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Capillary Monolithic Bioreactor of Immobilized Snake Venom Phosphodiesterase for Mass Spectrometry Based Oligodeoxynucleotide Sequencing

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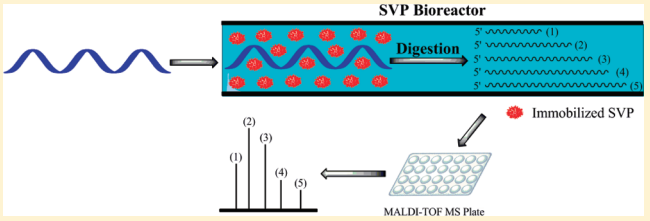
Supporting Information

ABSTRACT: A capillary monolithic bioreactor of snake venom phosphodiesterase (SVP) was constructed to generate different single-nucleotide mass ladders of oligodeoxynucleotides for mass spectrometry (MS)-based sequencing by immobilization. The immobilization of SVP in the porous silica monolith significantly enhances its stability for prolonged and repeated applications. The constructed capillary bioreactor has the advantages of handling (sub)microliter DNA samples and having good permeability. Benefiting from its good permeability, DNA solutions can be directly injected into the sequential digestion bioreactor simply by hand pushing or a low-pressure microinjection pump. Moreover, the immobilization of SVP facilitates the elimination or repression of the metal adducts of oligodeoxynucleotides, improving the analytical performance of MS sequencing. By the application of capillary bioreactor of immobilized SVP, the sequence-specific modification of single-stranded oligodeoxynucleotide induced by a ubiquitous pollutant acrolein (Acr) was identified, demonstrating its promising applications in identification of sequence-specific damage, which may further our understanding of DNA damage caused mutagenesis.



The rapid development in DNA sequencing has greatly stimulated the research in life sciences.[1−8] Modern mass spectrometry (MS) technologies can provide an accurate mass measurement and possess an extraordinary capacity of structural elucidation; therefore, these technologies are attractive in complement to high-throughput sequencing technologies.[9−13] In addition to the uncoding of normal sequence information, sequence-specific DNA damages can also be explicitly located and characterized by employing MS sequencing. In general, the mutations, which are largely caused by DNA damage, can be mapped and the mutational hot spots in genes can be identified using a variety of DNA sequencing, but the DNA damages in the sequence context and their chemical structures are only obtained by MS-based sequencing. By identification of the sequence-specific damages, the links between the damages and the mutations can be established, which are very helpful to the understanding of the selective formation and repair of DNA damages and consequent mutagenesis and carcinogenesis.[14−19]

In this work, we made an effort to construct a capillary monolithic bioreactor to generate mass ladders of oligodeoxynucleotides for MS-based sequencing and identification of sequence-specific damage through the immobilization of an exonuclease in a porous silica monolith. Previously, no attempt had been made to develop nuclease bioreactors for DNA sequencing. Snake venom phosphodiesterase (SVP) is chosen because of its unique ability as an exonuclease to sequentially cleave one nucleotide from single-stranded DNA. By taking advantage of this unique ability of SVP, the mass ladders of oligodeoxynucleotides with a single nucleotide difference can be produced, allowing for MS-based sequencing.[20−24] However, it is hard to control the sequence cleavage reaction of SVP due to its instability and too fast reaction kinetics in aqueous solution.[25] Meanwhile, the metal−oligodeoxynucleotide adducts that may deteriorate the performance of MS cannot be eliminated due to the unavoidable contamination of trace metals in enzyme preparation and buffers. Furthermore, the exonuclease used for free solution DNA cleavage will be present with the cleaved DNA fragments together; therefore, it may interfere with MS-based sequencing analysis.

Here we described the immobilization of the exonuclease SVP in porous silica monoliths and the construction of an SVP-based capillary bioreactor to overcome the bottleneck of free solution DNA digestion. Compared with a micrometer-particle packed capillary column, the porous monolith generated by in situ polymerization has excellent permeability. Therefore, the enzyme solution can be directly injected into the capillary without the need for any pump machine to allow further immobilization in the capillary (i.d. 75 μm). On the basis of the constructed bioreactor, we further developed a bioreactor off-line-coupled MS method for oligodeoxynucleotide sequencing, in which the metal−oligodeoxynucleotide adducts can be

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suppressed. The method is applicable for short oligodeoxynucleotides sequencing and identification of sequence-specific damage.

EXPERIMENTAL SECTION

Materials and Chemicals. *Crotalus atrox* SVP and poly(ethylene glycol) (PEG, 95%) were purchased from Sigma-Aldrich (St. Louis, MO). Nicotinic acid (NA, 99.5%), anthranilic acid (AA, 99.5%), and diammonium hydrogen citrate (DHC, 99%) were purchased from Fluka Analytical (Buchs, Switzerland). Tetramethoxysilane (TMOS, 98%) was purchased from J&K Chemica (Beijing, China). 3-Aminopropyl-trimethoxysilane (95%) and sodium cyanoborohydride ($NaCNBH_3$, 95%) were purchased from Acros Organics (Geel, Belgium). The oligodeoxynucleotides were synthesized at the Sangon Biotech Co., Ltd. (Shanghai, China). Other biochemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Preparation of the Capillary Silica Monolith. A fused-silica capillary (75 μm i.d., 360 μm o.d.) was rinsed with deionized water and methanol, and then, the capillary was treated with 0.1 M NaOH for 3 h, followed by rinsing with deionized water for 30 min. Subsequently, it was flushed with 0.1 M HCl for 1 h and deionized water for 2 h. At last, the capillary was purged with nitrogen at 60 °C prior to use. The conditioned capillary was used for the preparation of the silica monolith.

The procedure for the preparation of the capillary monolith was modified from previous work.[26] Briefly, 2.1 mL of TMOS was added to 5.0 mL of acetic acid solution (0.01 M) that contained 0.44 g of PEG (MW = 10 000) and 0. 45 g of urea (0.45 g), and the mixture was stirred at 0 °C for 35 min. The resultant mixture was injected into the fused-silica capillary to allow the overnight polymerization at 40 °C. The two ends of the capillary were sealed by silicon rubbers. After overnight polymerization, the capillary was then heated at the gradient temperature from 40 to 120 °C for 5 h and subsequently rinsed with deionized water and methanol.

Glutaraldehyde Activation and Exonuclease Immobilization. A solution of 3-aminopropyl-trimethoxysilane, ethanol, and water (5:10:90, v/v) was continuously injected into the prepared capillary silica monolith by air pressure at room temperature for 2 h twice, followed by 1 h of deionized water rinsing and nitrogen purge at 60 °C. Then, a solution of 20% (v/v) glutaraldehyde dissolved in 25 mM phosphate buffer (pH 6.9) was continuously injected into the monolithic capillary at 4 °C for 2 h twice and rinsed by 25 mM phosphate buffer (pH 6.9) for 20 min. Subsequently, 1 mg/mL SVP in 25 mM DHC−NH_4OH buffer (pH 9.4, adjusted by NH_4OH) was continuously injected into the monolithic capillary at 4 °C for 2 h twice. After the immobilization reaction, the capillary was washed by 25 mM DHC−NH_4OH buffer for 20 min, and the residual aldehyde groups on the surface of the support were depleted by injecting 2 mg/mL sodium cyanoborohydride ($NaCNBH_3$) for 1 h at 4 °C. The constructed capillary monolithic bioreactor was stored at 4 °C.

Exonuclease Digestion of DNA. The oligodeoxynucleotides was injected into the capillary SVP bioreactor and incubated at 37 °C for the desired time. After digestion, the digestion solution was pushed out for matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS analysis. Note: the bioreactor was rinsed with 25 mM DHC−NH_4OH buffer after each enzyme digestion.

MALDI-TOF MS Analysis. A Bruker Autoflex III Smartbeam mass spectrometer (Bruker Daltonics, Bremen, Germany) was used for MALDI-TOF MS analysis, equipped with a nitrogen laser operating at 337 nm and an ion source operating in delayed extraction mode. The total acceleration voltage was 20 kV. At the mass range of m/z 100−8000, the mass spectra were obtained with 70−80% laser energy, 2×10^{-6} mbar vacuum, 200 Hz trigger frequency, and the length of the linear flight path was 1.8 m. An average of 0−500 laser shots was accumulated for each spectrum. In order to obtain the best signal in terms of sensitivity and resolution, both ion mode and operation mode were tested. Linear positive-ion mode was better matched with the requirements; thus, it was used for MS sequencing. At the same time, the extraction delay times from 100 to 700 ns were examined for data acquisition, with 200 ns generating the best mass resolution and signal-to-noise ratio. Therefore, the extraction delay with 200 ns was used throughout the experiments. The instrument was internally calibrated using synthetic DNA of known molecular weight adjusting the m/z range of each analyte.

AA/NA/DHC matrix solution was prepared by dissolving 3.0 mg (0.02 mmol) of AA and 1.5 mg (0.01 mmol) of NA in 80 μL of acetonitrile/ultrapure water (5:3, v/v) and adding 6 μL of 10 mM DHC. The final molar ratio of AA, NA, and DHC was 2:1:0.006. The digest solution (1.0 μL) was mixed with 1.0−1.5 μL of the matrix solution and deposited onto a 384/400 μm Anchor Chip MALDI target plate (Bruker Daltonics, Billerica, MA, U.S.A.) immediately. The droplet was dried and crystallized in air.

Determination of Immobilized SVP Capacity. The amount of immobilized SVP in the bioreactor was determined using a Coomassie blue dye staining based Bradford assay.[27] Briefly, the bioreactor was treated by 0.1 N NaOH for 2 h at room temperature to release immobilized SVP completely. The released SVP solution (1.0 μL) was mixed with 100 μL of Bradford reagent (Bio-Rad, Richmond, CA.) and incubated at room temperature for 5 min. The absorbance of the formed SVP−dye complex was measured at 595 nm. By calibration with standard SVP solutions (0.01−0.24 μg/μL) the amount of the immobilized SVP was calculated.

SVP Activity Assay. The enzyme activity of the immobilized SVP (1.82 mg/mL) in the capillary bioreactor was evaluated by its hydrolysis of the substrate *p*-nitrophenyl thymidine-5′-phosphate (NTP). A solution of 5 mM NTP in 110 mM Tris−HCl buffer (pH 8.9) was injected into the capillary bioreactor for the substrate hydrolysis of 0.5, 1.0, 2.0, 3.0, and 4.0 min. The solutions were then pushed out, and the corresponding absorbance was measured at 400 nm using a NanoDrop 2000 spectrophotometer (Thermo Scientific Inc., Bremen, Germany). The enzyme activity of free SVP solution was also determined by the hydrolysis of 5 mM NTP in 110 mM Tris−HCl buffer (pH 8.9).

K^+ Measurement. The concentration of K^+ was measured using inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 2000DV, Perkin Elmer, Massachusetts, USA) analysis. First, a calibration curve was established using K^+ standard, and the buffer of 25 mM DHC−NH_4OH (pH 9.4) was used as the blank. Then, the K^+ in SVP solution (prepared in blank buffer) was measured and the corresponding concentration was calculated using the calibration curve.

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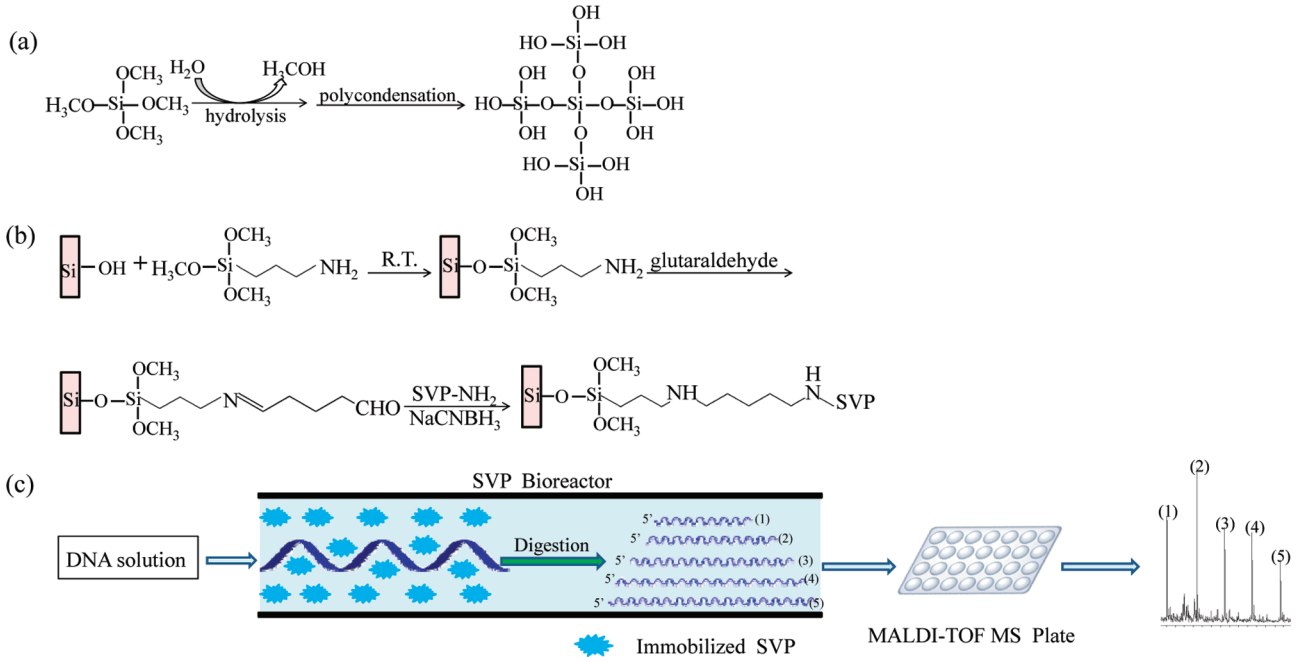
Scheme 1. Illustration of the Construction of the Capillary Monolith Bioreactor of SVP[a]



[a](a) Synthesis and the formation of silica monolith matrix; (b) the immobilization of SVP in the porous monolith; (c) the off-line coupling of the constructed capillary bioreactor with MALDI-TOF MS.

RESULTS AND DISCUSSION

Preparation and Characterization of the Immobilized SVP Bioreactor. The construction of a capillary monolithic bioreactor of SVP is illustrated in Scheme 1. First, a porous monolith in a fused-silica capillary was prepared through the polymerization of TMOS. Second, the synthesized silica monolith was modified with 3-aminopropyl-trimethoxysilane to introduce an amino group, which is further modified by the bifunctional glutaraldehyde, providing an appropriate arm and a reactive aldehyde group for SVP immobilization. Finally, the SVP was introduced into the monolithic capillary and was immobilized in situ. The constructed capillary bioreactor of SVP can be off-line-coupled with MALDI-TOF MS and is applicable to the oligodeoxynucleotide sequencing (Scheme 1).

The synthesized silica monolith in a fused-silica capillary (i.d. 75 μm) was imaged using scanning electron microscopy (SEM). The SEM imaging analysis shows the formation of a rigid skeleton and the even distribution of macropores within the capillary monolith (Figure 1). Due to the large porosity, the capillary reactor has low back pressure and good permeability. Benefiting from its good permeability, DNA solution can be directly injected into capillary monolithic reactor by hand pushing or a low-pressure microinjection pump.

The immobilization of SVP on the capillary monolith was characterized by fluorescence imaging. The immobilized protein was first stained by SYPRO orange dye (1:5000), and the stained capillary became fluorescently visible when excited by blue light (450–490 nm). The capillary monolith with the immobilized SVP shows strong fluorescence throughout the capillary (Figure 2d), showing the even distribution of the immobilized SVP. In contrast, the capillary monolith without protein immobilization shows no visible fluorescence dot throughout the capillary (Figure 2b).

The amount of immobilized SVP that was measured using Bradford assay is about 4.0 μg in a 50 cm long capillary monolith (with a total volume of 2.2 μL). In a word, the immobilized capacity of SVP is about 1.82 mg/mL, showing a high immobilization capacity. By using NTP as a substrate, the enzyme activity of SVP was measured through monitoring the absorbance at 400 nm. Then, the specific enzyme activity can be derived through dividing the measured enzyme activity by the corresponding concentration. The estimated specific enzyme activity for the immobilized SVP is about 0.0110 Units/mg, which is 14-fold higher than that for the free SVP in aqueous solution (0.0008 Units/mg). This may indicate that the immobilization of SVP on silica monolith can enhance rather than reduce its enzyme activity. This has been observed with the immobilized trypsin.[28] The increase of enzymatic

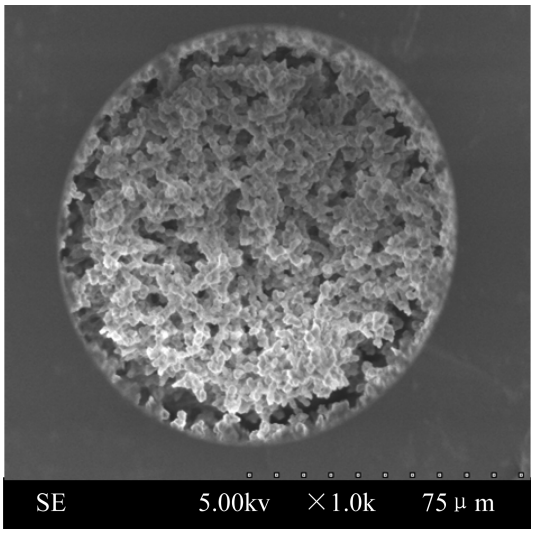


Figure 1. Scanning electron microscopic image of the monolith within the synthesized capillary bioreactor.

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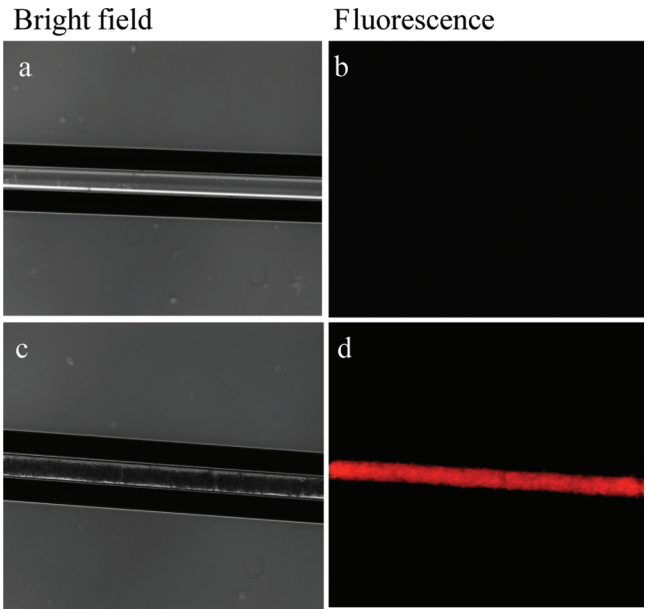

Figure 2. Bright field and fluorescence images of the capillary monolith without (a and b) and with (c and d) the immobilization of SVP. The capillaries were stained by SYPRO orange dye (1:5000) for 20 min and then rinsed by 25 mM phosphate buffer (pH 6.9) for 10 min to remove the excess protein-unbound dye molecules prior to imaging.

activity is probably attributed to the high local concentration of SVP in the bioreactor and enhanced stability.

Stability of the Immobilized SVP Bioreactor. We further investigated the stability and preserved activity of the immobilized SVP for repeated use. The preserved activity and stability of the immobilized SVP in one capillary bioreactor was qualitatively evaluated by its ability to generate the mass ladder from the digestion of a 22-mer oligodeoxynucleotide. The generated mass ladder was analyzed using MALDI-TOF MS under the same conditions. In between any two tests, the SVP bioreactor was stored at 4 °C. As shown in Figure 3a, for the immobilized SVP, the mass ladder can be generated, within 10 min, from the 3′-end (G) to the 19th nucleotide (G) on the first day, to the 17th nucleotide (T) on the fourth day and eighth day, and to the 16th nucleotide (A) on the 12th day until 30th day. With 30 min of digestion, the mass ladder corresponding to the sequence from the 3′-end to the 20th nucleotide (G) can be generated from the first to 30th day. It is evident that the enzyme activity of the immobilized SVP was well-maintained, rendering the SVP bioreactor suitable for a prolonged use. In contrast, the free SVP in solution, which can digest the oligodeoxynucleotide into dimers or trimmers by 30 min if freshly prepared, reluctantly cleaved two or three nucleotides from the whole oligodeoxynucleotide only after storage of 1 day at 4 °C (Figure 3b), suggesting the loss of enzymatic activity and the instability of free SVP in aqueous solution. It seems that the stability of SVP can be enhanced by immobilization in a porous monolith.

Repression of Metal Adducts for MS-Based Sequencing. It is known that the oligodeoxynucleotides could form metal adducts during ionization, mainly K^+ and Na^+ adducts. The formation of metal−oligodeoxynucleotide adducts significantly reduces the performance of MALDI-TOF MS analysis (e.g., detection sensitivity, mass resolution), deteriorating the quality of sequence reading.[29−31] To suppress the production of

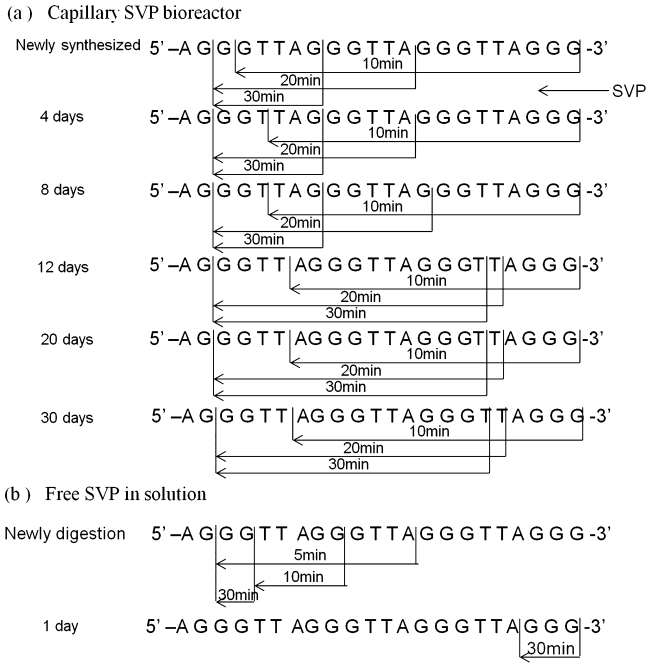

Figure 3. Qualitative assessment of enzymatic digestion of 22-mer by the immobilized SVP bioreactor after the storage of 4, 8, 12, 20, and 30 days (a) and by free SVP after the storage of 0 and 1 day (b). The buffer of 25 mM DHC−NH_4OH (pH 9.4) was used for storage and digestion. The SVP was stored at 4 °C. Arrows indicate the direction of enzymatic digestion and the produced mass ladder after the digestion for the indicated time.

metal adducts in MALDI-TOF MS analysis, one of the popular comatrixes,[12,21,23] DHC, was used together with AA and NA as the matrix for MALDI-TOF MS analysis. The ratio of AA/NA/DHC was maintained as 2:1:0.006. In such a matrix design, it has two advantages: AA can strongly absorb the light at the laser excitation wavelength (337 nm), whereas NA can cocrystallize well with oligodeoxynucleotides.[21]

Although the optimum matrix of AA/NA/DHC was used for MALDI-TOF MS analysis, K^+ adducts appeared with strong signal as shown in mass spectrum of the digest of 22-mer generated by free SVP in aqueous solution (Supporting Information Figure S1). The K^+ adducts were indicated by cross stars in all MS spectra. This observation may indicate that certain unknown metal K^+ sources may contribute to the formation of K^+−oligodeoxynucleotide adducts, probably due to the contamination of K^+ in SVP preparations. Indeed, K^+ (162.0 ppm) can be detected in the commercial product of SVP by ICP-OES analysis.

Compared with the unelimination of K^+ adducts in free solution digestion, the immobilization of SVP may facilitate the removal of the coexisted potassium in the SVP preparation. To this purpose, DHC was tested for the immobilization of SVP and the followed enzyme digestion.

Initially, the effect of the immobilizing buffer was investigated. Meanwhile, the buffer of 25 mM DHC−NH_4OH, pH 9.4 was chosen for the followed enzyme digestion. By keeping the immobilizing buffer at pH 6.9, the buffer of 25 mM DHC−NH_4OH can effectively reduce the signal of the K^+ adducts (Figure 4C), but the two buffers of 25 mM Tris−HCl and 25 mM phosphate increase the signal of K^+ adducts (Figure 4, parts A and B). Moreover, by adjusting the buffer of DHC−NH_4OH from pH 6.9 to pH 9.4, the K^+ adducts almost

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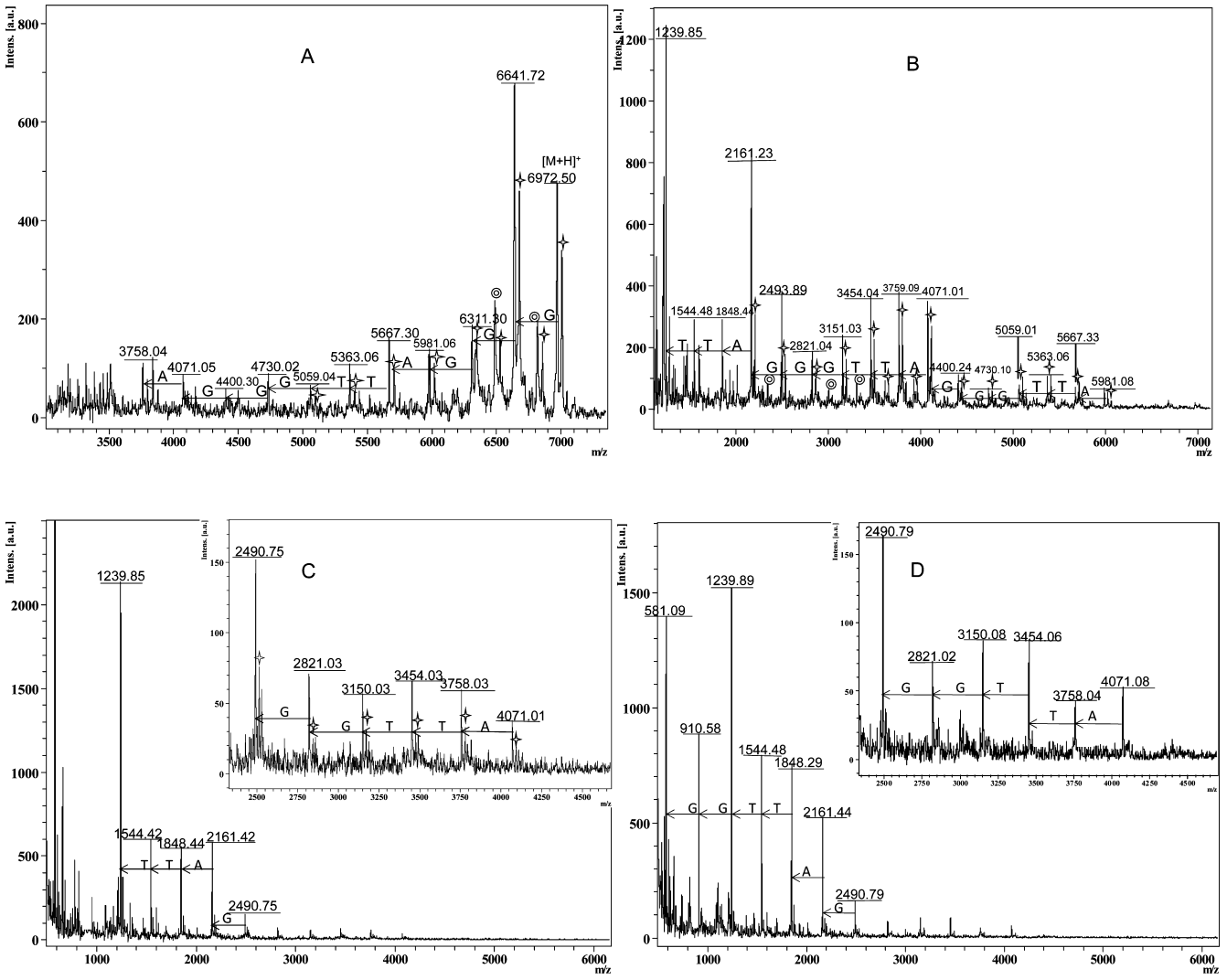

Figure 4. Effect of immobilizing buffer for the capillary SVP bioreactor on the MALDI-TOF MS analysis of digested 22-mer by the SVP bioreactor. The immobilizing buffer: (A) 25 mM Tris−HCl, pH 6.9; (B) 25 mM phosphate buffer, pH 6.9; (C) 25 mM, DHC−NH_4OH, pH 6.9; (D) 25 mM DHC−NH_4OH, pH 9.4. The 25 mM DHC−NH_4OH, pH 9.4 was kept as the digestion buffer. The cross stars and concentric circles indicate K^+−oligodeoxynucleotide adducts and unknown peaks, respectively.

disappeared (Figure 4D). These results consistently suggest the effective repression of K^+ adducts by DHC in combination of the SVP immobilization.

We further investigated the effect of the digestion buffer while the buffer of 25 mm DHC−NH_4OH (pH 9.4) was kept as an immobilizing buffer (Supporting Information Figure S2). Similarly, the buffer of DHC−NH_4OH (pH 9.4) displayed the best efficiency for the suppression of the signal of the metal adducts (Supporting Information Figure S2D). In contrast, the use of 25 mM Tris−HCl buffer (pH 6.9) and 25 mM phosphate buffer (pH 6.9) may increase the interfering signal of K^+ adducts (Supporting Information Figure S2, parts A and B).

Taken together, the buffer of DHC−NH_4OH (pH 9.4) is an optimum buffer for both the immobilization of SVP and the followed enzyme digestion in the capillary monolithic bioreactor, which can greatly suppress the signal of the K^+ adduct of the oligodeoxynucleotides in the mass spectrum. Interestingly, the use of the alkaline buffer (pH 9.4) will not compromise the enzyme activity of immobilized SVP (Figure 4, parts C and D).

As shown by MALDI-TOF MS analysis, the immobilized SVP in the capillary monolithic bioreactor under optimized conditions can efficiently digest an oligodeoxynucleotide of 22-mer (5′-AGG GTT AGG GTT AGG GTT AGG G-3′, m/z 6972.50) following the direction of 3′ → 5′ and the digested products are distributed as a series of the desired mass ladder with one nucleotide difference. The measured and calculated molecular weights of the digest of 22-mer are listed in Supporting Information Table S1. The accuracy values of the mass measurements are varied from 0.004% to 0.095%. Due to the distinct mass of the four nucleotides of dG (m/z = 329.20), dC (m/z = 289.20), T (m/z = 304.20), and dA (m/z = 313.20) in genomic DNA, the cleaved nucleotide can be accurately identified from the mass shift, and therefore, the corresponding sequence can be derived. The results clearly demonstrate the applicability of immobilized SVP to MS-based oligodeoxynucleotide sequencing.

Identification of Sequence-Specific Damage. Damage to DNA that can be caused by a variety of endogenous and exogenous factors (e.g., carcinogenic chemicals, ionizing radiation, and UV irradiation) is the key step to the

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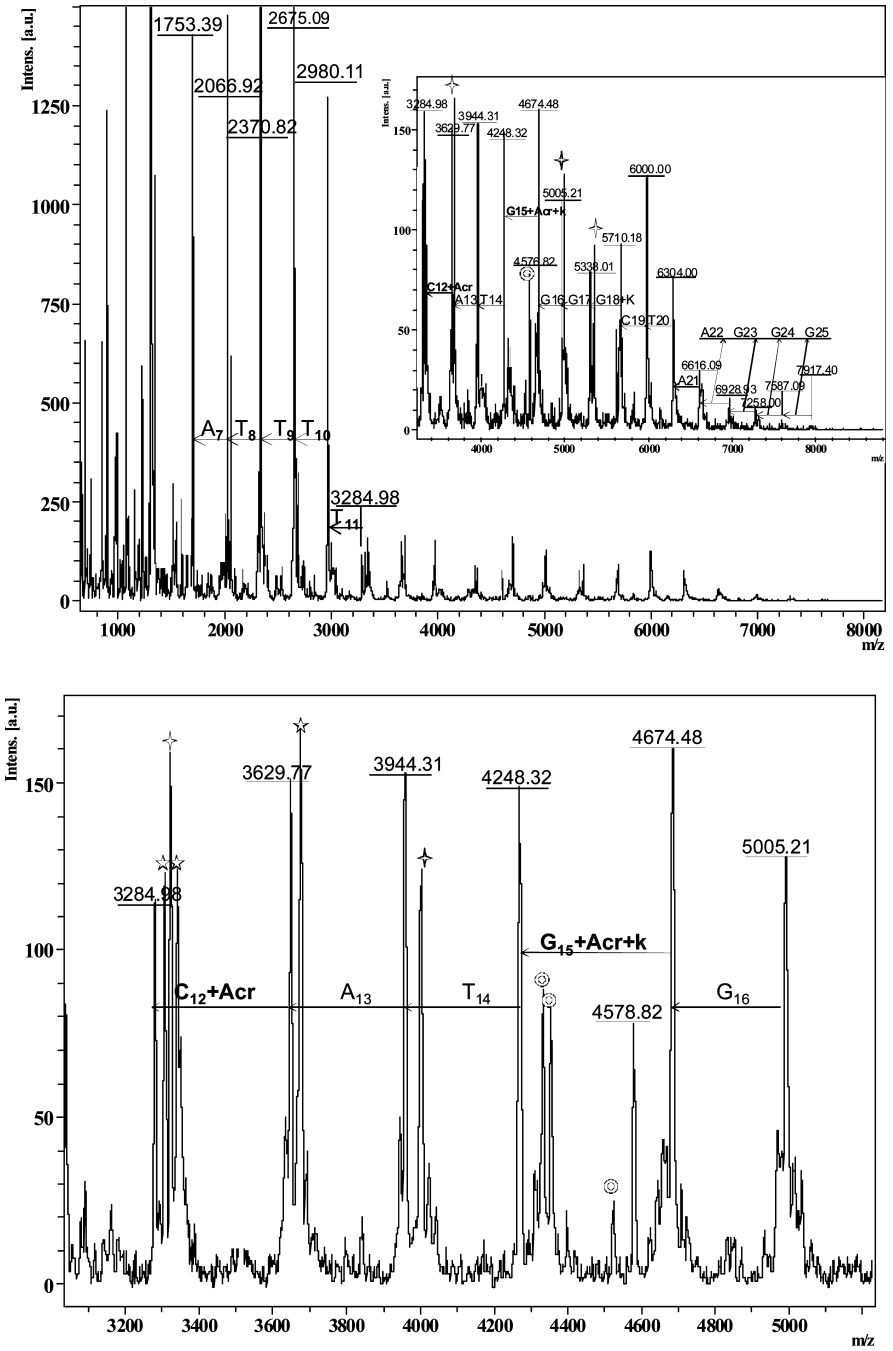

Figure 5. Sequencing of Acr−33-mer adducts by SVP capillary bioreactors coupled with MALDI-TOF MS analysis, showing the identification of base-specific and sequence-specific modification induced by Acr. The subscripts of nucleotides (e.g., C_{12} and G_{15}) were counted from the 5′-end. The cross stars, five-pointed stars, and concentric circles indicate K^+−oligodeoxynucleotide adducts, Na^+−oligodeoxynucleotide adducts, and unknown peaks, respectively. The Acr−33-mer adducts were directly obtained by incubation of 50 μM 33-mer with 1 mM Acr in 20 mM phosphate buffer (pH 7.4) at 37 °C over 48 h in the dark. The mixture was subjected to digestion and MALDI-TOF MS analysis without any purification.

mutagenesis and carcinogenesis.[32−34] Interestingly, different damaging agents may cause distinct sequence-specific mutation, which may be linked with the sequence-specific formation and repair of DNA damages.[35−37] Therefore, it is important to identify the sequence-specific damage for the understanding of the mutagenesis and carcinogenesis.

By the application of the constructed SVP bioreactor, we further demonstrate the applicability to determine the distribution and sequence specificity of the damage in single-stranded DNA using acrolein (Acr)−DNA adducts as an example. Acr is a major toxicant in cigarette smoke, with an amount of more than 1000-fold higher than that of polycyclic aromatic hydrocarbons (PAHs), and is proposed to make an important contribution to smoke-caused lung cancer.[38,39] Acr is a highly electrophilic α,β-unsaturated aldehyde and displays strong reactivity against all four normal nucleotides in DNA (dG,[40,41] dC,[42,43] T,[44,45] and dA[46,47]). By incubating a synthesized oligodeoxynucleotide of 33-mer (50 μM) with excess Acr (1.0 mM), a product of Acr−33-mer adducts was obtained. The product was subject to enzymatic digestion by the SVP bioreactor, and the formed fragments were further determined by MALDI-TOF MS analysis.

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Although the 33-mer (5′-GCC CTG ATT TTC ATG GGG CTA AGG GTC GCG GGA-3′) possesses all four nucleotides (13 dG, 7 dC, 5 dA, and 8T) and 20 times excess Acr was used for the reaction, only Acr−dG and Acr−dC adducts were observed in the adducted 33-mer (Figure 5 and Supporting Information Table S2). The Acr adducts of dG and dC were derived from their unique mass shifts of $\Delta m/z$ = 426.16 (theoretic mass of dG + Acr + K^+, 425.20) and $\Delta m/z$ = 344.79 (theoretic mass of dC + Acr, 345.20), respectively. The observation is consistent with previous mutation mapping of the *supF* gene showing that the base substitutions at G:C positions are the major type of mutations induced by Acr modification.[38,39] Interestingly, the Acr−dC adduct was only found at the 12th nucleotide and the Acr−dG was only observed at the 15th nucleotide (both counted from 5′-end), showing the preferential formation of Acr−dC and Acr−dG adducts in the respective sequence context of 5′-TC*A-3′ and 5′-TG*G-3′ (* represents the nucleotide that is being adducted). Coincidentally, this sequence preference is partly matched with the mutation mapping of the *supF* gene. The middle G of TGG is one of the mutational hot spots, and the middle C of TCA belongs to the few nucleotides that cannot form an Acr−dG adduct but cause mutation.[38,39]

CONCLUSION

By taking advantage of an in situ synthesized capillary monolith and enzyme immobilization, we demonstrate a reusable SVP bioreactor for MS-based DNA sequencing. Benefiting from the immobilization of SVP, metal adducts in the mass spectrum can be efficiently suppressed using DHC−NH_4OH (pH 9.4), improving the performance of MS sequencing. On the other hand, the immobilization rendering the SVP enhanced enzyme activity and stability. Furthermore, all the operations were performed in a single capillary with a 75 μm i.d., providing the possibility to handle microliter or submicroliter reaction volumes to generate a mass ladder for DNA sequencing. By the constructed capillary bioreactor of SVP and using Acr adducts as an example, we not only demonstrate the identification of the preferential bases for damage formation but also demonstrate the identification and mapping of DNA damage sites in the context of the DNA sequence.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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