



EDGEWOOD

RESEARCH, DEVELOPMENT & ENGINEERING CENTER

U.S. ARMY CHEMICAL AND BIOLOGICAL DEFENSE COMMAND

ERDEC-TR-373

**DETECTION OF CHOLERA TOXIN BY OPTICAL METHODS:
A MECHANISM-BASED APPROACH TO THE GENERIC DETECTION
OF PROTEIN TOXINS**

Ronald J. Young
Fu-Lien Hsu

RESEARCH AND TECHNOLOGY DIRECTORATE

April 1997

Approved for public release; distribution is unlimited.



19970731042

Aberdeen Proving Ground, MD 21010-5423

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

DEPARTMENT OF THE ARMY
U.S. Army Edgewood Research, Development and Engineering Center
Aberdeen Proving Ground, Maryland 21010-5423

ERRATUM SHEET

15 December 1997

REPORT NO. ERDEC-TR-373

TITLE DETECTION OF CHOLERA TOXIN BY OPTICAL
METHODS: A MECHANISM-BASED APPROACH
TO THE GENERIC DETECTION OF PROTEIN
TOXINS

AUTHORS Ronald J. Young, and Fu-Lien Hsu

DATE April 1997

CLASSIFICATION UNCLASSIFIED

Please remove the front cover from copies of ERDEC-TR-373 sent to you earlier in 1997 and attach the enclosed replacement cover. Previously printed covers were inadvertently printed with the incorrect activity name and logo.

Sandra J. Johnson

SANDRA J. JOHNSON
Chief, Technical Releases Office

19970721042

A327736

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE 1997 April	3. REPORT TYPE AND DATES COVERED Final, 96 Mar - 96 Sep		
4. TITLE AND SUBTITLE Detection of Cholera Toxin by Optical Methods: A Mechanism-Based Approach to the Generic Detection of Protein Toxins			5. FUNDING NUMBERS PR-10161101A91A	
6. AUTHOR(S) Young, Ronald J., and Hsu, Fu-Lien				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) DIR, ERDEC, ATTN: SCBRD-RTL, APG, MD 21010-5423			8. PERFORMING ORGANIZATION REPORT NUMBER ERDEC-TR-373	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) Methods to monitor the ADP-ribosyltransferase activity of cholera toxin fragment A were investigated. Ultraviolet spectroscopy was a suitable technique when the acceptor molecule contained a chromophore. Toxin catalyzed ADP-ribosylation of acceptor molecules, which do not contain a chromophore but do contain an amino group, can be monitored by labeling the acceptor molecule with a fluorescent probe after its ADP-ribosylation. The sensitive technique of fluorescence spectroscopy can be employed to monitor the action of cholera toxin without regards to the substituents on the acceptor molecule by use of eNAD, the fluorescent analogue of NAD.				
14. SUBJECT TERMS Toxin Spectroscopy Ultraviolet Optical Fluorescence ADP-ribosylation Cholera Biological detection ADP-ribosyltransferase			15. NUMBER OF PAGES 17	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL	

Blank

PREFACE

The work described in this report was authorized under Project No. 10161101A91A, In-House Laboratory Independent Research. This work was started in March 1996 and completed in September 1996.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release. Registered users should request additional copies from the Defense Technical Information Center; unregistered users should direct such requests to the National Technical Information Service.

Blank

CONTENTS

1.	INTRODUCTION	7
2.	METHODS	8
2.1	ADP-Ribosylation	8
2.2	UV Spectroscopy	8
2.3	Fluorescence Spectroscopy	8
2.3.1	Reaction with eNAD	8
2.3.2	Labeling with Fluorescent Probes	12
2.3.3	Reaction with NAD	12
2.3.4	Materials	12
3.	RESULTS	12
3.1	NBAG	12
3.2	eNAD	13
3.3	Labeling with Fluorescent Probes	14
4.	CONCLUSIONS	16
	LITERATURE CITED	17

FIGURES

1	Time Course of Reaction of NAD and NBAG	9
2	Time Course of ADP-Ribosylation of LAME and Agmatine with ϵ NAD and Cholera Toxin Fragment A	10
3	Time Course of Reaction of ADP-Ribosylation of LAME with Cholera Toxin Fragment A	11

TABLES

1	Retention of ϵ NAD by Dowex 1	13
2	Reaction of LAME and Agmatine with Fluorochromes	14
3	Sensitivity of Detection of LAME and Agmatine by Fluorescamine	15
4	Retention of LAME and Agmatine by Dowex 50	15

DETECTION OF CHOLERA TOXIN BY OPTICAL METHODS: A MECHANISM-BASED APPROACH TO THE GENERIC DETECTION OF PROTEIN TOXINS

1. INTRODUCTION

Biological detection systems currently fielded [e.g., Army Biological Integrated Detection System (BIDS) and the Navy Interim Biological Agent Detection System (IBADS)] are based on a highly specific interaction between the detector element and the suspect biological agent. These systems may be regarded as specific identification devices enabling the rapid identification of suspected biological agents. However, agents that are either structurally or immunologically unrelated to the detector element will not react and will escape detection by such a device. The number of biological materials (toxins and pathogens) of potential use as threat agents is quite large. A correspondingly large number of detection elements, each specific for one threat agent, will be required. However, this deficiency would be overcome by deploying a generic detection system able to recognize numerous toxins. A generic system would recognize a class of toxins rather than a specific toxin and may be preferred to a specific detection system to give early warning of the presence of a threat agent.

Toxins have been classified either according to their source (plants, bacteria, fungi, reptiles, amphibians, insects, and marine animals) or by the alteration in physiological functions they cause in infected animals.¹ Toxins grouped by these two criteria may be further organized on the basis of the mechanism by which they exert their toxic effects. A mechanism-based approach to toxin detection would produce a generic detection system capable of recognizing unrelated toxins from a variety of sources with different biological properties.

The threat agents cholera and diphtheria toxins² are two members of a family of 18 bacterial toxins with a similar action mechanism.³ All members of this family are composed of two subunits. The B subunit binds to the target cell and translocates the A subunit into the cell where it catalyzes the adenosine diphosphate (ADP)-ribosylation of a specific cellular protein, which is different for each toxin. Other members of this family include pertussis toxin, the C toxins of *Clostridium spp*, A toxin of *P. aeruginosa*, and the LT toxins of *E. coli*. The toxins can be further subdivided into four subtypes, depending on the amino acid in the protein that is ADP-ribosylated by toxin fragment A. *In vivo*, arginine is ADP-ribosylated by cholera toxin; but, *in vitro*, the amino acid itself, and some of its analogues can serve as the acceptor in place of the protein molecule.⁴⁻⁶ Incorporation of the *in vitro* reaction into a detection system would give early warning of the presence of either cholera toxin or its subtype. The *in vitro* reaction has been routinely monitored by radioisotopic methods.⁷ This report demonstrates that the reaction can be monitored by optical methods.

2. METHODS

2.1 ADP-Ribosylation.

The general conditions for the ADP-ribosylation reaction are as follows: cholera fragment A (20-40 $\mu\text{g/mL}$), either nicotinamide adenine dinucleotide (NAD) or nicotinamide 1, N^6 -etheno adenine dinucleotide (ϵNAD , 1-30 mM); acceptors [either agmatine, L-arginine methyl ester (LAME), or p-nitrobenzylideneaminoguanidine (NBAG), 2-60 mM]; DTT (20-30 mM); and phosphate buffer (pH 7.4, 150-200 mM) in a final volume of 125-250 μL . Incubation was at either 23 $^{\circ}\text{C}$ or 33-35 $^{\circ}\text{C}$. At 0, 0.25, 0.5, 1, 2, 4, and 6 hr, an aliquot was removed either for observation by either fluorescence or ultraviolet (UV) spectroscopy or measurement of emission intensity after reaction with either a fluorochrome, fluorescamine, or o-phthaldehyde/mercaptoethanol (OPA/ME). The blank did not contain fragment A. Conditions for experiments with individual ADP-ribosyl acceptors are given in the legends to Figures 1, 2, and 3.

2.2 UV Spectroscopy.

p-Nitrobenzylideneaminoguanidine was prepared as described by Soman and co-workers.⁸ Absorption maxima at pH 5, 7, and 10.8 were 316 nm ($\xi = 2.56 \times 10^4$), 317 nm ($\xi = 2.86 \times 10^4$), and 370 nm ($\xi = 1.8 \times 10^4$), respectively. Spectra were obtained with a Beckman (Columbia, MD) DU 640 spectrometer. The reaction mixture (110 μL) was placed in one compartment of the Beckman microcell, and the blank was placed in the adjacent compartment. The microcell was covered with parafilm and placed in a moist chamber at room temperature. At 0, 0.25, 0.5, 1, 2, 4, and 6 hr, spectra were taken. The reaction was followed by the difference in absorption at 370 nm.⁸

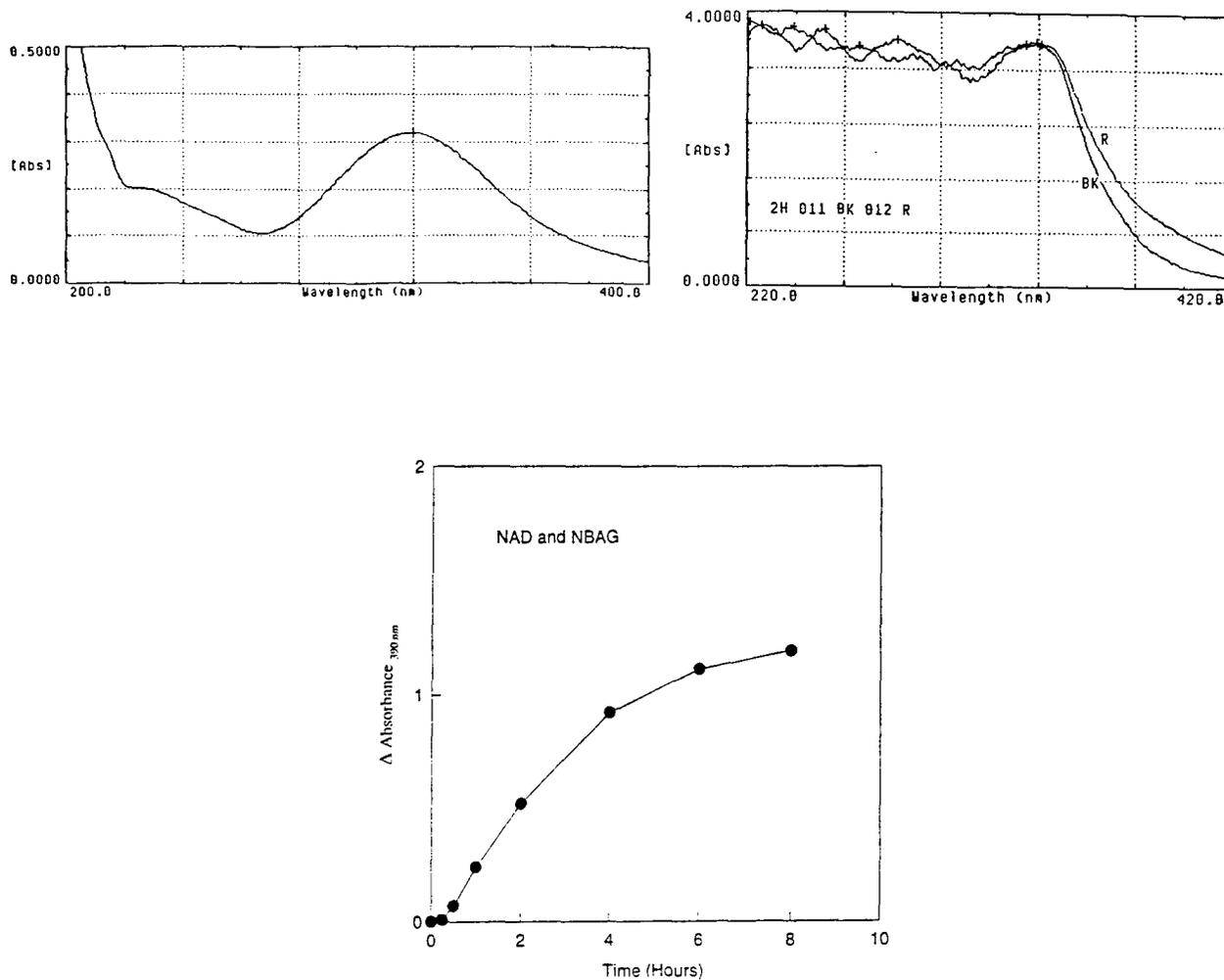
2.3 Fluorescence Spectroscopy.

ADP-ribosylation was followed either by reaction with the fluorescent analogue of NAD or by labeling of the acceptor with a fluorescent probe.

2.3.1 Reaction with ϵNAD .

Dowex 1 x 2-100 ion exchange resin was washed with 1M NaOH, water, 1M HCl and water until neutral, and stored under water. The resin was rinsed with water and blotted dry with tissue before use.

Reaction was carried out with agmatine and LAME. At 0, 0.25, 0.5, 1, 2, 4, and 6 hr, either 2, 5, or 10 μL of the reaction mixture was removed in either duplicate or triplicate, added to 150-200 mg of Dowex 1 suspended in water (600-800 μL), and vortexed for 1 min. The supernatant was removed, and the fluorescence was measured at 410 nm (390-nm filter) after excitation at 320 nm in a Perkin Elmer LS50B fluorescence spectrometer. The reaction mixture was incubated in a humidified incubator at 33 $^{\circ}\text{C}$.

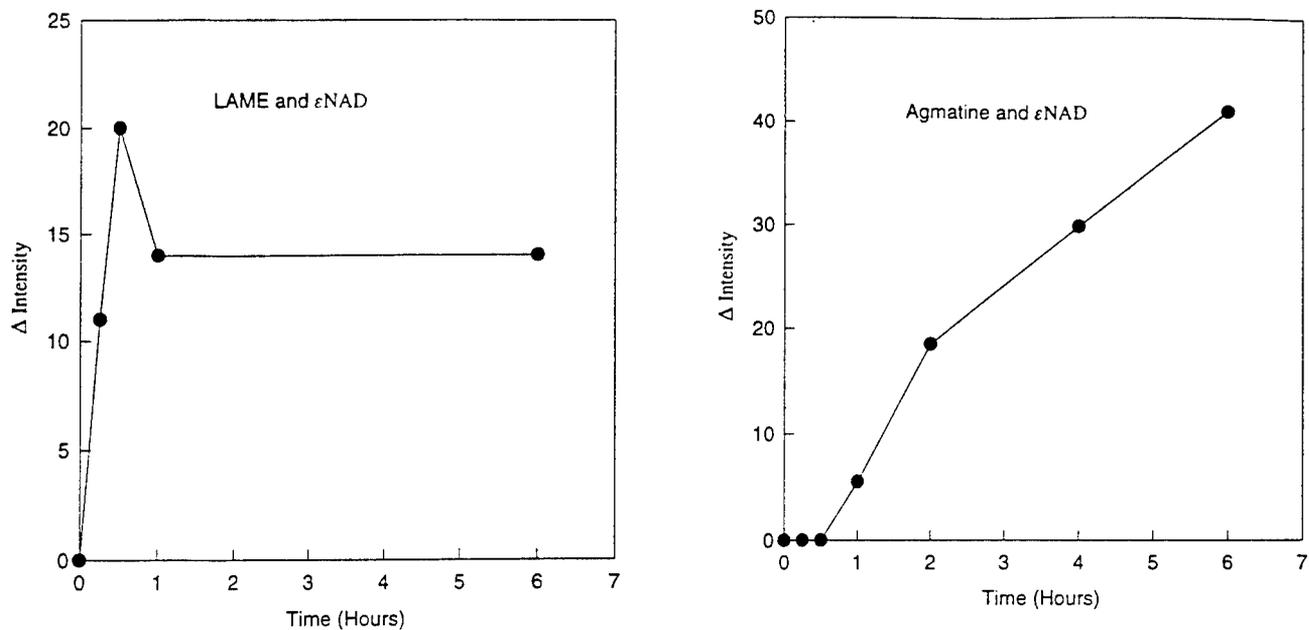


Top left: Ultraviolet spectrum of NBAG, pH 7.4

Top right: Ultraviolet spectrum of reaction at 2 hr

Bottom: NAD 9.9 mM, DTT 30 mM, NBAG 0.24 mM, PO_4^{3-} 200 mM, cholera fragment A 28.6 $\mu\text{g}/\text{mL}$. Final volume was 175 μL . Temperature 23 $^\circ\text{C}$. Reaction mixture and blank were placed in adjacent compartments of a Beckman microcell. The microcell was covered lightly with parafilm and placed in a moist, covered chamber. At time intervals, the microcell was removed from the chamber, and the ultraviolet spectrum was recorded.

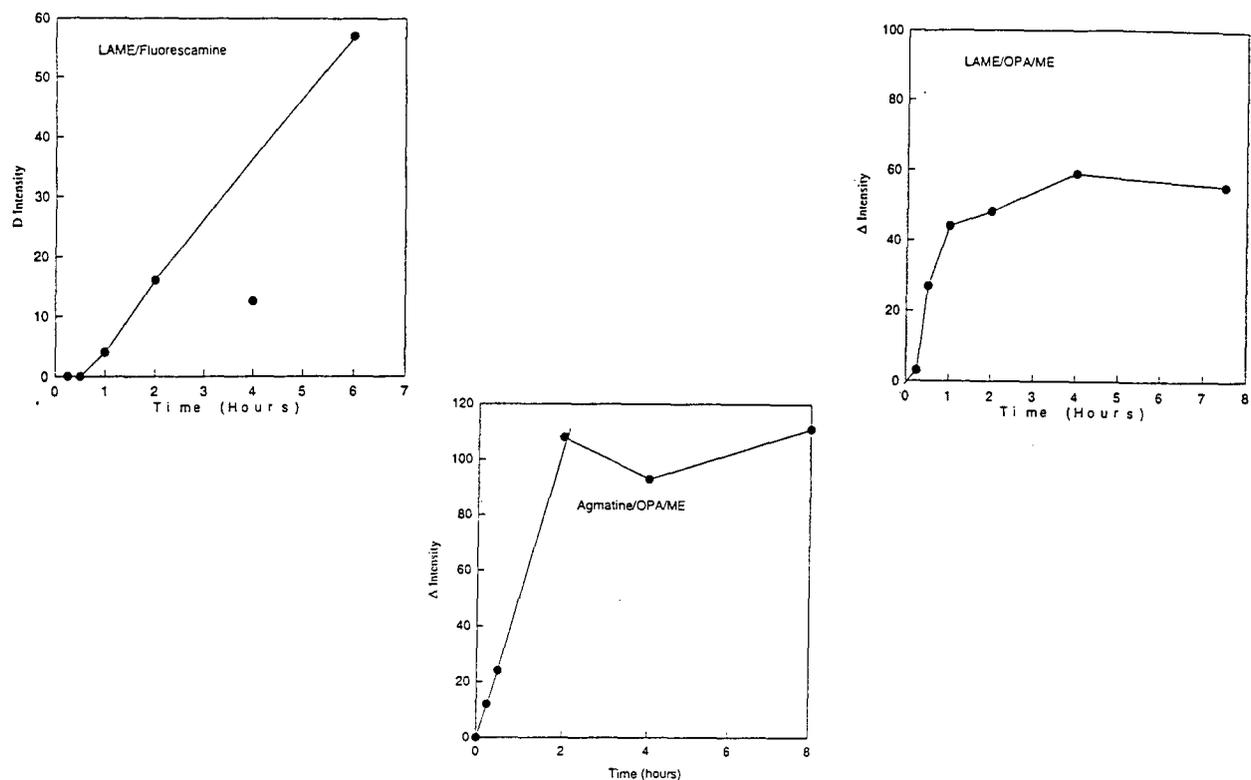
Figure 1. Time Course of Reaction of NAD and NBAG



Left: LAME 60 mM, DTT 20 mM, PO_4^{3-} 170 mM, ϵNAD 1 mM, cholera toxin fragment A 20 $\mu\text{g}/\text{mL}$. Temperature 35 °C. Final volume was 100 μL . At time intervals, 2 μL in duplicate was removed from the blank and reaction, added to 150 mg Dowex 50 suspended in 600 mL 20 mM PO_4^{3-} , and vortexed for 1 min. Emission in the supernatant was measured at 410 nm with a 390-nm filter. Excitation was at 320 nm.

Right: Agmatine 60 mM, DTT 30 mM, PO_4^{3-} 150 mM, ϵNAD 1 mM, cholera fragment A 30 $\mu\text{g}/\text{mL}$. Final volume was 100 μL . Temperature 35 °C. At time intervals, 5 μL of blank and reaction were removed in duplicate and added to a suspension of 200 mg Dowex 1 in 800 μL 15 mM PO_4^{3-} , and the suspension was vortexed for 1 min. Emission intensity of the supernatant was read as above.

Figure 2. Time Course of ADP-Ribosylation of LAME and Agmatine with ϵNAD and Cholera Toxin Fragment A



Top left: LAME 25.6 mM, DTT 30.8 mM, cholera fragment A 24 $\mu\text{g}/\text{mL}$, PO_4^{3-} 200 mM, NAD 9.2 mM. Temperature 30 °C. Final volume was 200 μL . At time intervals, 10 μL of reaction and blank was added in duplicate to 600 μL of water containing 200 mg Dowex 50 and vortexed for 1 min. The supernatant was reacted with fluorescamine. Emission intensity was measured at 475 nm (excitation 390 nm). Δ Intensity is the difference in emission between reaction and blank.

Right: LAME 20.6 mM, DTT 30 mM, PO_4^{3-} 200 mM, NAD 9.5 mM, cholera fragment A 20 $\mu\text{g}/\text{mL}$. Temperature 35 °C. Final volume was 250 μL . At time intervals, 10 μL reaction and blank were added in duplicate to 600 μL 4 mM PO_4^{3-} pH 7.4 and 150 mg Dowex 50 and vortexed for 1 min. The supernatant was reacted with OPA/ME. Emission intensity measured at 450 nm (excitation 320 nm). Δ Intensity is the difference in emission between reaction and blank.

Bottom: Agmatine 10.5 mM, DTT 30 mM, NAD 10.7 mM, PO_4^{3-} 200 mM, cholera toxin fragment A 20 $\mu\text{g}/\text{mL}$. Temperature 35 °C. Final volume was 250 μL . At time intervals, 10 μL reaction and blank was added in duplicate to 4 mM PO_4^{3-} pH 7.4 and 150 mg Dowex 50 and vortexed for 1 min. The supernatant was reacted with OPA/ME reagent. Emission intensity was measured as above.

Figure 3. Time Course of Reaction of ADP-Ribosylation of LAME with Cholera Toxin Fragment A

2.3.2 Labeling with Fluorescent Probes.

Labeling with OPA/ME was carried out as described by Larew and co-workers.⁹ Fluorescence was measured at 450 nm with a 430-nm filter after excitation at 340 nm.

The fluorescent probe fluorescamine was used as described by Udenfriend and co-workers.¹⁰ Excitation was at 390 nm, and the fluorescence was measured at 470 nm with a 430-nm filter.

2.3.3 Reaction with NAD.

Dowex 50W x 4-100 ion exchange resin was washed with 1M NaOH, water, 1M HCl, and water until neutral, then washed with NaCl to convert it to the Na⁺ form, and stored under water after further washes to remove Cl⁻.

The NAD, cholera fragment A, either LAME or agmatine, and DTT were incubated in phosphate buffer, pH 7.4 at 33 °C in a final volume of 200-300 μ L in a sealed culture tube in a humidified incubator. At intervals of 0, 0.25, 0.5, 1, 2, 4, and 6 hr, either 5 or 10 μ L was removed in either duplicate or triplicate, added to 150 mg of the resin suspended in 500-700 μ L of mM phosphate buffer (pH 7.4), and vortexed for 1 min. Three hundred microliters of the suspension was removed. The pH was adjusted to 9.3 with either phosphate or borate buffer (final molarity 25-50 mM), and 200 μ L of OPA/ME was added. The blank did not contain fragment A. When fluorescamine was used, 200 μ L of the resin supernatant was removed and added to 100 μ L of borate buffer (pH 8.6) before reaction with the fluorescent probe. The blank contained fragment A but not NAD. Emission intensity in both cases was measured at 30-s intervals after reagent addition.

2.3.4 Materials.

Cholera toxin fragment A was obtained from List Biological Laboratories (Campbell, CA). Fluorescamine was purchased from Molecular Probes (Eugene, OR). All other chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

3. RESULTS

3.1 NBAG.

The reaction of NAD with NBAG was monitored by the difference in absorbance at 390 nm between the blank and the reaction mixture.⁸ Figure 1 shows the UV spectrum of NBAG and a reaction mixture at 2 hr. The time course of toxin catalyzed ADP-ribosylation of NBAG is also shown in Figure 1. There was a lag of 0.25 hr. However, an increase in absorption at 390 nm was apparent by 0.5 hr when the reaction approached linearity. This result was also reported by Soman and co-workers.⁸

3.2 εNAD.

Excess εNAD, the fluorescent NAD analogue, was removed from the reaction with cholera toxin and either agmatine or LAME as the acceptor by vortexing the reaction mixture with Dowex 1 anionic exchange resin for 1 min. Table 1 shows that 70 nmol of εNAD in 20 mM PO₄³⁻ (pH 7.4) was removed from solution by vortexing with 100-150 mg of the resin. The product, εADP-acceptor with net zero charge, should not bind to the resin. At 410 nm, the difference in emission intensity between the reaction and the blank after vortexing both with Dowex 1 will be a measure of the extent of ADP-ribosylation. Figure 2 shows the time course of the reaction with agmatine and LAME. The reaction with LAME was rapid. An increase in the emission intensity in the reaction with agmatine was not observed until 0.5-1 hr, but the reaction was linear at 1 hr.

Table 1. Retention of εNAD by Dowex 1

Amount of Amine (nmol)	Phosphate or Acetate Conc. (nM)	pH	Relative Emission Intensity (410 nm)	Amount of Resin (mg)
70	20	7.4	74	None
70	20	7.4	13	100
70	20	7.4	7	200
70	20	4	12	100
70	20	4	6	200
5	2	7	986	None
5	2	7	80	100
5	2	7	61	200
Blank	2	7	60	None
5	15	7	253	None
5	15	7	57	100
5	15	7	58	150
Blank	15	7	42	None

A solution of either εNAD in phosphate buffer (either pH 7.4 or 7.0) or acetate buffer (pH 4) was vortexed for 1 min with Dowex 1 Cl⁻ form. The emission intensity of the supernatant was measured at 410 nm with a 390-nm filter in a Perkin Elmer LS 50B spectrometer. Excitation was at 320 nm.

3.3 Labeling with Fluorescent Probes.

Reaction of the fluorogenic reagents OPA/ME and fluorescamine with agmatine and LAME was rapid. The fluorescent adduct with both amines formed within 1 min and started to decay by 3 min (Table 2). This rapid formation and decay of the fluorescent adduct were also found with other amino acids.^{10,11} However, apparently, reaction of either the ADP-ribosylated agmatine or LAME was slower, because maximum emission in the fragment A catalyzed reaction was not observed until 10-13 min after addition of OPA/ME reagent and 3-4 min after addition of fluorescamine. Development of emission maximum was also delayed (1 min) in the reaction blank.

Table 2. Reaction of LAME and Agmatine with Fluorochromes

Compound	Reagent	Relative Emission Intensity	Time (Min)
LAME	OPA/ME	239	1
LAME	OPA/ME	236	2
LAME	OPA/ME	222	3
Agmatine	OPA/ME	135	1
Agmatine	OPA/ME	139	2
Agmatine	OPA/ME	132	3
LAME	Fluorescamine	152	1
LAME	Fluorescamine	148	2
LAME	Fluorescamine	148	3
Agmatine	Fluorescamine	187	1
Agmatine	Fluorescamine	182	2
Agmatine	Fluorescamine	181	3

The fluorescent adducts of LAME and agmatine with the reagents were formed as described.^{7,8} Emission was monitored at 450 nm for OPA/ME and 475 nm for fluorescamine.

The reagents could detect picomolar amounts of either LAME or agmatine (Table 3). Either agmatine or LAME, present in large excess in each reaction, was removed by absorption to Dowex 50 cationic exchange resin. Table 4 shows that 100-150 mg of resin was sufficient to remove 325 nmol of either agmatine or LAME. Either the ADP-ribosylated agmatine or LAME without a positive charge would not be retained by the resin,

Table 3. Sensitivity of Detection of LAME and Agmatine by Fluorescamine

Compound	Amount (pmoles)	Relative Emission Intensity
LAME	4.8	256
LAME	2.4	152
LAME	1.2	88
Agmatine	4.18	122
Agmatine	2.09	76
Agmatine	1.05	52

Serial dilutions of LAME and agmatine were reacted with fluorescamine as described.⁸ Emission was measured at 475 nm, with excitation at 390 nm. A 430-nm filter was used.

Table 4. Retention of LAME and Agmatine by Dowex 50

Compound	Amount (nmol)	Resin (mg)	Relative Emission Intensity	pH
LAME	325	--	665	5.0
LAME	325	100	169	5.0
LAME	325	200	177	5.0
Blank	--	--	241	5.0
Agmatine	85	--	353	7.4
Agmatine	85	100	126	7.4
Blank	--	--	105	7.4

Either LAME or agmatine was vortexed with either Dowex 50 suspended in PO_4^{3-} (pH 7.4) or acetate (pH 5) for 1 min. The supernatant was reacted with OPA/ME reagent, and the emission at 450 nm was measured. Excitation was at 349 nm, and a 430-nm filter was used.

and the emission intensity of the reaction mixture after vortexing with resin is a measure of the extent of ADP-ribosylation. Figure 3 shows the time course of ADP-ribosylation of LAME with cholera toxin fragment A as measured with either OPA/ME or fluorescamine. At 35 °C, ADP-ribosylation of LAME with NAD was apparent by 0.25 hr and was linear for 1 hr. ADP-ribosylation of agmatine was also rapid. The reaction remained linear for 2 hr.

4. CONCLUSIONS

Radiolabeled nicotinamide adenine dinucleotide (NAD) is usually used to monitor the action of cholera toxin. This is a very sensitive technique suitable for use in a laboratory environment, but not as a component in a detection device for field use. The demonstration¹² that aminoguanidines substituted with a chromophore will act as an acceptor for the adenosine diphosphate (ADP)-ribosyl moiety is an important advance in attempts to develop a detection system based on the mechanism of toxin action. Use of aminoguanidines either containing or labeled with a fluorescent group would increase the sensitivity of toxin detection if such compounds will serve as acceptors in the toxin catalyzed reaction.

The present work presents two alternative approaches. The commercially available fluorescent NAD analogue, nicotinamide 1,N⁶-etheno adenine dinucleotide (εNAD), is a substrate for cholera toxin, thereby providing a sensitive means to monitor toxin action by the appearance of a fluorescent ADP-ribosylated product. In the second alternative to the use of aminoguanidines, derivatives of arginine, the natural acceptor for cholera toxin, were labeled with a fluorescent probe after the ADP-ribosylation reaction. Under the reaction conditions used, a fluorescent product was detected in about 0.25 hr in either method. Monitoring cholera toxin action with εNAD requires fewer steps and is preferred over labeling the ADP-ribosylated product with a fluorescent probe. This method, and the use of a fluorescent aminoguanidine, is worthy of further study to determine reaction kinetics to obtain optimum detection conditions.

A device incorporating an optical system to monitor the fluorescent ADP-ribosylated product and/or a mass spectrometer to detect nicotinamide, the other product of toxin action, would provide a means for recognizing the presence of toxins whose action depends on an ADP-ribosyltransferase.

LITERATURE CITED

1. Tu, A.T., Ed., Handbook of Natural Toxins, Volumes 1-5, Marcel Dekker, New York, NY, 1983-1991.
2. Potential Military Chemical/Biological Agents and Compounds, Army Field Manual 3-9, Department of the Army, Washington, DC, December 1990.
3. Moss, J., Iglewski, B., Vaughan, M., Tu, A.T., Eds., Handbook of Natural Toxins, Volume 8, Marcel Dekker, New York, NY, 1995.
4. Moss, J., and Vaughan, M., "Mechanism of Action of Cholergen," J. Biol. Chem. Volume 252, pp 2455-2457 (1977).
5. Mekalanos, J.J., Collier, R.J., and Romig, W.R., "Enzymic Activity of Cholera Toxin," J. Biol. Chem. Volume 254, pp 5849-5854 (1979).
6. Murayama, T., Tasi, Su-C., Adamik, R., Moss, J., and Vaughan, M., "Effects of Temperature on ADP-Ribosylation Factor Stimulation of Cholera Toxin Activity," Biochemistry Volume 32, pp 561-566 (1993).
7. Moss, J., and Vaughan, M., "Toxin ADP-Ribosyltransferases that Act on Adenylate Cyclase Systems," Methods in Enzymology Volume 106, pp 411-418 (1984).
8. Soman, G., Tomer, K.B., and Graves, D.J., "Assay of Mono ADP-Ribosyltransferase Activity by Using Guanyldrazones," Anal. Biochem. Volume 134, pp 101-110 (1983).
9. Larew, J.S.-A., Peterson, J.E., and Graves, D.J., "Determination of the Kinetic Mechanism of Arginine-Specific ADP-Ribosyltransferases Using a High Performance Liquid Chromatographic Assay," J. Biol. Chem. Volume 266, pp 52-57 (1991).
10. Udenfriend, S., Stein, S., Böhlen, S., Dairman, W., Leimgruber W., and Weigle M., "Fluorescamine: A Reagent for Assay of Amino Acids, Peptides, Proteins, and Primary Amines in the Picomole Range," Science Volume 178, pp 871-872 (1972).
11. Alvarez-Coque, M.C.G., Hernández, M.J., Villanueva, R.M.C., and Fernández, C.M., "Formation and Instability of o-Phthalaldehyde Derivatives of Amino Acids," Anal. Biochem. Volume 178, pp 1-7 (1989).
12. Soman, G., Narayanan, J., Martin, B.L., and Grafes, D.J., "Use of Substituted (Benzylidineamino)guanidines in the Study of Guanidino Group Specific ADP-Ribosyltransferase," Biochemistry Volume 25, pp 4113-4119 (1986).