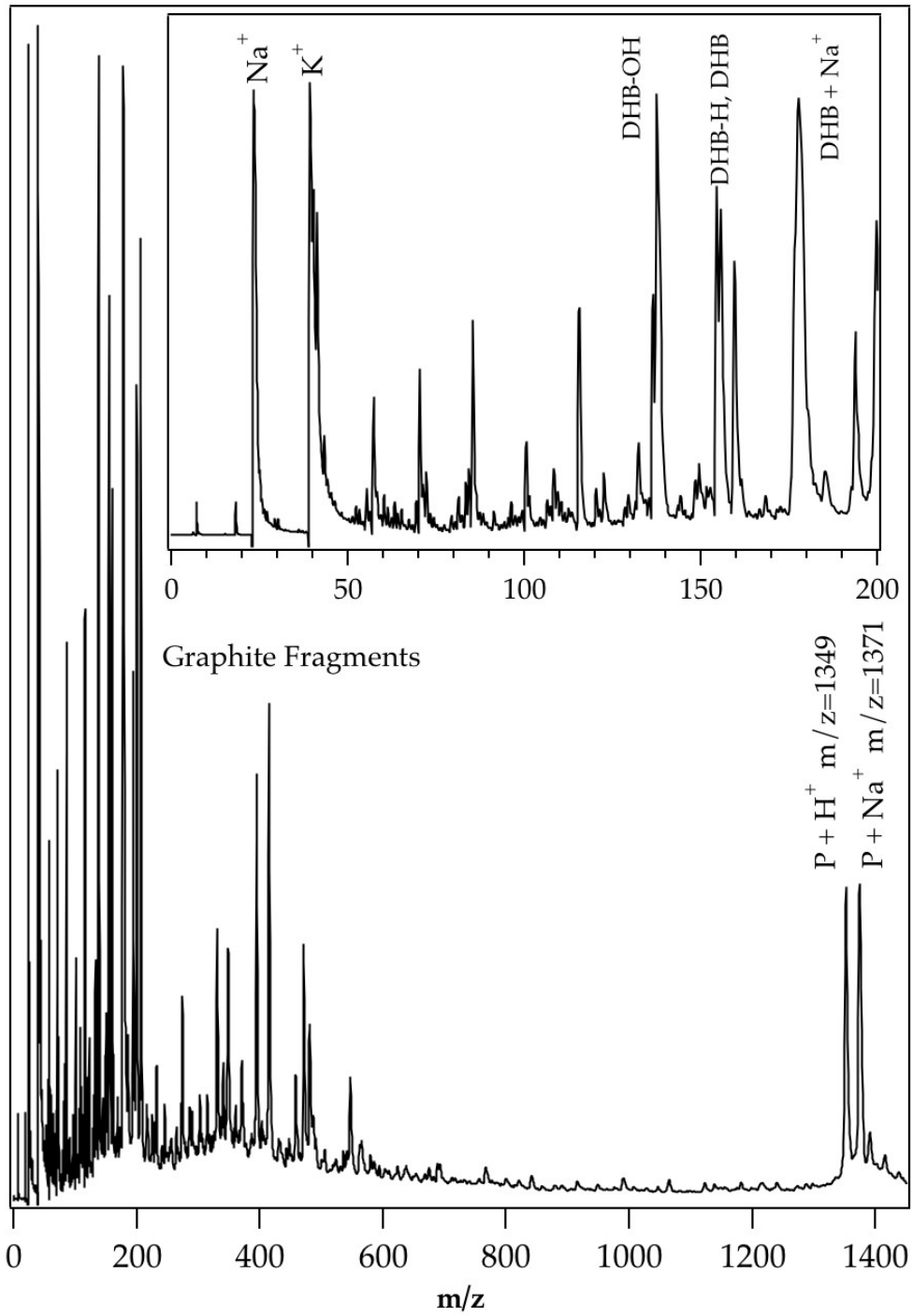

Figure 4. Surface assisted desorption ionization mass spectra of substance P and DHB on graphite. The sample was made from the same solution as used to demonstrate matrix suppression in Figure 1. Physical distribution of both DHB and analyte over the graphite surface leads to reappearance of positive matrix ion signals. This indicates that matrix excitations can coalesce in the absence of nearby analyte.

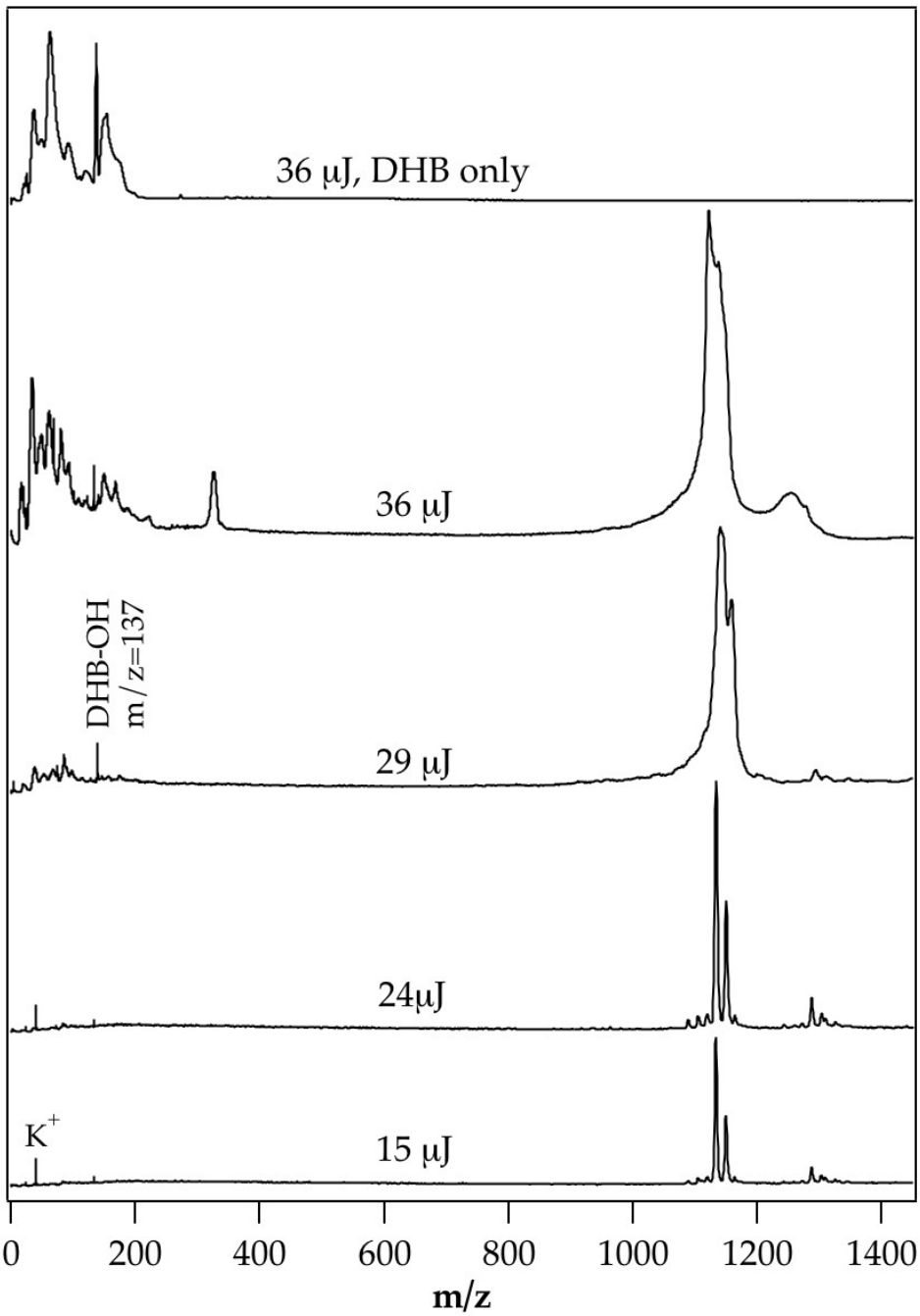

Figure 5. Laser pulse energy dependence of valinomycin MALDI signal in DHB. Except for the upper spectrum, the matrix:valinomycin ratio was 10. At usual MALDI pulse energies matrix suppression is observed. At higher energies typical matrix ions again appear, as do many smaller fragments. Resolution decreases with increased pulse energy also. These effects are consistent with high matrix excitation density at high pulse energies.

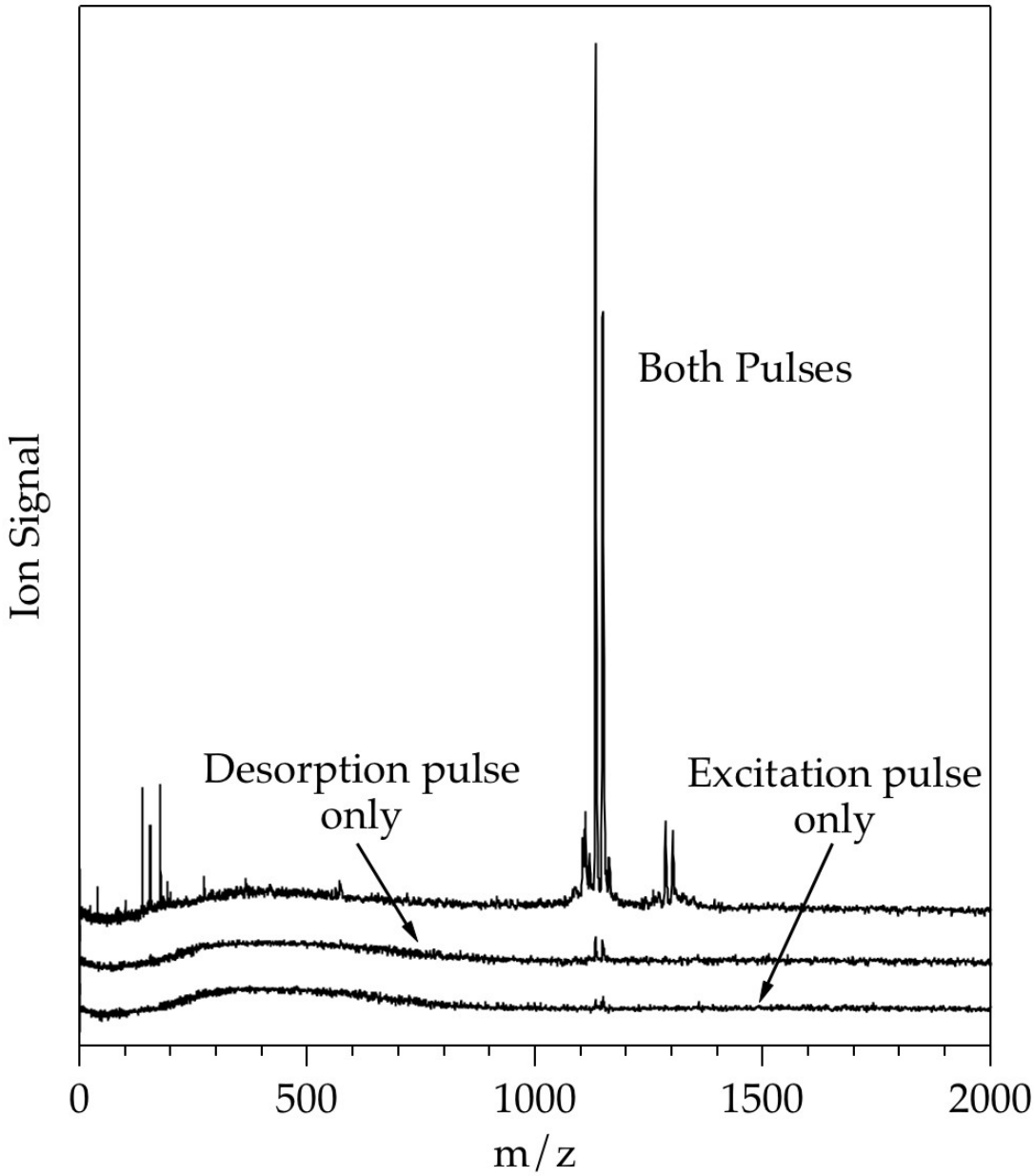

Figure 6. MALDI mass spectra of valinomycin in the two pulse experiment. Both the excitation and desorption pulses have been adjusted to near threshold fluences. Only when both pulses are present is a strong MALDI spectrum observed. The mixing ratio was 10. The UV pulse widths were 3.2 ns, the separation between pulses was 5.5 ns. The baseline curvature peaking at m/z=400 is an electronic artifact. See the text for further explanation.

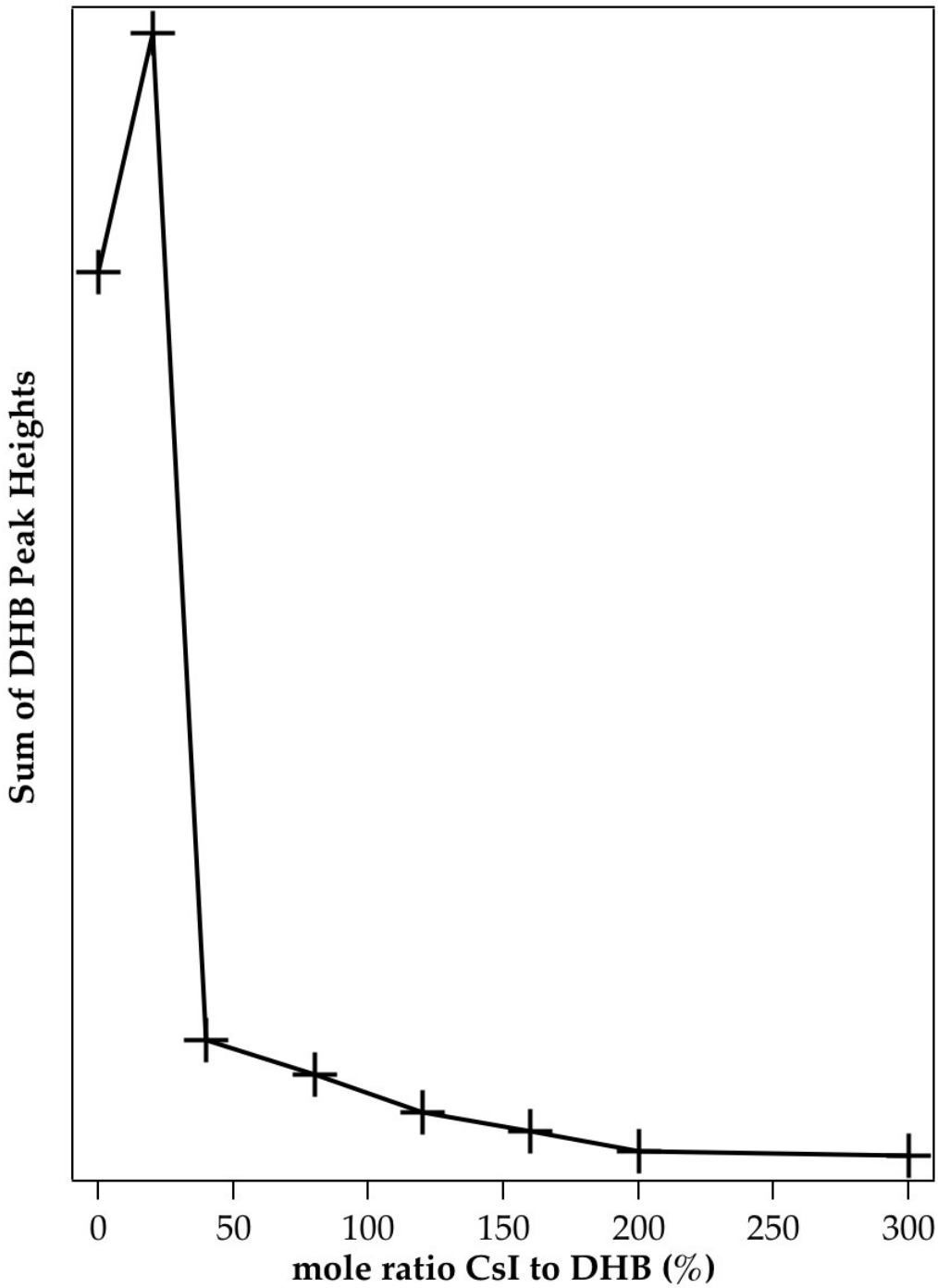

Figure 7. Matrix ion signals as a function of dilution with CsI. The largest matrix signals of all types were summed, including m/z=137 (DHB-OH), m/z=154 (DHB); m/z=155 ($DHB+H^+$), and m/z=177 ($DHB+Na^+$). The total matrix signal intensity drops dramatically with increasing mole fraction of about CsI, which is consistent with excitation pair production models.