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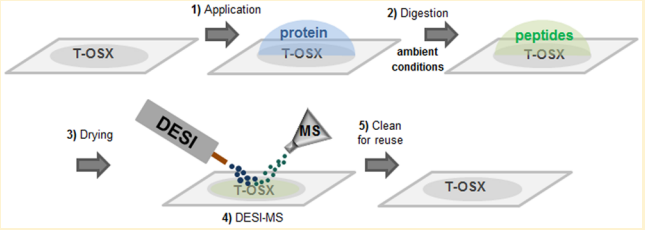
Protein Analysis by Ambient Ionization Mass Spectrometry Using Trypsin-Immobilized Organosiloxane Polymer Surfaces

Maria T. Dulay, Livia S. Eberlin, and Richard N. Zare*

Department of Chemistry, Stanford University, Stanford, California 94305, United States

ABSTRACT: In the growing field of proteomic research, rapid and simple protein analysis is a crucial component of protein identification. We report the use of immobilized trypsin on hybrid organic−inorganic organosiloxane (T-OSX) polymers for the on-surface, in situ digestion of four model proteins: melittin, cytochrome *c*, myoglobin, and bovine serum albumin. Tryptic digestion products were sampled, detected, and identified using desorption electrospray ionization mass spectrometry (DESI-MS) and nanoDESI-MS. These novel, reusable T-OSX arrays on glass slides allow for protein digestion in methanol:water solvents (1:1, v/v) and analysis directly from the same polymer surface without the need for sample preparation, high temperature, and pH conditions typically required for in-solution trypsin digestions. Digestion reactions were conducted with 2 μL protein sample droplets (0.35 mM) at incubation temperatures of 4, 25, 37, and 65 °C and digestion reaction times between 2 and 24 h. Sequence coverages were dependent on the hydrophobicity of the OSX polymer support and varied by temperature and digestion time. Under the best conditions, the sequence coverages, determined by DESI-MS, were 100% for melittin, 100% for cytochrome *c*, 90% for myoglobin, and 65% for bovine serum albumin.



Shotgun proteomics, an important technique in modern biological and medical research, relies significantly on protein sequence analysis. Traditionally, proteins are first digested into their complementary peptides and then separated before their analysis by mass spectrometry (MS). In-solution digestion is the conventional approach, which tends to be time-consuming and may be prone to artifacts, reducing the dynamic range of MS detection in complex samples. An alternative approach is the use of immobilized enzymes attached to an inert support.

Trypsin is the most highly utilized protease for the digestion of proteins. There is a rich literature on the use of immobilized trypsin, demonstrating its advantages, including high enzyme-to-substrate ratio, smaller sample volumes, little to no trypsin autolysis, shorter incubation times, and reusability.[1−5] Immobilization by covalent attachment of trypsin has been achieved using organic, inorganic, and hybrid matrices.[1−6] Previously, we prepared immobilized trypsin on a hydrophilic photopolymerized silica-based hybrid inorganic−organic polymer for online separation by capillary electrochromatography.[6] We found that immobilized trypsin was stable in high pH, achieving bioactivity approximately 2,000 times higher than in bulk solution at room temperature. This previous work motivated the present study.

There are several reports on the use of immobilized trypsin for protein identification followed by MS analysis.[7−11] Although some of the disadvantages of in-solution trypsin digestion have been eliminated in these immobilized trypsin platforms, some characteristics of in-solution trypsin digestion remain, such as the need for high-temperature digestion and product separation prior to MS analysis. For example, Dovichi and co-workers[8] demonstrated that digestion of proteomes at 37 °C by immobilized trypsin on magnetic microspheres was 2 orders of magnitude more rapid than in-solution digestion; however, the digestion fragments still required separation prior to MS analysis.

Matrix-assisted laser desorption ionization mass spectrometry (MALDI) is the method of choice in the analyses of the digestion products from immobilized trypsin. MALDI, however, is limited in that sample preparation remains a requirement prior to analysis and the sample must be placed in vacuum for analysis.

Ambient ionization techniques, such as desorption electrospray ionization mass spectrometry (DESI-MS), minimize sample preparation by allowing spectra to be recorded on samples in their native states in open air at room temperature. DESI-MS has become an important method for the rapid analysis of a variety of analytes, including pharmaceuticals, drugs of abuse, biological compounds,[12] and intact tissues.[13] In DESI experiments, desorption and ionization of analytes from a sample occur through the interaction of charged microdroplets that are generated in the electrospray with the sample surface. Two studies on the use of ambient ionization mass spectrometry for the analysis of in situ tryptic digests have been reported. Rao and co-workers[14] described a modified DESI and liquid extraction surface analysis (LESA) MS for the detection and identification of proteins adsorbed onto biomaterial surfaces, which were then subsequently digested

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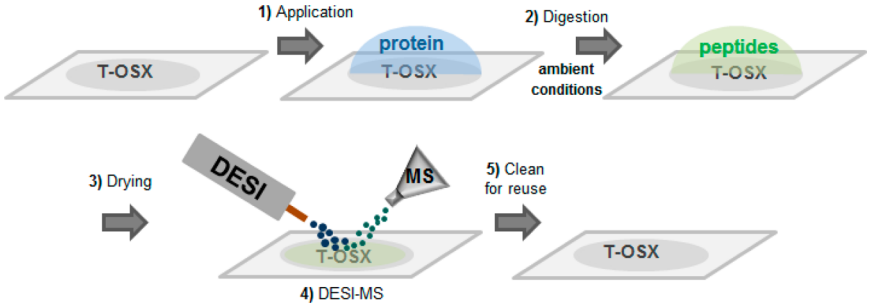

Figure 1. Schematic representation of the experimental procedure for protein digestion using the T-OSX material followed by DESI-MS detection and characterization of the digestion products. First, a small volume (~2 μL) of protein solution is deposited onto the T-OSX material (step 1). Tryptic digestion of the protein into small peptides occurs in ambient conditions for 5 min or longer (step 2). The material is dried for 5 min (step 3) and directly subjected to DESI-MS analysis (step 4). The T-OSX material can be cleaned and reused for other experiments (step 5).

by a solution of trypsin prior to mass analysis. Montowska and co-workers[15] demonstrated the analysis of skeletal muscle proteins deposited onto a glass slide and subsequent digestion with the addition of trypsin solution to the glass slide followed by DESI and LESA-MS analysis. However, no effort has been made to couple immobilized enzymes to ambient ionization techniques for the analysis of proteins. It is this combination that we report here.

EXPERIMENTAL SECTION

Procedure. Figure 1 presents a schematic of the experimental setup for analyzing peptides by using immobilized trypsin on an OSX polymer surface by ambient ionization, either DESI-MS or nanoDESI-MS. We performed on-surface in situ digestion of pure samples of four standard proteins: melittin, cytochrome *c*, myoglobin, and bovine serum albumin (BSA). Fresh stock solutions of individual protein solutions were prepared as either 1 mg/mL or 0.35 mM in an organic-aqueous solvent system containing 1% (v/v) formic acid before every DESI-MS experiment. The organic solvents are methanol, ethanol, and isopropanol. A volume of 2 μL of protein solution was deposited directly onto the surface of a T-OSX polymer, and the protein was allowed to react on the polymer surface by being incubated in a humidified chamber (a Petri dish lined at the bottom with a Kimwipe wetted with 4 mL of distilled H_2O) to limit the evaporation of the sample solvent. After drying for 5−10 min, the surface of the T-OSX polymer was directly sampled by DESI-MS or nanoDESI. After each use, T-OSX arrays were rinsed gently with distilled water and stored dry at 4 °C for reuse. Bulk solution digestion of 0.35 mM melittin or 0.35 mM myoglobin and 12 ng of trypsin in 50% methanol showed no digestion of melittin after even 12 h at room temperature. In what follows, we describe the individual components found in Figure 1.

DESI-MS. We used a lab-built DESI-MS source coupled to an LTQ-Orbitrap XL mass spectrometer (Thermo Scientific, San Jose, CA). DESI-MS was performed in positive ion mode from m/z 100−2000 using an orbitrap as the mass analyzer. The spray solvent MeOH:H_2O 1:1 (v/v) was used for analysis at a flow rate of 5 μL/min; the N_2 pressure was set to 150 psi for the nebulizing gas, and the spray voltage was set to 5 kV.

NanoDESI-MS. We interfaced a nanoDESI source, operated in positive ion mode, with an X-Y sample stage and coupled this to the LTQ-Orbitrap XL mass spectrometer using the orbitrap as the mass analyzer. The outlets of two capillary tubes are approximately 60 μm O.D. The spray solvent was CH_3CN:H_2O 2:1 (v/v), which was delivered at a flow rate between 2 and 4 μL/min and selected to match the self-aspiration rate of the carrier solvent through a secondary capillary (350 μm O.D. × 250 μm I.D.). The spray voltage was set to 2.4 kV.

MALDI-TOF-MS. All measurements were performed at the Canary Center at Stanford (Palo Alto, CA) on an AB Sciex 5800 MALDI TOF mass spectrometer (Framingham, MA). Alpha-cyano-4-hydroxycinnamic acid (Agilent Technologies, Santa Clara, CA) was used as the matrix. MS data were acquired with TOF/TOF Series Explorer software, and imaging data were acquired with TOF/TOF Imaging software both on the AB Sciex mass spectrometer. Just prior to MALDI analysis, the matrix was spotted by hand onto each T-OSX polymer after the trypsin digestion reactions. We coupled T-OSX-1 and T-OSX-2 to MALDI-TOF-MS. We were only able to observe 1 to 2 digestion products of melittin, myoglobin, and BSA because the insulating property of the OSX polymer interfered with the MALDI process. Consequently, we did not pursue further MALDI studies.

Reagents and Chemicals. Methyltrimethoxysilane (MTMS), dimethyl-dimethoxysilane (DMDMS), bovine trypsin (TPCK-treated), melittin (from honey bee venom), lysozyme (from chicken egg white), myoglobin (equine heart), cytochrome *c* (equine), bovine serum albumin (BSA), phosphate-buffered saline (PBS 1X), acetic acid (HOAc), methanol, and acetonitrile were purchased from Sigma-Aldrich (Milwaukee, WI) and used as received. Trimethoxysilylbutyraldehyde (TMSB) was purchased from Gelest (Morisville, PA) and used without further purification.

Organosiloxane (OSX) Polymer Preparation. We prepared two types of OSX polymers. The reaction solution for OSX-1 was prepared by stirring 500 μL of MTMS and 225 μL of DMDMS with 600 μL of 0.12 N HOAc at room temperature for 30 min. The reaction solution for OSX-2 was made by stirring 730 μL of MTMS and 600 μL of 0.12 N HOAc at room temperature for 30 min. Each of the 12 5 mm round wells on a Teflon-printed glass slide (Electron Microscopy Sciences, Hatfield, PA) was filled with 10 μL of reaction solution. The glass slide was kept in a covered Petri dish during the curing stage of the polymerization in a 65 °C oven for approximately 21 h. The resulting OSX polymers were rinsed free of unreacted starting materials and alcohol byproduct by immersing the slides in a container of acetonitrile and agitating for 45 min. Any acetonitrile remaining on the glass slides and polymers were allowed to evaporate under ambient conditions before further modification. For MALDI-TOF-MS experiments, OSX polymers were prepared in a

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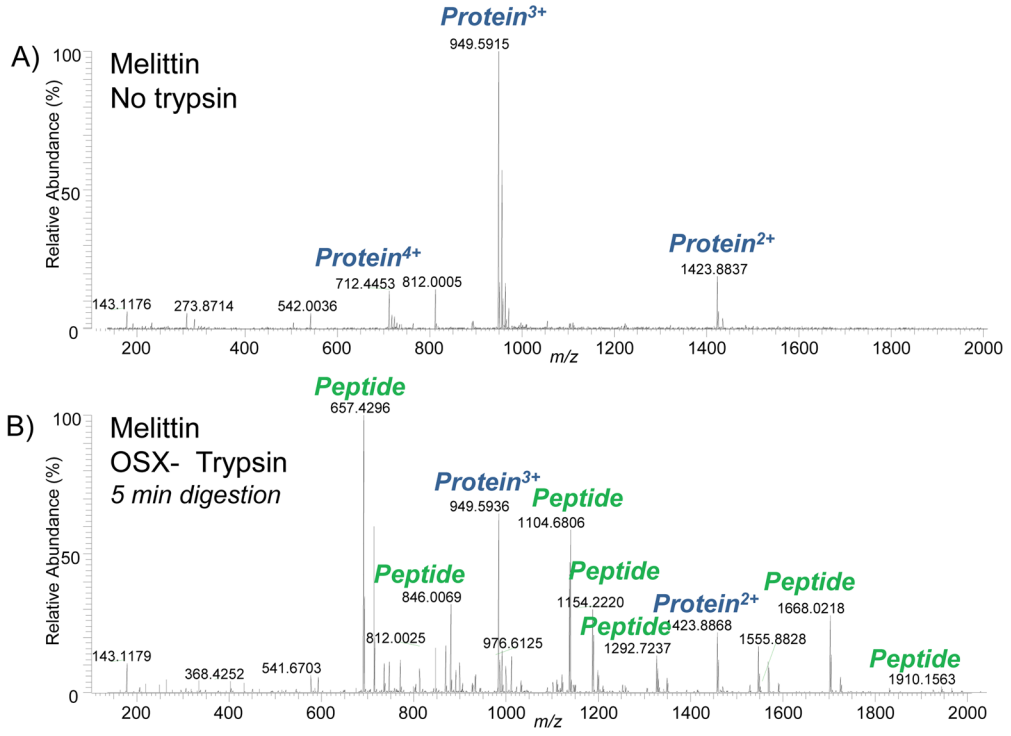

Figure 2. Positive ion mode DESI mass spectra obtained from 3 μL of melittin solution (0.1 mg/mL in 40% methanol) deposited onto (A) T-OSX material and (B) nonderivatized OSX material after 5 min of digestion and 5 min of drying time. Trypsin digestion fragments of melittin are labeled in green, and the intact multiply charged ions are labeled in blue.

similar fashion on ITO-coated glass slides. A PAP pen (Sigma-Aldrich, St. Louis, MO) was used to draw 5 mm diameter reaction areas on the ITO-coated glass slides. The PAP markings around the OSX polymers were not removed.

Trypsin Immobilization. Trypsin was immobilized on OSX polymers following a previously reported procedure[6] with modifications. OSX polymers were first derivatized with 8 μL of 20% (v/v) TMSB in acetonitrile for 1 h at room temperature. Unreacted TMSB was removed by immersion of OSX polymers in acetonitrile for 30 min. Aldehyde-functionalized OSX was stored dry at room temperature until ready to immobilize with trypsin.

Before trypsin immobilization, aldehyde-functionalized OSX polymers were rinsed with PBS. A volume of 8 μL of a fresh solution of trypsin (4 mg or 10 mg) in 1 mL of PBS buffer was deposited onto each OSX polymer to completely cover the polymer surface. With the glass slides containing the polymers in a covered Petri dish, the reaction was allowed to proceed for 24 h at 4 °C after which the reaction was stopped by immersing the polymers in PBS buffer to remove any unreacted trypsin. When not in use, the trypsin-modified OSX polymers were stored dry at 4 °C. It was found that the loading at 4 mg of trypsin gave optimal results. It is speculated that at higher loading the proximity of the trypsin strands interferes with the enzymatic digestion.

The amount of trypsin bound to the OSX surface was determined using fluorescamine, which binds to lysine residues. The resulting fluorescamine−trypsin complex is detected and quantitatively measured using a PerkinElmer fluorimeter. Prior to the assay, trypsin from each of the 12 OSX polymers on a single slide was cleaved by exposure to 0.1 N NaOH. A volume of 20 μL of 0.1 N NaOH was deposited onto each of the 12 OSX polymers on a glass slide. Exposure to NaOH was allowed to proceed at room temperature for approximately 2 h. Trypsin standards were prepared in 0.1 N NaOH. Each of the trypsin standards and the cleaved trypsin from 12 OSX polymers was placed in individual wells of a 96-well microtiter plate. To each solution was added 100 μL of fluorescamine. The samples were incubated at room temperature for 5−30 min. We found that the trypsin amount on a single OSX-1 and OSX-2 polymer was 12 ± 3 ng (n = 3 slides each) for an immobilization reaction solution containing 4 mg/mL of trypsin in buffer. Assuming that trypsin coverage is homogeneous across the surface, each disk has a trypsin surface coverage of 0.61 ng/mm^2.

It was expected for OSX-2, with the higher surface hydroxyl groups as compared to OSX-1, that the surface would be activated with more aldehyde groups, which follows that there would be more trypsin bound to the surface.

RESULTS AND DISCUSSION

T-OSX Polymers. The formation of cracks in polymers prepared by sol−gel chemistry is a significant problem that reduces the mechanical stability of the polymer. The OSX polymers that we prepared with tetramethoxysilane exhibited cracks after being deposited on glass slides. To avoid this problem, methyl-substituted alkoxysilanes were used in the sol−gel reactions to prepare two OSX polymers, OSX-1 and OSX-2, as crack-free supports for the surface immobilization of trypsin. OSX-1 is a hydrophobic OSX polymer that was prepared with monomethyl-functionalized silane (MTMS) and dimethyl-functionalized silane (DMDMS). OSX-2, which was prepared with MTMS only, has a lower density of methyl groups than OSX-1 and therefore has lower hydrophobicity than OSX-1. We immobilized trypsin on OSX polymers T-OSX-1 and T-OSX-2 using Schiff base chemistry. Four different proteins, melittin, cytochrome c, myoglobin, and BSA were

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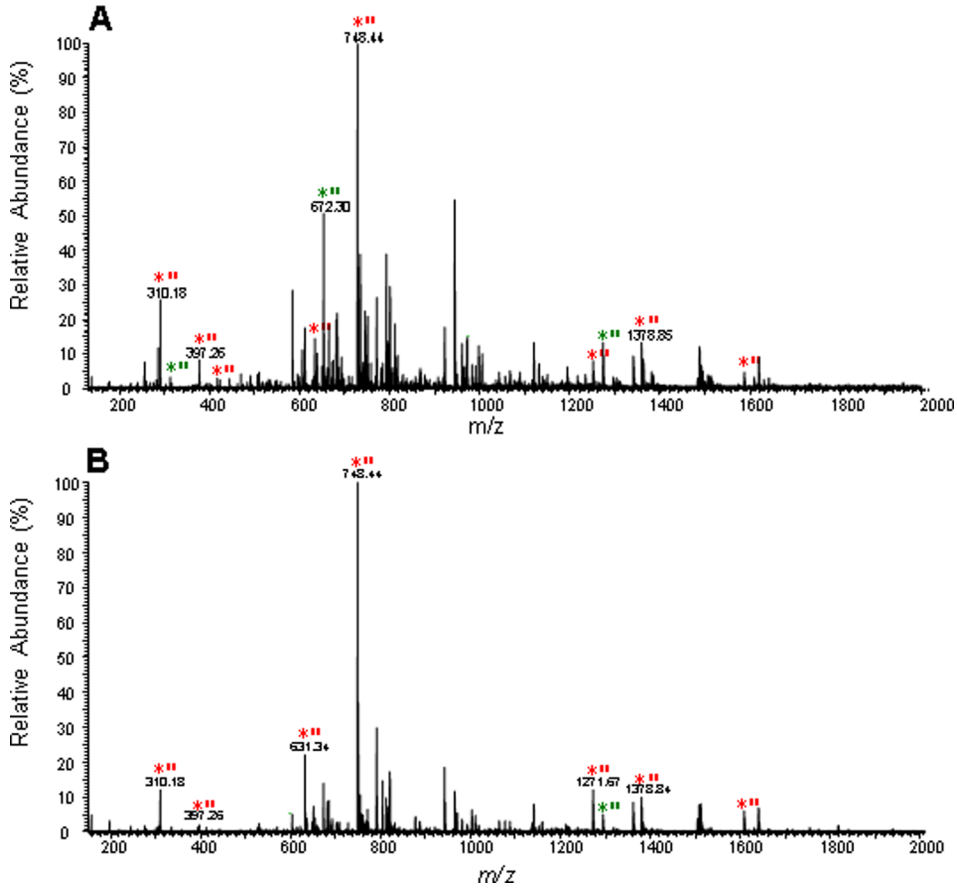

Figure 3. Positive ion mode DESI mass spectra obtained from 2 μL of myoglobin solution (0.35 mM in 50% methanol) deposited onto (A) T-OSX-1 after 2 h and (B) T-OSX-2 after 2 h of 37 °C digestion. The most abundant trypsin digestion fragments of myoglobin, $[M + H]^+$, are labeled in red, and the sodium adducts, $[M + Na]^+$, are labeled in green.

digested by the trypsin, and the resulting peptide fragments were detected by DESI-MS and nanoDESI-MS.

Proteolytic Activity of T-OSX for the Digestion of Melittin. The digestion of melittin in methanol:water (1:1, v/v) was used initially to determine the performance of the T-OSX-1 and T-OSX-2 materials, each prepared with 4 mg of trypsin. Partial digestion of melittin at room temperature in 5 min was determined by the presence of one of the peptide fragments (amino acid sequence, GIGAVLK) with m/z 657 (Figure 2A), whereas the absence of immobilized trypsin on the T-OSX surface did not result in any digestion of melittin (Figure 2B). We did not observe any missed-cleavage products during the digestion of melittin, which rules out partial digestion. Remarkably, no peaks corresponding to the digestion products of trypsin or intact trypsin were observed in any of the mass spectra recorded throughout all of our experiments.

Effect of OSX Polymer Hydrophobicity on the Proteolytic Activity of Immobilized Trypsin. T-OSX-1 and T-OSX-2 polymers were compared for their digestion of myoglobin in a 50% aqueous methanol solution at room temperature for 2 h (Figure 3). There is enhanced digestion on T-OSX-1 as compared to T-OSX-2. Higher sequence coverage of 65.7 ± 9.8% (n = 3) with 14−17 peptide fragments identified was achieved for the digestion of myoglobin employing the more hydrophobic T-OSX-1 polymer as compared to the less hydrophobic T-OSX-2 that afforded only 34.0 ± 12.5% (n = 2) sequence coverage with 6−10 peptide fragments identified (see Table 1). Using nanoDESI-MS, we observed the enhanced digestion of myglobin and BSA after 2 h of digestion at 37 °C on T-OSX-1 (Table 1). For myoglobin, there is more than a 30% increase in sequence coverage on T-OSX-1 (73%, 14 peptides) as compared to the sequence coverage on T-OSX-2 (37%, 7 peptides). The enhancement in sequence coverage for the digestion of BSA on T-OSX-1 (28.3%, 38 peptides) is more modest when compared to T-OSX-2 (25%, 30 peptides). The extent of enzyme digestion may result from a difference in hydrophobicity between T-OSX-1 and T-OSX-2. OSX-1 polymers have higher methyl group content than those of OSX-2. There are conflicting reports on the extent of proteolysis bound on different hydrophobic surfaces. Studies have shown that protein unfolding may be induced by protein−

Table 1. Two-Hour Digestion of Proteins in 50% Methanol on T-OSX Polymers

protein	polymer	T (°C)	sequence coverage (%)	# peptides detected
myoglobin	T-OSX-1	25	65.7 ± 9.8	14−17
myoglobin	T-OSX-2	25	34.0 ± 12.5	6−10
myoglobin	T-OSX-1	37	73	14
myoglobin	T-OSX-2	37	37	7
BSA	T-OSX-1	37	28.3	38
BSA	T-OSX-2	37	25	30
melittin	T-OSX-1	25	100	5
myoglobin	T-OSX-1	25	61	14
BSA	T-OSX-1	25	37	24
cytochrome c	T-OSX-1	25	14	10

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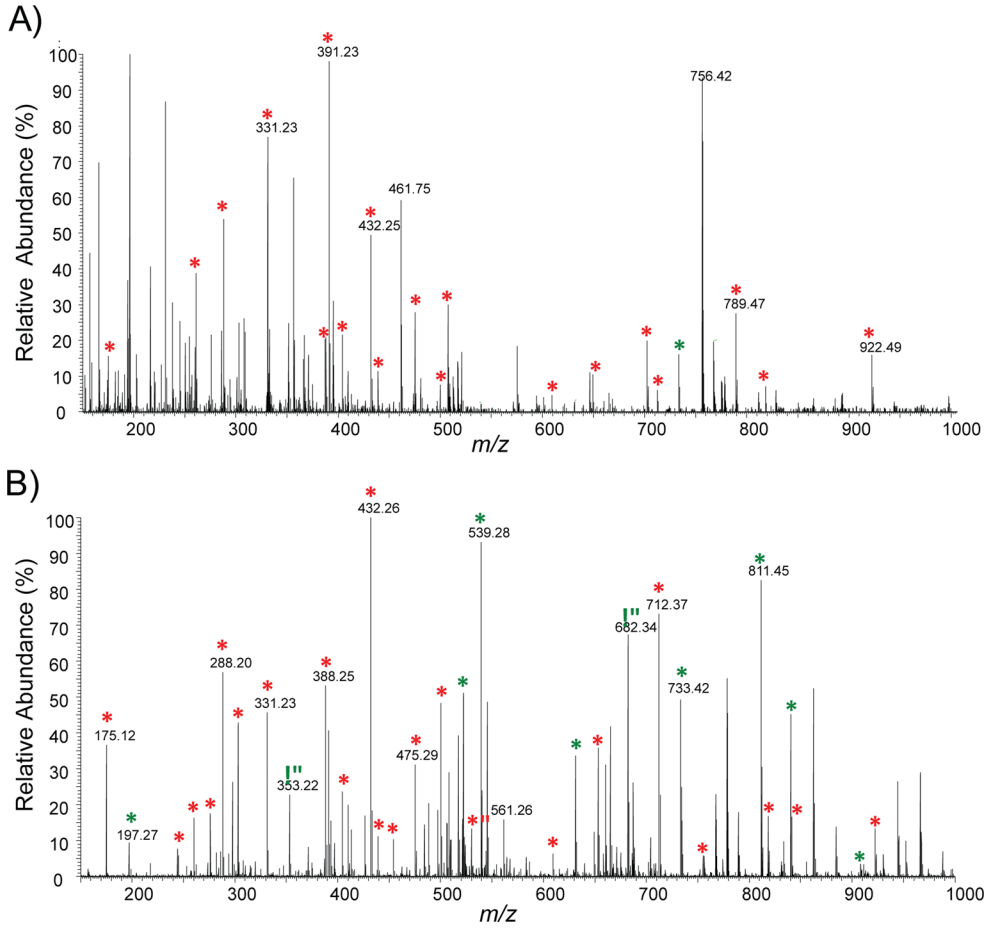

Figure 4. Positive ion mode nanoDESI mass spectra obtained from 2 μL of myoglobin solution (0.35 mM in 50% methanol) deposited onto T-OSX-1 after (A) 2 h of 37 °C digestion and (B) 24 h of 37 °C digestion. The most abundant trypsin digestion fragments of myoglobin, $[M + H]^+$, are labeled in red, and sodium adducts, $[M + Na]^+$, are labeled in green.

surface interactions. Partial unfolding of an enzyme can occur when the polymer surface is hydrophobic enough, allowing for interaction between the enzyme and the hydrophobic domains of the polymer surface and decreasing enzyme activity.[16,17] Previously, we demonstrated that the immobilized trypsin activity on a monolithic hydrophilic OSX polymer in a capillary increased by more than 2,000 times as compared to comparable in-solution trypsin digestion.[6] It has been reported that in situ digestion on a hydrophobic surface provided better digestion than on a hydrophilic surface.[18] Optimized protein digestion conditions were identified by studying the effect of T-OSX composition, solvent composition, digestion temperature, and storage requirements.

Effect of Digestion Solvent Composition on the Proteolytic Activity of T-OSX-1. We studied the effects of different compositions of organic-aqueous solvent systems on protein digestion on T-OSX-1. Initial studies compared the digestion of melittin (0.1 mg/mL, 5 min, RT) in different digestion solvents containing water and 92% isopropyl alcohol, 76% ethanol, 50 or 60% methanol, and in 100% water where no precipitation of melittin was observed. The 5 min digestion of melittin under these different solvent conditions was monitored using the peak m/z 657 (amino acid sequence, GIGAVLK). The digestion of melittin was determined using the peak abundance ratio of a peptide fragment (m/z 657) and intact protein peak (m/z 949). Nearly complete digestion of melittin in 5 min at room temperature was achieved in 50% methanol with a peak abundance ratio of 3.0. The peak abundance ratios for 76% ethanol, 60% methanol, and 100% water are 0.35, 2.5, and 1.4, respectively. No digestion was observed in 92% isopropyl alcohol. Denaturation of proteins is an important step in the digestion of proteins, and this task is typically achieved by the use of chemical denaturants or high concentrations of organic solvents. In solvent-assisted digestion, tryptic digestion is enhanced beyond that of pure water because the organic solvent decreases the insulating effects of water, thereby increasing the ionic interaction between the protein and the immobilized enzyme.[19−21] In addition, polar organic solvents are able to remove the water shell surrounding a protein, resulting in its denaturation and accelerated digestion.[8] In the presence of high concentrations of organic solvent systems, immobilized trypsin retains its bioactivity, whereas the proteins are denatured in organic solvents.[6,22] We found that preparation of proteins in 1:1 (v/v) methanol:water with 0.1% (v/v) formic acid significantly improves protein digestion. These facts explain the choice of the solvents we used.

Proteolytic Activity of T-OSX-1 for the Digestion of Proteins. We followed the digestion of the proteins on T-OSX-1 in 50% methanol for 2 h at room temperature by DESI-MS. Table 1 lists the sequence coverages for each protein. High sequence coverage was achieved even for the protease-resistant protein myoglobin. Sequence coverages of 100% (melittin), 61% (myoglobin), 37% (BSA), and 14% (cytochrome c) were achieved. Sequence coverage of digested myoglobin using the

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low hydrophobic T-OSX-2 under the same conditions is 12%. Digestion of myoglobin in free solution under the same experimental conditions was not observed. No trypsin autolysis products were detected in the mass spectra in any of the experiments performed. The enhancement in the digestion efficiency of immobilized trypsin compared to in-solution catalysis can be ascribed to (1) minimal to no trypsin autolysis and (2) possible stabilization of trypsin, affording higher accessibility of the protein substrate to the active site of the enzyme. Increases in the catalytic activity of immobilized enzymes can also arise from a high enzyme-to-substrate (E/S) ratio (i.e., 20:1−1:1).[23] In contrast, the E/S ratio is kept low (i.e., 1:100−1:20) for in-solution digestion to minimize the autolysis of free trypsin. The E/S ratio in our experiments is 1:167 for both T-OSX materials. Previously, we reported an E/S ratio of 1:3220 for trypsin immobilized in a hydrophilic polymer formed in a capillary and a trypsin activity 2,000 times that in free solution.[6] Similarly, digestion of the standard proteins was achieved despite the low E/S ratios of T-OSX-1, suggesting that covalent attachment of trypsin to the surface of this hydrophobic OSX polymer offers a favorable microenvironment that can help to maintain the active three-dimensional structure of the enzyme.[6]

Effect of Temperature on Protein Digestion and Sequence Coverage. A temperature increase in the digestion from room temperature to 37 °C enhanced trypsin digestion of the proteins by 5−20%. Increasing the digestion time from 2 h up to 24 h also enhanced trypsin digestion of the proteins, especially BSA, which did not show any digestion at 2 h at room temperature or 37 °C. At 24 h digestion time, increased sequence coverages were obtained for some of the proteins digested on T-OSX-1 at room temperature (100% melittin, 78% cytochrome *c*, 58% myoglobin, and 38% BSA) and at 37 °C (100% melittin, 100% cytochrome *c*, 90% myoglobin, and 65% BSA). Figure 4 shows mass spectra from the nanoDESI-MS analysis of the digestion of BSA on T-OSX-1 at 37 °C at 2 and 24 h. Thirty-eight peptide peaks for a sequence coverage of 28.3% from a 2 h digestion of BSA were observed under nanoDESI-MS conditions (Figure 4A), whereas no peaks were observed when BSA digestion was analyzed by DESI-MS. Figure 4B shows the 45 peptide peaks from a 24 h digestion of BSA for a sequence coverage of 31.8%. Interestingly, DESI-MS analysis of the digestion of the proteins occurred on T-OSX-1 at 4 °C for 24 h (100% melittin, 76% cytochrome *c*, 58% myoglobin, 21% BSA). Sequence coverages for the digestion of myoglobin on other immobilized trypsin materials have ranged between 17 and 100%.[24] Sequence coverages for BSA were reported as 46% on a Sigma Trypsin Spin column at room temperature[25] and as high as 84% on trypsin bound to nylon membranes at 37 °C.[3]

Effect of T-OSX Storage Conditions. We determined the best storage conditions for T-OSX-1 and T-OSX-2. We studied the effects of four different storage conditions on the activity of T-OSX-1 by monitoring the digestion of melittin and myoglobin at 37 °C for 2 h. No melittin digestion fragments were detected when T-OSX-1 was stored wet in PBS at 4 °C or room temperature. Complete digestion of melittin (100% sequence coverage with no intact melittin protein) was achieved using T-OSX-1 stored dry at 4 °C or at room temperature. Similar results were obtained when myoglobin was digested on T-OSX-1 under these storage conditions. The digestion of myoglobin on T-OSX-1 polymers stored under different conditions was monitored by monitoring the abundance of m/z 748 (amino acid sequence, ALELFR), the most prominent digestion fragment for myoglobin. It was not further determined if storage at 4 °C is better than at room temperature; all T-OSX materials were stored at 4 °C. Trypsin is covalently bound to the OSX surface by the formation of an imine bond between amine groups on trypsin and the butyraldehyde functional groups on OSX-1. It is known that imine bonds, in the presence of water, undergo hydrolysis to reform the aldehyde and free the trypsin even in the absence of an acid catalyst. The imine bond can be reduced with sodium borohydride to avoid this hydrolysis. We followed this approach but found no significant improvement.

T-OSX arrays are reusable by simply washing the disks with water after each use and storing them dry at 4 °C when not in use to preserve the activity of T-OSX-1 and T-OSX-2. The activity of T-OSX-1 decreased to 50% for melittin over 6 weeks.

Presently, the DESI-MS and the nanoDESI-MS detection of peptide fragments is carried out at one spot on the T-OSX polymer. As time progresses, we observe a depletion of the fragment signals, as expected, but if the T-OSX polymer is moved, the signal returns. This behavior suggests that more data could be collected by scanning across the polymer surface and summing the collected fragment signals if needed. At present, the limit of sensitivity for the detection of melittin, for example, is 0.3 μg/mm^2 with a spray spot size of approximately 300 μm in diameter. It would be anticipated that this detection limit would be significantly lowered by scanning over the entire polymer surface.

■ CONCLUSIONS

We have demonstrated the in situ digestion of proteins using a novel approach based on immobilizing trypsin on a hybrid organosiloxane polymer followed by DESI-MS or nanoDESI-MS for identification and characterization of the digestion products. Despite the low enzyme-to-substrate ratios, digestion is still achieved with the T-OSX polymers. The results demonstrate that better sequence coverage was achieved with the more hydrophobic T-OSX-1 as compared to the less hydrophobic T-OSX-2. No sample preparation was necessary prior to digestion. It is suggested that the T-OSX arrays with DESI and other MS surface sampling analysis techniques might open new possibilities for high throughput and rapid protein analysis desorbed in their native states directly from the T-OSX surface.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: zare@stanford.edu.

Notes

The authors declare no competing financial interest.

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