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Online Monitoring of Enzymatic Reactions Using Time-Resolved Desorption Electrospray Ionization Mass Spectrometry

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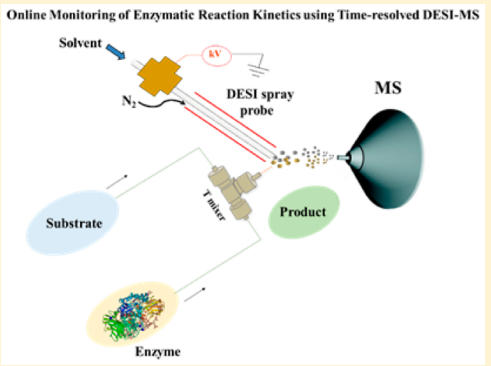
Supporting Information

ABSTRACT: Electrospray ionization mass spectrometry (ESI-MS) is powerful for determining enzymatic reaction kinetics because of its soft ionization nature. However, it is limited to use ESI-favored solvents containing volatile buffers (e.g., ammonium acetate). In addition, lack of a quenching step for online ESI-MS reaction monitoring might introduce inaccuracy, due to the possible acceleration of reaction in the sprayed microdroplets. To overcome these issues, this study presents a new approach for online measuring enzymatic reaction kinetics using desorption electrospray ionization mass spectrometry (DESI-MS). By using DESI-MS, enzymatic reaction products in a buffered aqueous media (e.g., a solution containing Tris buffer or high concentration of inorganic salts) could be directly detected. Furthermore, by adjusting the pH and solvent composition of the DESI spray, reaction can be online quenched to avoid the postionization reaction event, leading to fast and accurate measurement of kinetic constants. Reaction time control can be obtained simply by adjusting the injection flow rates of enzyme and substrate solutions. Enzymatic reactions examined in this study include hydrolysis of 2-nitrophenyl-β-D-galactopyranoside by β-galactosidase and hydrolysis of acetylcholine by acetylcholinesterase. Derived Michaelis−Menten constants K_m for these two reactions were determined to be 214 μM and 172 μM, respectively, which are in good agreement with the values of 300 μM and 230 μM reported in literature, validating the DESI-MS approach. Furthermore, this time-resolved DESI-MS also allowed us to determine K_m and turnover number k_{cat} for trypsin digestion of angiotensin II (K_m and k_{cat} are determined to be 6.4 mM and 1.3 s^{-1}, respectively).



Kinetic studies play an essential role in the elucidation of chemical and biochemical reaction mechanisms.[1] Typically, kinetic measurements are carried out using optical methods, such as fluorescence and absorption spectroscopies. Nowadays mass spectrometry (MS) has become a powerful tool for the study of reaction kinetics.[2−15] The advantage of using MS over spectroscopic techniques is that MS has a high chemical specificity and does not require chromophoric substrates. It can also provide the molecular weight and structure information for reaction products and intermediates. With the advances in the field of MS, various elegant MS-based approaches, particularly those employing the soft electrospray ionization (ESI) technique,[12,16−22] have been reported for online reaction kinetic studies. However, the reports about online measurement of enzymatic reaction kinetics using ESI-MS are still limited.[10,12,22] In general, there are conflicts of ESI ionization condition with enzymatic reaction media. For instance, enzymatic reactions occur in buffered aqueous environments with certain pH and ionic strength (containing inorganic salts or nonvolatile buffers), while ESI usually needs the addition of acid and organic additives into samples for efficient ionization. These additives would denature enzymes. One compromised solution is to use volatile buffers, such as ammonium acetate for both enzymatic reaction and for ionization. Another challenge is the necessity of online reaction quenching, which is critical for obtaining accurate kinetics measurement. Recent studies showed that the reaction could be accelerated in the charged droplets generated from the spray.[23−26] It was reported that reaction rate in microdroplets could be significantly accelerated by a factor of a thousand compared to the one in bulk solution.[27] Therefore, if the reaction were not quenched prior to MS analysis, it could be accelerated during the spray process of ionization, which would make it difficult to obtain accurate kinetics measurements. However, online quenching of reaction prior to ESI-MS analysis is inconvenient and was not reported before. To tackle these issues, we proposed a new approach of using desorption electrospray ionization mass spectrometry (DESI) for online measurement of enzymatic reaction kinetics.

DESI, a representative spray-based ambient ionization technique, was introduced in 2004 by Cooks and co-workers as a milestone in the field of MS.[28,29] Numerous of applications

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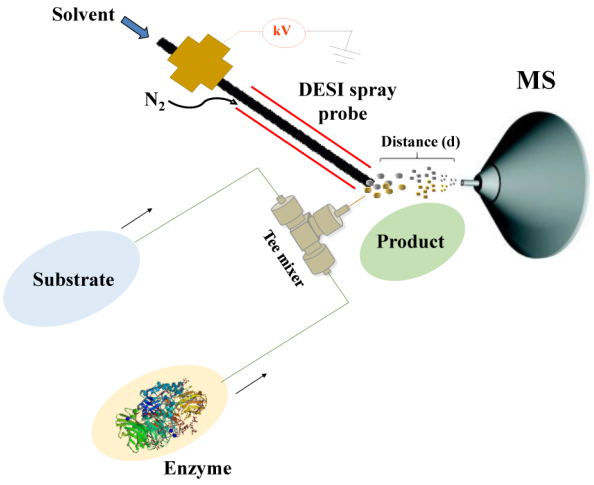
of DESI-MS have been reported, ranging from drug detection to tissue imaging.[28−31] Traditionally DESI addresses solid samples deposited onto a surface. In our and other's laboratories, DESI was extended for the analysis of liquid samples.[32−36] Analyte ionization by liquid sample DESI occurs via interaction of the liquid sample with charged droplets generated by the DESI spray and the resulting ions are collected and analyzed by a nearby mass spectrometer. It is useful for directly analyzing samples including large proteins/protein complexes from their native environments.[33,37−42] Furthermore, it is also possible to use liquid sample DESI-MS for capturing transient organometallic or electrochemical reaction intermediates.[43−46] On the basis of the capability of DESI for ionizing a high-speed liquid jet, generated from fast mixing of reactants, at different positions, kinetics measurement of fast reaction with a high time resolution (300 μs) was achieved.[47]

Scheme 1 illustrates the principle underlying the DESI-MS method for online measurement of enzymatic reaction kinetics,

Scheme 1. Schematic Showing the Apparatus of Time-Resolved DESI-MS



in which enzyme and substrate solutions undergo mixing in a micro-Tee mixer that is connected with a short piece of silica capillary to serve as a microreactor for reaction. After exiting the microreactor capillary, the reaction mixture is directly ionized by DESI for MS detection. The reaction time can be varied by changing injection flow rates of enzyme and substrate solutions. There are several advantages of using DESI-MS for enzymatic reaction monitoring. First, unlike ESI which requires organic solvent/acid additives to be added into the sample for obtaining optimal sensitivity, DESI allows the direct ionization of sample from aqueous solution with good sensitivity.[38] This is important for enzymatic reaction analysis because the enzyme could be deactivated with these additives, as mentioned above. This feature of DESI is ascribed to the freedom to adjust its spray solvent composition, for example, adding acid, base or acetonitrile into the spray solvent. Meanwhile, addition of these additives into the spray solvent could be used for online reaction quenching during the ionization step. Thus, the potential postionization reaction in the spray droplets could be avoided. Furthermore, DESI has high salt tolerance[47−49] and is suitable for directly detecting enzymatic reaction product or monitoring the consumption of the substrate in the buffered solution which is typically required media for enzymatic reaction (e.g., a solution containing Tris buffer or high concentration of inorganic salts). Also, different from our previous kinetics experiment,[47] the formation of a liquid jet is not required in this case. Therefore, the consumption of substrate sample could be greatly reduced. In this study, three hydrolysis reactions catalyzed by β-galactosidase, acetylcholinesterase and trypsin were chosen as enzymatic reaction models to validate the proposed DESI-MS method.

EXPERIMENTAL SECTION

Chemicals. 2-Nitrophenyl-β-D-galactopyranoside (ONPG), bovine serum albumin (BSA), acetylcholine (Ach), acetylcholinesterase (AchE), trypsin, magnesium chloride ($MgCl_2$), potassium chloride (KCl), trizma, and choline were purchased from Sigma-Aldrich (St louis, MO, USA). β-Galactosidase from *Escherichia coli* and acetonitrile (ACN) were purchased from EMD Millipore (Billerica, MA, USA). Angiotensin II and angiotensin II (3−8) were bought from Genway (San Diego, CA, USA). Angiotensin III antipeptide was purchased from CPC Scientific (Sunnyvale, CA, USA). Ammonia ($NH_3{\cdot}H_2O$), methanol (MeOH), and acetic acid (HOAc) were purchased from Fisher Scientific (Hampton, NH, USA). 2-Nitrophenol-3,4,5,6-d_4 (ONP-d_4) and choline-d_4 were purchased from CDN isotope (Pointe-Claire, Quebec, Canada). Silica capillary was purchased from IDEX (Lake forest, IL, USA). P890 micro-Tee mixer was purchased from Upchurch Company (Oak Harbor, WA, USA).

Method. Experiments were performed using either a DECA MAX LCQ ion trap mass spectrometer (Thermo Finigan) or an LTQ Orbitrap mass spectrometer (Thermo Scientific) equipped with a homemade liquid sample DESI source. Scheme 1 shows the configuration of the online time-resolved DESI-MS apparatus. The nebulization nitrogen gas pressure was set at 160 psi. The high voltage applied to the DESI sprayer was +5 kV for the positive ion mode and −5 kV for the negative ion mode. The enzyme and the substrate were introduced by two syringes separately (driven by a single syringe pump with two channels) and mixed in a micro-Tee mixer that is connected with a piece of silica capillary (ID 100 μm, 3.5 cm long) to serve as a microreactor for reaction.

The reaction buffer for hydrolysis of 2-nitrophenyl-β-D-galactopyranoside by β-galactosidase consisted of 2.0 mM KCl, 0.1 mM $MgCl_2$, and 0.1% BSA (w/w) and its pH was adjusted to 7.8 with aqueous ammonia. The enzyme was dissolved in the reaction buffer to prepare a 9 nM enzyme solution. The substrate was dissolved in the same reaction buffer at different concentrations (91, 182, 373, 455, 545 μM) with addition of 10 μM ONP-d_4 as an internal standard (IS) for the product quantification purpose. The enzyme solution and the substrate solution injection flow rates were kept the same and were varied from 10 to 1 μL/min (corresponding to the reaction time from 0.99 to 9.96 s). The spray solvent for hydrolysis of 2-nitropenyl-β-D-galactopyranoside by β-galactosidase was $NH_3{\cdot}H_2O$/ACN/H_2O (0.1:50:50 by volume, ammonia concentration is 15 mM) for enhancing the product ionization efficiency in the negative ion mode and also for quenching the reaction (see the quenching test result in the discussion below and in Figure S4).

Acetylcholinesterase was dissolved in 10 mM Tris buffer with the final enzyme concentration of 1.4 μM. Acetylcholine was dissolved in the same buffer at different concentrations (0.14, 0.35, 0.56, 0.7, 1.4 mM) with addition of 70 μM choline-d_4 as an internal standard. The same sample introduction system

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(Scheme 1) was used for studying the hydrolysis of acetylcholine catalyzed by acetylcholinesterase. ACN was used as the spray solvent for quenching the reaction as it is an organic solvent.[14]

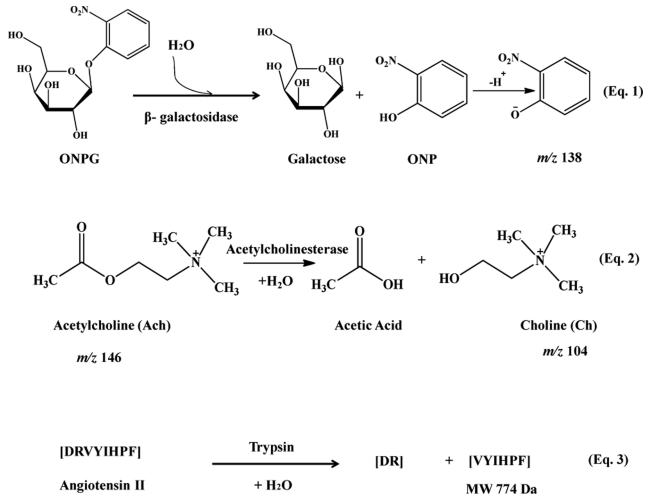
For the study of trypsin digestion kinetics, angiotensin II was chose as the reaction substrate. Different concentrations of angiotensin II (1.5, 2, 2.5, 3, 3.5 mM) and 0.06 mM trypsin were prepared in 25 mM ammonium bicarbonate. 40 μM angiotensin III antipeptide was added to the substrate solution as an internal standard. The same sample introduction system (Scheme 1) was used for examining the hydrolysis of angiotensin II by trypsin. The DESI spray solvent for this reaction was MeOH/H_2O/HOAc (50:50:3, by volume) for enhancing sample ionization efficiency and for quenching the reaction (see the quenching test result in the discussion below and in Figure S6).

RESULTS AND DISCUSSION

Enzymes plays very important role in many disciplines, such as biochemistry, medicine, and pharmacology. One key issue in enzyme-based assays is the accurate characterization of the enzymatic reaction constants such as Michaelis–Menten constant K_m and turnover number k_{cat}.[50,51] The Michaelis–Menten kinetics is one of the most commonly used models to describe the bulk reaction of enzymes,[52,53] and K_m and k_{cat} are two of the important constants in enzymatic reactions. K_m quantifies the affinity between enzyme and substrate and k_{cat} reveals the turnover rate of the reaction. A typical strategy used to calculate K_m and k_{cat} is the Lineweaver–Burk plot (L–B plot),[54] which is based on relationship between two reciprocals of both the initial reaction rate (v_0) and the substrate concentration (C_0).

Scheme 2 shows three enzymatic reactions that were examined in this study. Hydrolysis of ONPG by β-galactosidase produces 2-nitrophenol (ONP) and galactose (eq 1, Scheme 2). In the negative ion mode, the product ONP can be deprotonated to form the ONP anion and detected at m/z 138 by DESI-MS. Hydrolysis of acetylcholine by acetylcholinesterase produces choline and acetic acid (eq 2, Scheme 2). The choline can be detected at m/z 104 by DESI-MS in the positive ion mode. Hydrolysis of angiotensin II (sequence DRVYIHPF) by trypsin produces angiotensin II (3–8) (sequence VYIHPF, eq 3, Scheme 2), which can be detected at m/z 775 by DESI-MS in the positive ion mode.

Scheme 2. Equations Showing Three Enzymatic Reactions That Were Studied: Hydrolysis of 2-Nitrophenyl-β-D-galactopyranoside by β-Galactosidase (Eq 1), Hydrolysis of Acetylcholine by Acetylcholinesterase (Eq 2), and Digestion of Angiotensin II by Trypsin (Eq 3)



The enzyme β-galactosidase dissolved in a reaction buffer consisting of 2.0 mM KCl, 0.1 mM $MgCl_2$, and 0.1% BSA (w/w, pH 7.8). Monovalent potassium ions (K^+), as well as divalent magnesium ions (Mg^{2+}), are required for obtaining the enzyme's optimal activity[55] and the optimal working pH of β-galactosidase is 6–8.[56] Both enzyme and ONPG dissolved in the same buffer were infused (at the same flow rates) into the micro-Tee mixer for reaction followed by direct DESI-MS detection. By changing the injection flow rates from 10 to 1 μL/min, different reaction times varying from 0.99 to 9.96 s were resulted which affected the yield of the reaction product ONP. Figure 1 displays the acquired DESI-MS spectra showing

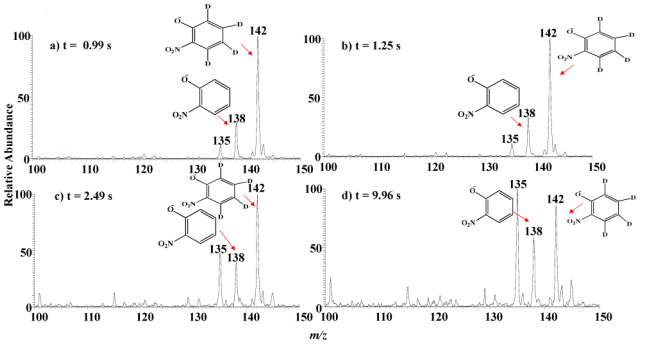


Figure 1. Time-resolved DESI-MS spectra showing the hydrolysis reaction of ONPG (545 μM) catalyzed by β-galactosidase (9 nM, pH at 7.8) sampled by DESI-MS at (a) 0.99, (b) 1.25, (c) 2.49, and (d) 9.96 s, respectively.

the enzymatic reaction of 545 μM substrate and 9 nM enzyme sampled at different reaction times. In this experiment, for quantification of the product ONP, ONP-d_4 was added into the substrate solution to serve as an internal standard. Our test results showed that ONP and ONP-d_4 have the same DESI ionization efficiency, even at different sample injection flow rates, becaue of the similarity of their structures (Figure S1). Therefore, the concentration of ONP from the enzymatic reaction can be calculated based on the relative intensity of the ONP peak (m/z 138) relative to the IS peak (m/z 142) and the known concentration of ONP-d_4. To confirm whether the addition of internal standard has influence on the reaction kinetics, experiments using different concentrations of internal standard were carried out. The result showed that the product concentration was the same when the concentration of internal standard ONP-d_4 changed from 10 to 5 μM (Figure S2), indicating that the added internal standard has no effect on reaction kinetics.

As shown in the acquired DESI-MS spectra (Figure 1), the intensity of m/z 138 relative to m/z 142 increased with the increased reaction time, showing the progress of the β-galactosidase-catalyzed hydrolysis with time. In this case, although the reaction sample contains inorganic salts such as KCl and $MgCl_2$, the product ONP can be well ionized by DESI-MS (Figures 1 and S3-a). In comparison, when ESI was used for the ionization of the same sample, weaker signal (by 4-fold) of m/z 138 was observed and the background of the ESI-

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MS spectrum was quite high (Figure S3-b), probably because of the salt suppression effect. In Figure 1, a peak at m/z 135 was observed, which was from the DESI spray background.

The spray solvent used in this case was $NH_3{\cdot}H_2O$/ACN/H_2O (0.1:50:50 by volume), which was chosen for two reasons. First, because of the acidity of ONP, ammonia was added into the spray solvent to facilitate the deprotonation of ONP into an anion. Second, such a basic solvent would be able to quench the enzymatic reaction, which is important and necessary for preventing the possible postionization reaction process. To test this hypothesis, DESI-MS spectra were recorded for the ionization of the reaction mixture under the condition that the distance between the microreactor capillary outlet tip and the inlet of mass spectrometer (d, Scheme 1) was changed. Our data showed that the intensity ratios of m/z 138 to m/z 142 was nearly kept the same (8.6−8.7%, Figure S4-a−c) with changing distances, indicating the reaction was well quenched when the basic spray solvent was used for ionization. In contrast, when the spray solvent was changed to ACN/H_2O (50:50 by volume) without ammonia, the ratio increased from 14% to 21% when the distance d increased from 0.2 to 1 cm (Figure S4-d−f). It is very likely that the reaction had not been completely quenched upon DESI ionization with the spray solvent of ACN/H_2O. The results above tell that simultaneous reaction quenching and efficient ionization could be achieved by DESI-MS with the use of an appropriate spray solvent. By contrast, carrying out online reaction quenching is inconvenient for ESI-MS, since the quenching solution needs to be infused through a second Tee-mixer, which would increase the complexity of the apparatus and delay the MS detection of the reaction product (therefore not suitable for fast reaction monitoring). In addition, we carried out an additional experiment to show that our DESI quenching could be more effective and faster than the traditional offline quenching. When the reaction solution in the capillary reactor was directed into a 10 μL quenching solution of $NH_3{\cdot}H_2O$/ACN/H_2O (0.1:50:50 by volume), we found that the reaction product yield was slightly higher than that from online DESI quenching, based on the subsequent MS measurement. It is likely that the mixing of reaction sample with the DESI sprayed droplets in a "confined volume" is faster than mixing with the bulk quenching solution, leading to faster quenching. Similar phenomenon was reported in a recent study reporting that ultrafast mixing of reactants in microsecond time scale can be achieved using microdroplets instead of using bulk solutions.[57]

Figure 2 displays the change of the reaction product concentration vs the reaction time. The substrate concentration varied at 91, 182, 373, 455, and 545 μM. Each concentration was sampled at 5 different reaction times in triplicate measurements. The reaction time was calculated by using the total dead volume of Tee mixer and the microreactor capillary (0.332 μL) divided by the total flow rate of substrate and enzyme solutions. Once the reaction commences, the intensity ratio of the product ion to the internal standard ion increases linearly in accordance with the classical Michaelis−Menten kinetics (this initial part of the enzyme reaction has a linear trend and the slope of the linear fit is the initial reaction velocity v_0). Using the obtained values of v_0 for different substrate concentrations (from Figure 2), the reaction constant K_m can be obtained, using the L−B plot. The L−B plot is described by eq 4 where $1/v_0$ has a linear relationship with $1/C_0$, in which the slope is K_m/V_{max} and the y-intercept is $1/V_{max}$. (where V_{max} is the maximum reaction rate and C_0 is the initial substrate concentration)

$$\frac{1}{v_0} = \frac{K_m}{V_{max}}\frac{1}{C_0} + \frac{1}{V_{max}} \quad (4)$$

$$V_{max} = k_{cat}[E] \quad (5)$$

The reciprocal of v_0 (i.e., the slope of the curve shown in Figure 2) was plotted against the reciprocal of C_0 (Figure 3). A least-squares linear fit provides the slope of the fitting line (K_m/V_{max}) and the y-intercept ($1/V_{max}$), which determines K_m to be 214 μM. This result of K_m measurement is in good agreement with the value of 300 μM reported before using spectroscopy.[58] Furthermore, based on the measured value of V_{max} from Figure 3 and eq 5 where [E] is the concentration of the enzyme, the value of k_{cat} was calculated to be 97 s^{-1}. Compared to spectroscopic methods, the method based on MS measurement has advantage of no need for chromophoric substrates. The success in measuring the key kinetics constants of this high-speed enzymatic reaction is ascribed to the capability of DESI-MS for both efficient ionization of high salt-containing samples and online quenching of reaction. To the best of our knowledge, no literature was found for using MS to study this reaction kinetics in the past.

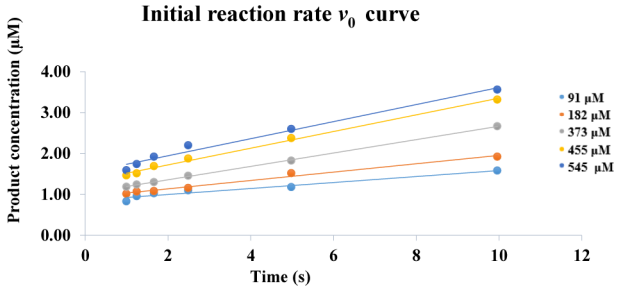

Figure 2. Plot showing the change of the reaction product concentration with the reaction time. The substrate ONPG concentration varied from 91 to 545 μM. The β-galactosidase concentration of enzyme was set as 9 nM.

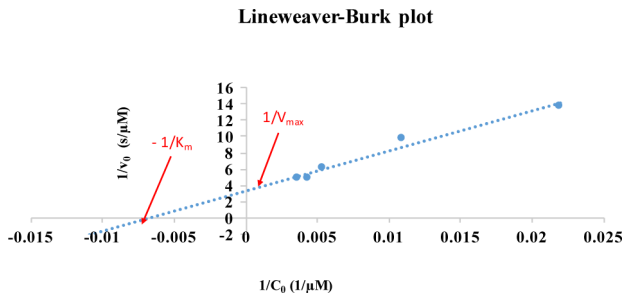

Figure 3. Lineweaver−Burk plot for characterizing K_m.

To further verify the applicability of this DESI-MS method, the hydrolysis of acetylcholine by acetylcholinesterase was also studied as another test example. Ach is a neurotransmitter in peripheral and central nervous systems.[59] AchE could reduce the Ach level in neurosystem by hydrolyzing Ach to choline and acetic acid. In the DESI-MS experiment, both AchE and Ach were dissolved in 10 mM Tris buffer and then underwent mixing for reaction in the microreactor, following by direct DESI-MS analysis. The DESI spray solvent used was ACN for aiding the ionization and quenching the reaction. Figure 4

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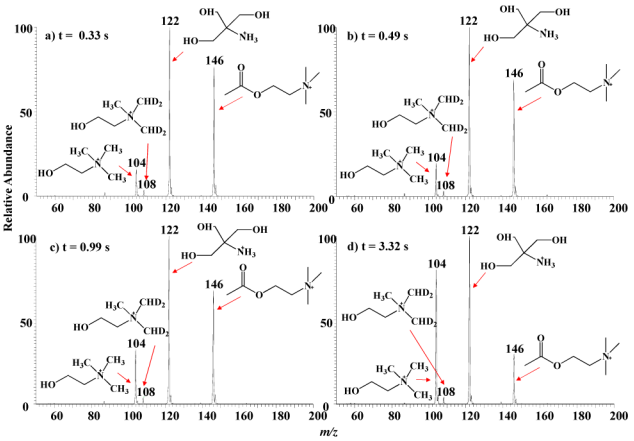

Figure 4. Time-resolved DESI-MS spectra showing the reaction between 1.4 mM Ach and 1.4 μM AchE sampled by DESI-MS at (a) 0.33, (b) 0.49, (c) 0.99, and (d) 3.32 s, respectively.

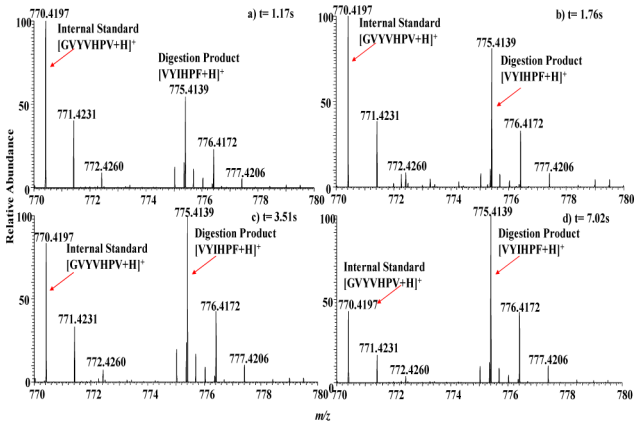

Figure 5. Time-resolved DESI-MS spectra showing the reaction between 3.5 mM angiotensin II and 0.06 mM trypsin sampled by DESI-MS at (a) 1.17, (b) 1.76, (c) 3.51, and (d) 7.02 s, respectively.

displays DESI-MS spectra showing the enzymatic reaction of 1.4 mM substrate and 1.4 μM enzyme in 10 mM Tris buffer sampled at different times. The choline-d_4 was added as the internal standard (seen at m/z 108) for assisting quantification of the reaction product choline (seen at m/z 104). According to the time-dependent DESI-MS spectra, m/z 104 (relative to m/z 108) increased with increasing reaction time. At the same time, the peak of the substrate (m/z 146) decreased with time. As displayed in the spectrum (Figure 4), the ion signal for m/z 104 is good, even in the presence of 10 mM Tris buffer. Again, this shows the high tolerance of DESI to matrices.[33,48] Figure S5-a and S5-b shows the change of the reaction product with the reaction time for five different substrate concentration measurements (0.14, 0.35, 0.56, 0.7, and 1.4 mM) and the L−B plot, respectively. The K_m was found to be 172 μM. Again, this result is in line with the value reported in the previous ESI-MS study (230 μM),[14] in which the reaction was offline quenched by ACN solution. Compared to the ESI-MS approach, our method has features with simpler apparatus and faster detection. Also, based on eq 5, k_{cat} was found to be 234 s^{-1}.

Trypsin is an important protease for protein digestion in typical MS-based shotgun proteomics experiments because of its high specificity, widespread availability, and ease of use.[60] This pancreatic serine protease cleaves peptides on the C-terminal side of lysine and arginine residues. Surprisingly, although trypsin is widely used for MS-based research, the reaction kinetics study for the digestion of proteins/peptides by trypsin using MS is rare.[12] Because of the success of the measurements of kinetics parameters of the aforementioned two hydrolysis reactions, we believe that our DESI-MS can serve for this purpose. In this experiment, peptide angiotensin II (sequence DRVYIHPF) was chosen as the substrate. Angiotensin II solutions at different concentrations containing 40 μM internal standard of angiotensin III antipeptide (sequence GVYVHPV, chosen because of its similar structure to the trypsin digestion product VYIHPF) was prepared in 25 mM ammonium bicarbonate and infused into the micro-Tee mixer via one inlet. 0.06 mM trypsin dissolved in 25 mM ammonium bicarbonate was infused into the micro-Tee mixer via the other inlet. Figure 5 shows the DESI-MS spectra of the enzymatic reaction of 3.5 mM substrate and 0.06 mM enzyme sampled at different reaction times. In Figure 5, the peak at m/z 775 corresponding to the protonated digestion product $[VYIHPF + H]^+$ as a result of the c-terminal cleavage of the substrate's arginine residue. The internal standard ion $[GVYVHPV + H]^+$ appears at m/z 770. A separate experiment showed the ionization efficiency of the product vs the internal stand is 0.77 (data not shown), which was used for measurement of the product concentrations at different reaction times. As also shown in Figure 5, with increasing reaction time, the intensity of the product ion increased. In this experiment, MeOH/H_2O/HOAc (50:50:3 by volume) was chosen as DESI spray solvent, for enhancing the ionization efficiency and quenching the reaction. As displayed in Figure 5, the product ion is clearly seen in the positive ion mode with high intensity. Furthermore, another separate experiment showed that the reaction was well quenched with this chosen solvent (Figure S6). DESI-MS spectra were recorded for the ionization of the reaction mixture under the condition that the distance between the microreactor capillary outlet tip and the inlet of mass spectrometer was changed. Our data showed that the intensity ratio of m/z 775 to m/z 770 remained constant (220%, Figure S6-a−c) when distance increased from 0.5 to 1 cm. In contrast, when the spray solvent was changed to MeOH/H_2O (50:50 by volume), the ratio increased significantly from 310% to 500% with changing distances (Figure S6-d−f).

Figure S7-a and S7-b displays the change of the reaction product concentration with the reaction time for different substrate concentration measurements (1.5, 2, 2.5, 3, and 3.5 mM) and the L−B plot, respectively. By the L−B plot, K_m was measured to be 6.4 mM, indicating the weak affinity of trypsin toward this peptide. Also, k_{cat} was calculated to be 1.3 s^{-1}. Under the conditions used in this experiment, this trypsin digestion turnover rate is the slowest among the three hydrolysis reactions measured in this study.

CONCLUSIONS

In summary, we present a new online DESI-MS approach for measuring enzymatic reaction kinetic constants. Two striking features of DESI-MS method in comparison to the traditional ESI-MS approaches are revealed. First, by choosing a proper spray solvent, the reaction product could be well ionized from aqueous solution containing high salt content or nonvolatile buffer which are required for optimal activity of enzymes. Second, the online reaction quenching could be realized, again through the judicious selection of the spray solvent. Three

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enzymatic reactions with different turnover rates were explored and demonstrated the capability of DESI-MS for online enzymatic reaction monitoring. We believe that DESI-MS would be of great value for measuring many other enzymatic reaction kinetics, in particular for fast reactions as DESI tolerates high sample injection rates.[61]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b03975.

MS spectra and kinetics data (PDF)

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Notes

The authors declare no competing financial interest.

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