Monitoring Protein-Drug Interactions with Mass Spectrometry and Proteolysis Protein Mass Mapping as a Drug Assay

J. Kathleen Lewis†, Jay Chiang, and Gary Siuzdak*
†Kratos Analytical, Inc., 100 Red Schoolhouse Rd., Building A, Chestnut Ridge, NY 10977
and
The Scripps Research Institute
10550 North Torrey Pines Road
La Jolla, CA 92037

* to whom correspondence should be addressed (siuzdak@scripps.edu).

ABSTRACT

Presented is a new approach for assaying the effects of drug candidates against protein targets. Human rhinovirus, HRV14, has been analyzed in the presence of both known and potential antiviral drug agents using enzymatically active mass spectrometer sample plates. The combination of automated mass spectrometry with proteolytic digestion provides for the rapid, high-throughput, and sensitive screening of protein-drug interactions. Multiple drug candidates can be assayed from MALDI sample plates containing 100 wells and, to further increase the number of drugs assayed, multiple drugs can be pooled into each solution (10 candidates per well, 1000 candidates per plate). Wells producing spectra indicative of drug activity can be further analyzed to discern the active compound or be tested with alternative assays.

INTRODUCTION:

The rapid growth of combinatorial libraries has created a need for faster, more accurate, and more sensitive analytical techniques capable of large-scale (high-throughput) screening. Numerous improvements in speed, sensitivity and accuracy, together with innovations in both automation and quantitation place mass spectrometry among the most powerful analytical techniques available today. Crucial to distinguishing the most active component or obtaining structure-activity relationships of compounds in a library is an efficient assay. Toward this end, electrospray ionization and matrix-assisted laser desorption/ionization have been useful for both qualitative[1-3]and, more recently, the quantitative screening of combinatorial libraries[4].

The combination of mass spectrometry with protease mapping[5] has recently been used to examine the binding effects of the antiviral agent WIN 52084 on the structure of the human rhinovirus (HRV14). HRV14, the causative agent of the common cold[6], consists of a protein shell or capsid surrounding an RNA core. WIN 52084 is a member of a family of experimental drugs designed to inhibit the lifecycle of picornaviruses[7]. In these studies viral-protein digestion was dramatically inhibited by the presence of the antiviral agent WIN 52084 (**figure 1**), while digestion of other viral systems was not inhibited (indicating that interaction of the inhibitor with the virus HRV14, and not the enzyme, caused the inhibition). Based on these results and other evidence it is believed that binding of WIN 52084 to HRV14 inhibits capsid dynamics[6,8].

This protein mapping approach provides a rapid and sensitive mass-based assay for screening libraries of potential antiviral agents. Those compounds which show anti-viral potential (very little to no viral-protein digestion), can be further tested for activity or modified to improve their activity. The conventional assay means of screening for antiviral drug activity is the plaque assay, an assay that requires significantly more time (days) than does the mass mapping approach[10]. Ultimately, plaque assays must be performed to confirm antiviral activity, yet the protein mass mapping method acts as a preliminary screening procedure which can significantly reduce the number of drugs that must undergo the plaque assay. As an automated mass spectrometric method for screening libraries of antiviral agents, protein mass mapping offers a novel and viable alternative.

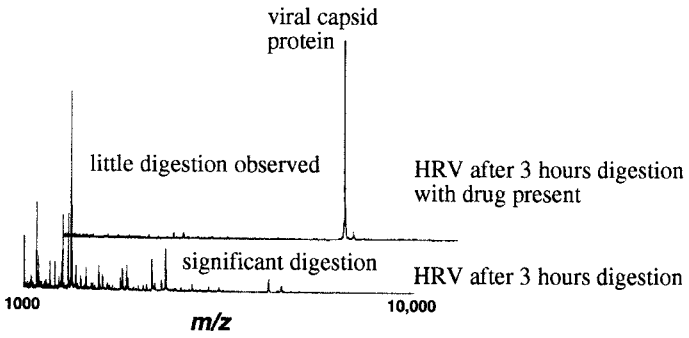

Figure 1. Mass spectra resulting from a 3 hour on-plate digestion of 4 femtomoles of HRV14 in the presence (top) and absence (bottom) of WIN52084. The presence of the WIN compound is found to stabilize the virus toward trypsin digestion. Inset is an electron micrograph of HRV (J. Struct. Biol., 1997, 120, 11).

EXPERIMENTAL:

HRV14 was prepared as previously described[10] to a final concentration of 3mg/ml in 10mM TRIS buffer at pH 7.6. On-plate trypsin digests were performed at 25° C in a high humidity environment. Aliquots of 1.0 µL of the digest solutions containing 25uM drugs (10 drugs per digest solution for a 250 µM total drug