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In-situ nanoelectrospray for high-throughput screening of enzymes and real-time monitoring of reactions

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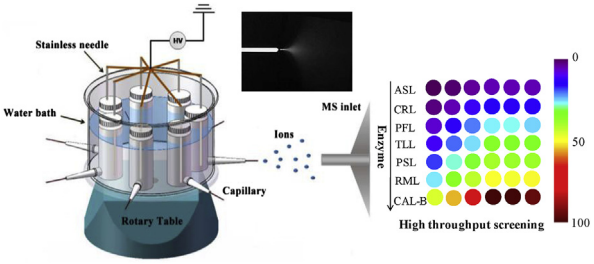
HIGHLIGHTS

- In-situ nanoelectrospray was designed for direct sampling and ionization for MS.
- It was fabricated by only inserting capillary into high-voltage applied liquid phase.
- Analytes in liquid reaction phase were directly extracted and ionized for MS.
- It achieved high-throughput enzyme evaluation and fast optimizations.
- It has been used for real-time monitoring of enzyme catalyzed reactions.

GRAPHICAL ABSTRACT



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ABSTRACT

The in-situ and high-throughput evaluation of enzymes and real-time monitoring of enzyme catalyzed reactions in liquid phase is quite significant in the catalysis industry. In-situ nanoelectrospray, the direct sampling and ionization method for mass spectrometry, has been applied for high-throughput evaluation of enzymes, as well as the on-line monitoring of reactions. Simply inserting a capillary into a liquid system with high-voltage applied, analytes in liquid reaction system can be directly ionized at the capillary tip with small volume consumption. With no sample pre-treatment or injection procedure, different analytes such as saccharides, amino acids, alkaloids, peptides and proteins can be rapidly and directly extracted from liquid phase and ionized at the capillary tip. Taking irreversible transesterification reaction of vinyl acetate and ethanol as an example, this technique has been used for the high-throughput evaluation of enzymes, fast optimizations, as well as real-time monitoring of reaction catalyzed by different enzymes. In addition, it is even softer than traditional electrospray ionization. The present method can also be used for the monitoring of other homogenous and heterogeneous reactions in liquid phases, which will show potentials in the catalysis industry.

1. Introduction

Compared with conventional chemical catalysts, enzymes offer several advantages, such as their environmentally friendly derivation from renewable resources, well biodegradation, typically stereoselectivity or substrate specificity, and the capability of operating under relatively mild conditions with respect to temperature or pH [1]. Efforts have been employed for achieving the evaluation of enzyme reaction with the low sample consumption [2,3]. However, due to the complexity of enzymes industry, including extensive engineering and optimization efforts, high-throughput screening (HTS) system is needed for efficiently screening processes [4]. Optical methods applied to HTS were almost based on changes of optical signals generated from gas phase reaction [5–9]. While many enzyme reactions are carried out in liquid phases, these gas phase-based methods are not suitable for HTS of enzymes or real-time monitoring of reactions, and could not

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provide molecular information of the products directly.

With the development of mass spectrometry techniques [10,11], ambient ionization methods for mass spectrometry have been developed rapidly, which enabled fast obtaining of molecular information from gas, liquid [12–14] or solid-phase samples [15–20]. For liquid samples, some techniques have been reported to directly ionize samples from the liquid phase, which would be significant for the enzyme evaluations. However, extractive electrospray ionization (EESI) [21,22], Venturi-easy ambient sonic-spray ionization (V-EASI) [23,24] as well as continuous-introduction ion-spray interface [25] need high-velocity (sonic) nebulizing gas to sample relatively high amount of analytes with relative low signal intensity. Segment flow ESI-MS [26–28] and probe electrospray ionization [29] have been reported the new strategies for the rapid and real time detection, the much simpler methods without driving pump or inserted probe are still encouraged. Low-temperature plasma (LTP) [30], glow discharge ionization [31] or dielectric barrier discharge ionization (DBDI) [17,32,33] are still not satisfactory for the in-situ monitoring of liquid catalytic reactions, because it could not provide reliable molecular changes below liquid level [34]. Therefore, some adaption is still needed for the high-throughput screening as well as the monitoring of enzyme catalyzed reactions with the small sample consumption.

Nanoelectrospray is extremely significant for the detection with low sample consumption, which has achieved analysis of samples with the assistance of solvent supplier [35–37], assisted gas flow [38] or other pathways [39]. Interestingly, without nebulizing gas, sampling and emitting of analytes can be obtained through a capillary inserting into fresh plants [40] or fruit slice on a spoon [41] under a high voltage. Similarly, spray consisting of the solvent can also be obtained from a short-tapered capillary tip, which can react with analytes desorbed from solid surfaces [42]. Therefore, the nanospray-based technique might be helpful for the on-line monitoring of reactions in liquid phases by simply inserting a capillary into the liquid reaction systems.

Here, an in-situ nanoelectrospray has been designed for the direct sampling and ionization of samples from liquid catalytic reaction system. Without the assistance of added solvent or assisted gas flow, this system is therefore applied for high-throughput evaluation of enzymes, fast optimizations as well as the on-line monitoring of reactions.

2. Experimental

2.1. Regents

Vinyl acetate (99%) was purchased from J&K Technology Co. Ltd. (Beijing, China). Ethanol (analytical grade) and D-glucose were supplied by Beijing Chemical Works (Beijing, China). The powdered enzymes were freeze-dried or immobilized on resins or silica gel. CAL-B (Lipozyme 435, immobilized on macroporous acrylic resin), RML (Lipozyme RM IM, immobilized on macroporous anion exchange resin), PSL (Lipase PS “Amano” IM), TLL (Lipozyme TL IM, immobilized on silica gel), PFL (Lipase AK “Amano”), CRL (Lipase AYS “Amano”), ASL (Lipase AS “Amano”) were obtained from novocata (Hangzhou, China). Cytochrome c and Angiotensin I were from Sigma (St. Louis, MO, USA). Berberine chloride hydrate and L-lysine was purchased from Aladdin (Beijing, China) and Beijing XinKeZhongjing Biological Technology Co. Ltd. (Beijing, China), respectively. 8-Quinolinol was from Beijing Xizhong Chemical Works (Beijing, China).

2.2. Instruments

All MS and MS/MS data were obtained by using a Thermo LCQ mass spectrometer (Thermo Scientific, San Jose CA). The capillary temperature was 200 °C, capillary voltage and tube lens voltage were set to 10 V and 75 V, respectively. The sheath gas flow rate is 8 arb. The distance between the capillary tip and MS inlet was 5–20 mm. Deionized water was supplied by Milli-Q water purification system (Millipore, Milford, MA). The electric rotation stage was purchase from Beijing Science & Technology development Billiton Limited (Beijing, China). For GC experiment, GC-14C gas chromatography (Shimadzu) was equipped with a glass column and a thermal conductivity detector. CS-Light Postrun Analysis was used for all GC analyses. 2 μL of the reaction liquid with 4 μL air was injected into gas chromatography. The conversion of vinyl acetate was calculated according to the peak area synchronously. The injector was kept at 100 °C, column temperature was set at 85 °C.

2.3. Fabrication of in-situ nanoelectrospray

As shown in Fig. 1A, a quartz capillary (50 μm i.d., 190–365 μm o.d., length: 2.50 cm) is inserted into an insulating Narrow-Mouth Bottle (Nalgene, USA) filled with the liquid sample. Liquid samples are extracted from the liquid system to the capillary tip under the capillary effect. A stainless needle is inserted into the liquid system with a high DC voltage (4.5 kV) applied. Then, a spray of charged droplets from liquid system is generated at the tip of the capillary toward the inlet of mass spectrometer for analysis. According to the inset of Fig. 1A, the stable spray at the capillary tip is clearly demonstrated. Fig. 1B shows the picture of the ionization set-up. As the simple-cut capillary could obtain the well ionization of samples, we used this simple-cut capillary for the following experiment.

3. Results and discussion

3.1. Fast ionization of samples from liquid phases

To evaluate the performance of the in-situ nanoelectrospray for liquid sample detection, different kinds of analytes including saccharides, amino acids, alkaloids, peptides and proteins have been detected (The optimizations are shown in Fig. S1, see Supporting Information). For the small molecules of L-lysine and 8-quinolinol, the molecular ions of $[M + H]^+$ (m/z 147 and m/z 146) can be easily obtained, whose signals are confirmed by tandem mass spectrum via collision-induced dissociation (CID) (Fig. 2A and B). The adduct ions such as $[M + NH_4]^+$ (m/z 198) and $[M + NH_4–H_2O]^+$ (m/z 180) can be detected for D-glucose with the confirmation of CID data (Fig. 2C), which is in accordance with the reports [43,44]. Furthermore, the radical molecular ion of M^{*+} (m/z 336) for berberine was also recorded (Fig. 2D). Taking the detection of angiotensin I (Fig. 2E) and cytochrome c (Fig. 2F) as examples, singly charged, doubly charged ions as well as cluster of multiply protonated molecules $[M + nH]^{n+}$ can all be detected for peptides and proteins. Therefore, the in-situ nanoelectrospray can be well used for the rapid detection of liquid samples.

3.2. Effect of sampling height on reaction monitoring

Considering the in-situ nanoelectrospray is the extraction of analytes from the liquid system, sampling at different heights might have effect on in-situ and on-line monitoring of the reaction in the liquid system. Thus, three in-situ nanoelectrospray systems with different sampling heights (1.0 mm below the liquid level, middle of the liquid phase, 1.0 mm above the bottle bottom) were fabricated for comparison. In the study, an irreversible transesterification reaction of vinyl acetate and ethanol catalyzed by immobilized lipase was selected as a model, whose temperatures were controlled by the water bath (the reaction is shown in the inset of Fig. 3A). During the reaction, the enzyme powder (1.0–5.0 mg) was