Ricin Activity Assay by Direct Analysis in Real Time Mass Spectrometry Detection of Adenine Release

U.S. Army Edgewood Chemical Biological Center, 5183 Black Hawk Road, Aberdeen Proving Ground, MD, 21010-5424

Approved for public release; distribution unlimited

Report Documentation Page

1. REPORT DATE
   2009

2. REPORT TYPE

3. DATES COVERED
   00-00-2009 to 00-00-2009

4. TITLE AND SUBTITLE
   Ricin Activity Assay by Direct Analysis in Real Time Mass Spectrometry Detection of Adenine Release

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHOR(S)

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
   U.S. Army Edgewood Chemical Biological Center, 5183 Black Hawk Road, Aberdeen Proving Ground, MD, 21010-5424

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR’S ACRONYM(S)

11. SPONSOR/MONITOR’S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
   Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:

   a. REPORT
      unclassified

   b. ABSTRACT
      unclassified

   c. THIS PAGE
      unclassified

17. LIMITATION OF ABSTRACT
   Same as Report (SAR)

18. NUMBER OF PAGES
   3

19a. NAME OF RESPONSIBLE PERSON

Standard Form 298 (Rev. 8-98)
Prepared by ANSI Std Z39-18
Ricin Activity Assay by Direct Analysis in Real Time Mass Spectrometry Detection of Adenine Release

Vicky L. H. Bevilacqua,⁎† J. Michael Nilles,‡,§ Jeffrey S. Rice,†,‡ Theresa R. Connell,†,§ Amanda M. Schenning,‡ Lisa M. Reilly,†,‡ and H. Dupont Durst†

U.S. Army Edgewood Chemical Biological Center, 5183 Black Hawk Road, Aberdeen Proving Ground, Maryland 21010-5424, and SAIC, Gunpowder Branch, P.O. Box 68, Gunpowder, Maryland 21010-0068

Biotoxin activity assays typically involve multistep sample preparation, multicomponent reactions, multistep analysis, or a combination thereof. We report a single-step, real-time ricin activity assay that requires little or no sample preparation and employs direct analysis in real time mass spectrometry. The release of adenine from the homogeneous substrate herring sperm DNA by ricin was determined to be 53 ± 2 pmol adenine per picomole of ricin per hour. This procedure can be readily adapted to any enzyme for which a reactant or product of low molecular weight (up to ~600) can be identified.

Biotoxin activity assays typically involve multistep sample preparation, multicomponent reactions, multistep analysis, or a combination thereof. We report a single-step, real-time ricin activity assay that requires little or no sample preparation and employs direct analysis in real time (DART) mass spectrometry (MS). Like the high-performance liquid chromatography (HPLC)–MS protocol reported by Hines et al.⁴ involving only ricin, substrate, and internal standard, our approach does not require multiple components, cell cultures, coupled enzyme reactions, radioactive materials, or chemical modification of the substrate.⁵–⁸ In addition, this procedure improves upon the HPLC–MS procedure in that no isotopic labeling of the internal standard is required, no introduction of ricin into the instrument occurs, and no quenching of the reaction occurs (i.e., monitoring is truly real-time). Furthermore, with little modification, the procedure could be applied to many possible enzymes.

Ricin is an enzyme of molecular weight above 60 000 composed of A- and B-chains of approximately equal molecular weights linked by a disulfide bridge. The B-chain binds to galactose moieties on the eukaryotic cell surface, thereby facilitating entry into the cell.⁹ Once inside the cell, the A-chain catalyzes cleavage at adenosine 4324 (in rat RNA) to release adenine.¹⁰ This action inhibits protein synthesis, leading to cell death. In addition to RNA, herring sperm DNA (hsDNA) is a substrate for ricin.¹¹ We chose to employ hsDNA for this assay because it is relatively stable and widely available. The procedure here applies to many possible enzymes.

The DART is an ion source used to analyze solids, liquids, gases, and solutions under ambient conditions.³ Mass spectra are collected within a few seconds. It is therefore well suited to time critical analyses such as enzyme activity studies. Mass spectral

Analytical Chemistry, Vol. xxx, No. xx, Month XX, XXXX  A
interpretation is minimal because little or no fragmentation occurs and the DART has been used for MS analysis of a wide range of polar and nonpolar molecules including peptides, organometallics, and chemical warfare agents.

Experimenatal Section

Reagents. Ricin (150 mM NaCl, 10 mM PO₄, 0.08% NaN₃, pH 7.8) was purchased from Vector Laboratories (Burlingame, CA) and the concentration determined (4.46 mg/mL) using an ε₂₈₀ of 1.434 L g⁻¹ cm⁻¹. The ricin Mₜ was ascertained based on nondenaturing sodium-dodecyl sulfate polyacrylamide gel electrophoresis using Kaleidoscope Prestained Standards and Tris-HCl gradient (4−20%) Ready Gels (Bio-Rad, Hercules, CA). The average Mₜ as determined from three gels was 65,000. Purified hsDNA (10 mg/mL) in DNase-free, RNase-free, distilled, deionized water was from Invitrogen (Carlsbad, CA). Adenine, cytosine, uracil, sodium acetate, and bovine serum albumin (BSA) were from Sigma-Aldrich (St. Louis, MO).

Activity Assay. The hsDNA (200 µg) was heated in a Fisher Scientific dry bath incubator at 95 °C for 5 min to shear the DNA, followed by cooling on ice for another 5 min. The sheared DNA was diluted with 10 mM ammonium acetate pH 4.7 containing follow by cooling on ice for another 5 min. The sheared DNA

Figure 1. Adenine concentration over time. • = reaction mixture containing 89 pmol of ricin with an initial concentration of 2 mg/mL hsDNA. ▲ = reaction mixture containing 88 pmol BSA and hsDNA. ■ = blank reaction mixture containing only hsDNA.

Figure 2. Adenine concentration over time for the initial 4 h in a reaction mixture containing 89 pmol of ricin and having an initial hsDNA concentration of 2 mg/mL.

(temperature = 180 °C, flow rate = 11.05 L/min). Temperatures above 200 °C resulted in hsDNA breakdown.

Linear Regression of Adenine Response. The response for adenine in the presence of 2 mg/mL hsDNA with uracil as an internal standard (892 µM) was linear over the range analyzed (2.9−740 µM, R² = 0.986). Herring sperm DNA produced a low-intensity interfering peak. The lowest adenine concentration (2.9 µM) evaluated could be readily detected above this peak with a signal‐to‐noise greater than 350.

Ricin Activity. Once linearity for adenine was ascertained, monitoring of adenine release from hsDNA by ricin was conducted. The reaction was followed for 50 h (Figure 1). The rate of adenine production was linear over the first 4 h and was determined to be 53 ± 2 pmol adenine/pmol of ricin/h (Figure 2). This value is on the same order as values of 70 pmol/pmol of ricin/h and 43 pmol/pmol of ricin A-chain/h obtained with hsDNA by Heisler et al. employing a colorimetric coupled enzyme assay and to ~60 pmol/pmol of ricin A-chain/h obtained with a synthetic 14-mer RNA by Hines et al. employing LC–MS. In comparison, the adenine concentration for hsDNA alone and for hsDNA in the presence of BSA (a nonenzymatic protein of similar molecular weight to ricin) was constant (Figure 1).

Conclusions

A new procedure using DART-MS for the determination of reaction rates of enzymes having low-molecular weight products or reactants has been demonstrated with the protein toxin ricin.

The procedure can be employed to measure reaction rates of an enzyme in the presence of an inhomogeneous substrate such as hsDNA. While it is impossible to predict the character of all possible samples, this method, like many other DART methods, is expected to be applicable to many complex or real-world matrixes. One complicating factor would be gross pre-existing sample contamination by adenine. In that event, a purification step may be necessary. The amount of ricin required (~90 pmol), on the order of 100 pmol rather than the 10 pmol required for the Hines et al. procedure, remains well below a level toxic to humans. The advantage of a very simple, straightforward measurement procedure combined with the potential for direct continuous measurement under inhomogeneous conditions presents an attractive alternative to reported assays.

ACKNOWLEDGMENT

We thank Drs. James A. Laramée and Rabih Jabbour for manuscript review, Dr. Steven R. Channel and Mr. Alan Zulich for administrative support, and the Defense Threat Reduction Agency for funding (Grant Program Element 0602384BP).

Received for review November 12, 2009. Accepted December 16, 2009.

AC9025972