Noncovalent electrospray ionization mass spectrometry: a powerful tool in drug discovery

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Introduction

The vast number of new drug candidates produced by high throughput chemistry (HTC) requires fast and efficient methods both for characterization of the individual compounds and their affinity to target molecules. One way to accelerate drug discovery and thus decrease the time for the overall drug development process is to perform drug screening at an early stage of the process. A prerequisite for such an approach is the availability of sensitive analytical methods.

The advantages of using electrospray mass spectrometry (ESI-MS) for analysis is performed with or without microdialysis device. Many proteins easily form metal adduct ions which impair their analysis by MS. The microdialysis device eliminates unwanted adducts with enhance sensitivity and interpretation of spectra as shown in Figure 2.

Noncovalent electrospray ionization mass spectrometry (ESI-MS) has successfully been introduced at Biovitrum as a powerful routine based screening tool in drug research. The principle is simple, the analyses is fast and consumes low amount of target protein. Protein and ligand are mixed prior to injection into the MS-instrument. Binding is confirmed by detecting the noncovalent protein-ligand complex in the gas-phase.

\[ P + L \rightleftharpoons PL \]

Ligand screening

Depending on the nature of the sample, analysis is performed with or without a microdialysis device. Many proteins easily form metal adduct ions which impair their analysis by MS. The microdialysis device eliminates unwanted adducts with enhance sensitivity and interpretation of spectra as shown in Figure 2.

Chip-based ligand screening

The core of the system consisted of a chip-based platform for automated sample delivery from a 96-well plate (Advion Bioscience) and subsequent analysis by nano-ESI based on non-covalent interactions.1

Samples are introduced to the reservoir inlet of the chip using conductive pipette tips.

- Sample capacity approximate 430 samples/day.
- Protein consumption in the range of 100 picomoles/sample.

Fig. 1. Ligand screening instrument set-up with microdialysis device

Fig. 2. ESI mass spectra of horse heart myoglobin (27 µM) with NaCN solution added to give a final concentration of 0.05 µM (a) without the on-line microdialysis, and (b) with on-line microdialysis.

Determination of dissociation constants

We are using a novel approach for the calculation of the dissociation constants of weakly bound complexes. The method is based on competitive binding using a dose-response titration followed by calculation of \( K_d \) from a non-linear regression curve.

For more details see poster number: ThC-138 by A. Tjernberg et al.

Conclusions

- Noncovalent ESI/MS has had great impact in many research projects, especially in projects where other screening methods were difficult to develop.
- Automatic nano-ESI/MS has great potential to serve as a complementary screening method to conventional HTS. Alternatively, it could be used as a first screening method in an early phase of drug development programs, where only small amounts of purified protein are available.
- Determinations of dissociation constants of protein-ligand complexes are done on routine basis using a novel approach.

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