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High-throughput detection of drugs binding to proteins using desorption electrospray ionization mass spectrometry

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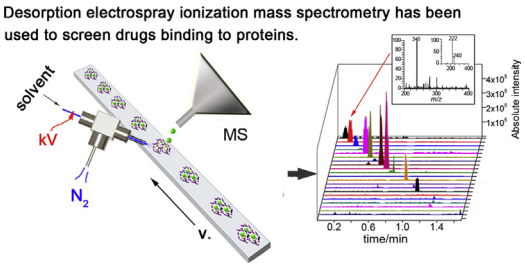
HIGHLIGHTS

- DESI-MS was applied to screen drugs binding to proteins.
- Relative binding affinities of Topo I inhibitors for Topo I were determined.
- High-throughput screening of 21 drugs binding to HSA was achieved within 1.75 min.
- Impact of spray solvent composition on the DESI process was studied.

GRAPHICAL ABSTRACT



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ABSTRACT

In this paper, we present a strategy for screening drugs that bind to proteins by combining centrifugal filtration with desorption electrospray ionization mass spectrometry (DESI-MS). Membrane filtration was used to remove any unbound drugs. Then, drug–protein complexes deposited on the DESI substrate were dissociated during the DESI-MS analytical process, and the liberated drugs were measured. To validate the methodology, the screening of a series of drugs against two types of proteins was performed. Three DNA topoisomerase I (Topo I) inhibitors (camptothecin (CPT), hydroxycamptothecin (OHCPT) and 7-ethyl-10-hydroxycamptothecin (SN-38)) were screened against Topo I and the DNA-Topo I complex using DESI-MS. The results indicated that none of the inhibitors bound to Topo I, because the inhibitors had binding affinities only to the DNA-Topo I complex. Among the three drugs that bound to the DNA-Topo I complex, SN-38 had the strongest relative binding affinity, and CPT had the weakest relative binding affinity. The impact of the DESI spray solvent composition on the analysis of drug–protein complex binding was evaluated. Seven alkaloid drugs were also screened against Topo I using DESI-MS. Berberine and palmatine had good binding affinities. A screening of 21 types of drugs was carried out to determine whether the drugs bound to human serum albumin (HSA). The DESI-MS screening process could be achieved within 1.75 min. The study provides a method to qualitatively detect compounds that bind to proteins, showing great potential in drug design and screening.

1. Introduction

Many small molecules, including synthetic and natural organic molecules, are important agonists or antagonists of specific biological targets. Most small molecule drugs take effect by interacting with biological molecules. An essential property for a drug candidate is to bind to appropriate biomacromolecules such as proteins [1]. Simple drug candidate–protein interaction systems include drug–plasma protein interactions and drug–target protein interactions [2,3]. For the pharmaceutical industry, it is important to gain a rapid understanding of drugs that have affinity for a particular protein target [4]. Developing a method of rapidly and

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sensitively screening drugs and drug-like small molecules binding to proteins is necessary.

A variety of studies have been performed to investigate the binding of compounds to proteins, mainly including surface plasmon resonance (SPR) [5], X-ray crystallography [6], calorimetric methods [7], fluorescence-based detection technologies [8] and mass spectrometry (MS) methods [9–11]. SPR provides affinity and kinetic data. X-ray crystallography provides the atomic-scale structural information of protein–ligand complexes. The main calorimetric method is isothermal titration calorimetry, which yields thermodynamic information for drugs binding to proteins. Fluorescence-based detection technologies, such as fluorescence intensity and fluorescence polarization, are widely used for high-throughput screening analyses because of their high sensitivity. Mass spectrometry is a highly selective and high-throughput analytical technique that plays a key role in nearly every stage of the drug development process [12]. Electrospray ionization mass spectrometry (ESI-MS) [9,13] and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) [14,15] are conventional mass spectrometry methods that have been widely used in the drug–protein binding analysis. ESI-MS is capable of releasing the intact drug–protein complex from its native solution state into the gas phase in the form of multiply charged ion. Special care must be taken with the source orifice potential and the solution pH and organic cosolvents must be avoided. MALDI-MS, a powerful tool for high-throughput screening, has led to significant advances in mass spectrometry for the direct characterization of biopolymers and their complexes. Hannewald et al. have studied drug–protein binding by MALDI-MS and centrifugal filtration [16], a new method for screening drugs binding to proteins. Although a matrix is necessarily required for MALDI-MS, the technique is still mature and sensitive.

Desorption electrospray ionization (DESI), a type of ambient ionization technique for MS, is used for surface sampling [17–21] and high-throughput analysis [22,23]. DESI allows for the direct analysis of trace amounts of sample under ambient conditions. The major advantage of DESI-MS is that it can analyze samples on surface. No matrix is needed can reduce the time-consuming sample preparation and avoid the disturbing of matrix in the detection. Besides, soft ionization, high-throughput and molecular specificity are the main characteristics of DESI-MS [24]. Many studies have reported drug detection by DESI-MS in different sample matrices [25–27]. Nielen et al. analyzed the antibiotic enrofloxacin captured on an immunoaffinity surface-plasmon resonance chip by DESI-MS [28,29], which was the first time drugs binding to proteins had been measured by DESI-MS. DESI-MS may therefore be a potential technique for the prescreening of drug-like molecules binding to biological targets.

In this study, DESI-MS was applied to the screening of drugs binding to proteins. Drug–protein complex was deposited on desired substrate in an array type. Drugs were dissociated and liberated from the complex when the DESI screening across the samples. Since surface sampling is the advantage for DESI-MS. The screening is rapid and high-sensitive. The proteins we focused on in this study were DNA topoisomerase I (Topo I) and human serum albumin (HSA). Three Topo I inhibitors (camptothecin (CPT), hydroxycamptothecin (OHCPT) and 7-ethyl-10-hydroxycamptothecin (SN-38)) were screened by DESI-MS after they interacted with Topo I, pUC19 DNA-Topo I complex and pUC19 DNA, respectively. The results showed that DESI-MS could qualitatively determine whether a drug binds to a target protein. At the same time, the relative binding affinities of the three inhibitors for pUC19 DNA-Topo I complex were also discussed. We also applied this method to screen seven other alkaloid drugs against Topo I. The result showed that berberine and palmatine had good binding affinities to Topo I. Rapid screening was achieved by screening 21 types of drugs against HSA. DESI-MS was therefore demonstrated to be a useful tool for rapidly and efficiently detection of compounds binding to proteins.

2. Experimental

2.1. Reagents and materials

All reagents were of analytical reagent grade. The commercially available drug compounds selected for this study were all purchased from Aladdin Reagent Company (Beijing, China). DNA topoisomerase I was purchased from SinoBio Biotech Ltd. (Shanghai, China). pUC19 DNA was purchased from Thermo Scientific Fermentas (Vilnius, Lithuania). The purity of human serum albumin purchased from Dingguo Biotechnology (Beijing, China) is 96–98%. Methanol and acetonitrile (analytical grade) were purchased from Beijing Chemical Factory (Beijing, China). Water was deionized and further purified with a Milli-Q water purification system (Millipore, Milford, MA). The nitrogen (99.999%, ultra high purity) purchased from Qianxi Gas Co. Ltd. (Beijing, China) was used as a desolvation gas. For the separation step, we utilized the Amicon Ultra-0.5 Centrifugal Filter Unit (0.5 mL) with Ultracel-10 membrane (Millipore, MA, USA) with a mass cutoff of 10,000 Da. The centrifugation equipment is an ultracentrifuge with the maximum number of revolutions of 14,800 rpm (Sartorius AG, Germany).

2.2. Desorption electrospray ionization mass spectrometry

MS experiments were performed using a homemade DESI ion source with an automatic sample moving stage coupled to a Thermo LTQ linear ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA) operated in the positive ion mode. The moving stage, MS inlet and desorption electrospray ionization source were shown as parts a, b and c in Fig. S-1A. The automatic moving stage can move at different rates (from 0 mm s^{-1} to 2 mm s^{-1}) in the *XY* directions, contributing to the rapid screening.

The DESI ion source utilized in this study was prepared as previously reported [30], and its operating parameters were as follows: spray voltage, 4 kV; solvent flow rate, 3 μL min^{-1}; nebulizing gas (nitrogen, 99.999%) pressure, 100 psi; incident angle α, 50°; incident angle β, 5°; tip-to-surface distance (d_1), 3 mm; inlet-to-surface distance (d_2), 1 mm and tip-to-inlet distance (d_3), 4 mm as displayed in Fig. S-1B. The DESI spray solvent was 1:1 (v/v) methanol/water. The instrument was set to a maximum ion trap injection time of 50 ms with 2 microscans per spectrum. Samples were deposited on a plain glass slide. Measurements were carried out by continuously scanning the DESI spray across the surface of the glass slide at different sampling rates. The data were acquired via the Xcalibur software.

2.3. Binding of inhibitors to DNA-Topo I complex and their screening

In the first stage, different inhibitors (CPT, OHCPT and SN-38) were incubated with Topo I and pUC19 DNA at 37 °C for 30 min in a polyethylene test tube. Binding reactions were prepared by mixing 2.5 μg of pUC19 DNA, 20 μL of Topo I containing 5 units of Topo I in its buffer [20 mM Tris–HCl (pH 7.9), 50 mM NaCl, 50 mM KCl, 0.01 mM EDTA, and 1 mM dithiothreitol], 2 μL of 5 mM inhibitors and 150 μL of ultrapure water. One unit of Topo I activity was defined as the amount of enzyme required to catalyze more than 95% of 0.5 μg of pUC19 DNA (negative supercoiling) in a volume of 25 μL, 37 °C for 25 min. Subsequently, a centrifugal filter unit with a mass cutoff of 10,000 Da was used to remove unbound drugs from those binding to the pUC19 DNA-Topo I complex as previously reported [16,31]. The sample was added