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Development of a Novel GC/MS Method for the Detection of Nicotinamide and Activity of ADP-Ribosylating Toxins

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Abstract / Introduction

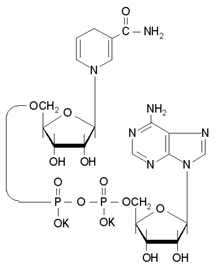

Figure 1 – Nicotinamide adenine diphosphate is broken by ADP-ribosylating toxins to release nicotinamide.

ADP-ribosylating toxin enzymes break NADH (Figure 1) and transfer the ADP-ribosyl group to a residue on a target protein, permanently inactivating or denaturing the protein. This activity is typically detected with a radioassay, which is expensive and requires radioactive materials.

ADP-ribosylation corresponds with the release of nicotinamide. It is possible to detect nicotinamide with a Gas Chromatograph/Mass Spectrometer (GC/MS) (Jacobson, Dame, Pyrek & Jacobson, 1995). The purpose of this study is to measure ADP-ribosylation activity using GC/MS by detecting the liberated nicotinamide. By derivatizing the nicotinamide, the detection limit was lowered to 0.5 ng/µl. Control measurements of ADP-ribosylation activity by a cholera toxin protein found low levels of nicotinamde contamination.

Materials

All GC/MS scans were performed on a HP GC/MS (Model 5890 gas chromatograph and 5972 mass selective detector with a 7673 GC/SFC auto-injector and a DB-5 column by J&W Scientific. Agilent Technologies, Inc.)

Abbreviations used:
BSTFA + TMCS: (N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane
Ctx: Cholera toxin

Nicotinamide Detection

Extensive testing was done to analyze the lowest concentration of nicotinamide that could be detected on a GC/MS.

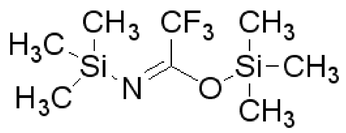

Figure 2 – BSTFA silanizing reagent

It was determined that optimum detection was found when the nicotinamide was derivatized with a silyl group (Figure 2) and dissolved in methylene chloride.

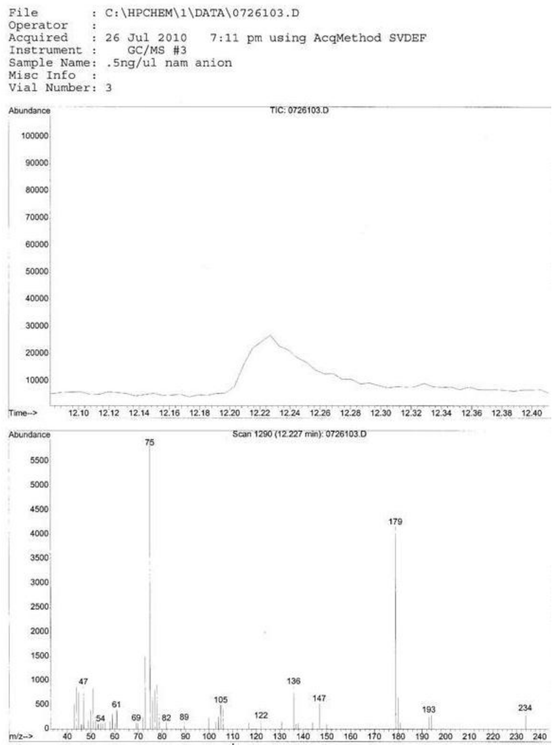

Figure 3 – Nicotinamide detection with standard volatiles method at a .5 ng/µl concentration.

Results/Discussion

Nicotinamide is detectable on the GC/MS with the proper solvent and derivatizing steps.

Nicotinamide detection can be improved by the use of selected ion monitoring methods

ADP-ribosylation detection

After developing a GC/MS system for the detection of low levels of nicotinamide, we began to pursue a method for the detection of ADP-ribosylation. Toxin protein and substrate concentrations used in the initial assay were based upon literature review (Moss, Maganiello & Vaughn, 1976) Cholera toxin (Ctx) was chosen as a model ADP-ribosyltransferase protein.

-Mix the toxin with the substrate in the correct quantities in a screw-top GC/MS target vial.
-Incubate RT 2 hr to allow reaction to proceed
-Dessicate in a drying oven at 100°C to remove water.
-Dissolve remnants in 150µL pyridine and derivatize with BSTFA. - Bring to 1 mL with methylene chloride.
-Analyze on GC/MS.

Results are compared in Figure 4.

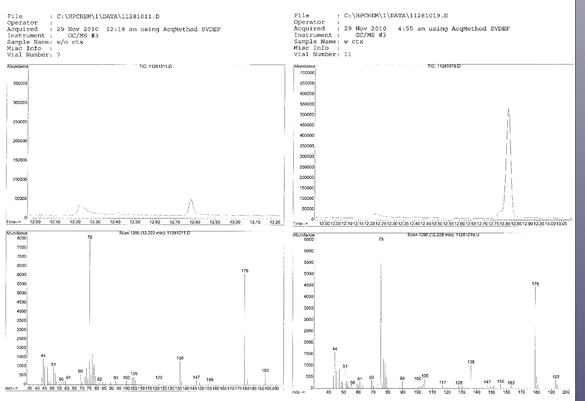

Figure 4 – A comparison of the GC/MS information. The image on the left is nicotinamide detected in a vial that did not contain Ctx. On the right is nicotinamide detected in a vial containing Ctx and NADH.

These results show that there is nicotinamide contamination in the control reaction. It appears the NAD may be hydrolyzing in the absence of enzyme or contaminated with nicotinamide.

Future Research

The method will be improved by determining and mitigating the source of nicotinamide contamination. Research is being conducted using higher purity NAD, as well as adjusting aspects of the procedure to reduce possible non-enzymatic hydrolysis of the NADH. Hopefully, these methods will reduce the level of nicotinamide present in the control reactions and allow for the measurement of low levels of nicotinamide release by ADP-ribosylating toxins.

The measurement of cholera toxin activity will serve as a model system for other ADP-ribosylating toxins.

GC/MS nicotinamide detection may be useful in a variety of clinical and laboratory applications, especially when a radioactive assay is not possible. Our goal is to provide a non-radioactive method for the detection and measurement of the activity of ADP-ribosylating toxins.

References

Jacobson, E. L., Dame, A. J., Pyrek, J. S., & Jacobson, M. K. (1995). Evaluating the role of niacin in human carcinogenesis. *Biochimie, 77*, 394-398.

Moss, J., Manganiello, V. C., & Vaughan, M. (1976). Hydrolysis of nicotinamide adenine dinucleotide by choleragen and its A protomer: Possible role in the activation of adenylate cyclase. *Proceedings of the National Academy of Science, USA, 73*, 4424-4427.

Thanks

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