GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: 2/24/81

Project Title: Tritiation of Proteins and Other Biomolecules

Project No: G-33-J04

Project Director: Dr. James C. Powers

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences

Agreement Period: From 4/1/81 Until 3/31/82 (04 Year)

Type Agreement: Grant No. 5-R01-GM25181-04

Amount: $ 98,919 New PHS Funds (G-33-J04)
10,369 GIT Contribution (G-33-358)
$109,288

Reports Required: Annual Progress Reports with Continuation Applications;
Terminal Progress Report upon Grant Expiration

Sponsor Contact Person (s):

Technical Matters

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(thru OCA)

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NOTE: Follow-On Project to G-33-J03 (03 Year)

Defense Priority Rating: None

Assigned to: Chemistry (School/~~Laboratory~~)

COPIES TO:

Project Director
Division Chief (EES)
School/Laboratory Director
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Project Code (GTRI)
Other OCA Research Project Coordinator

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION SHEET

Date 6/22/82

Project Title: Tritiation of Proteins and Other Biomolecules

Project No: G-33-J04

Project Director: Dr. James C. Powers

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences

Effective Termination Date: 3/31/82 (end of 04 year)

Clearance of Accounting Charges: ----

Grant/Contract Closeout Actions Remaining:

- ☐ Final Invoice and Closing Documents
- ☐ Final Fiscal Report
- ☐ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☒ Other Annual Report of Expenditures due by 6/30/82

NOTE: Follow-on project (05 year) is G-33-J05

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

Research Admin. Network	Research Security Services	EES Public Relations (2)
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FORM OCA 10:781

G-33-J04

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1	GRANT NUMBER
SECTION IV—SUMMARY PROGRESS REPORT	GM 25181-05
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT
Powers, James C.	FROM: 4/1/81 THROUGH: 1/15/82
NAME OF ORGANIZATION	
Georgia Institute of Technology	

TITLE (Repeat title shown in Item 1 on first page)

Tritiation of Proteins and Other Biomolecules G33-J04/Powers

1. List all publications, not previously reported, resulting from work supported by this grant (author(s), title, page numbers, year, journal or book). List manuscripts separately as submitted for publication or accepted for publication.
2. Provide two reprints of publications not previously submitted to the awarding unit.
3. Progress Report. (See instructions)

1. G.A. Bush, N. Yoshida, M.O. Lively, B.P. Mathur, M. Rust, T.F. Moran, and J.C. Powers, "Ion Beam Tritium Labeling of Proteins and Peptides", J. Biol. Chem. 256, 12213-12221 (1981).

2. Progress Report.

Scientific Goals. This research is directed toward the development of a general method of tritium labeling of proteins and other molecules of biological interest with carefully controlled particle beams composed of T_3^+ and T_2^+ ions and fast T_2 molecules. We have now tritiated over 10 different proteins and over 5 different peptides and small molecules. After removing the readily exchangeable tritium, we have obtained specific activities of 300-900 Curie/mole with proteins and 20-40 Curie/mole with small molecules. The method labels all amino acid residues and has worked with every organic molecule which we have tried.

Tritiation Apparatus. A high performance ion beam system for the tritiation of proteins has been designed and constructed during the past year. The original ion beam apparatus has been kept fully operational and working while the new, high performance apparatus has been constructed. This new system incorporates several major advances which include the following: a) magnetic confinement of the electron beam to give an intense collimated electron beam, b) separate focussing and acceleration system for the ion beam, c) magnetic analysis of the reactant T_3^+ ion beam. With this spatially confined electron beam, we have a very "bright" source of ionization which produces an intense well defined ion beam. This ion beam is accelerated, focussed and passed into a state-of-the-art, high field, Co-Sm permanent magnet which has been built for momentum (mass) analysis of the tritium ion beam. This specially configured magnet incorporates both high transmission and resolution so that only mass selected T_3^+ ion beams bombard the protein sample. A small post-acceleration/deceleration and ion beam steering system delivers the T_3^+ onto the protein sample. Electronic power supplies for the electron beam and for ion beam acceleration/focussing have been designed and are operational. The flux/cm^2 of ions from our new source is 100 times that of the previous system. We are presently involved in the determination of the precise geometrical shape of the ion beam as it exits the magnetic field and is focussed on the solid sample. In order to facilitate rapid, inexpensive testing and modifications of the new system on a day-to-day basis we have used non-radioactive deuterium ion beams. Once the optimum experimental parameters for matching of intense ion beams to the lyophilized protein sample area have been established, tritium will be used and we expect significantly increased incorporation of tritium into proteins at non-exchangeable sites.

Biochemical Studies. Our first paper on the ion beam tritiation of proteins and peptides has recently been published and we describe the tritiation and characterization of ribonuclease A, porcine pancreatic elastase, thermolysin, soybean trypsin inhibitor, α_1-protease inhibitor, and the peptide aldehydes leupeptin and antipain. After removal of all readily exchangeable tritium, the products were obtained in 32-83% yields with specific radioactivities of 18-856 Ci/mol. The products were chemically pure and had complete biological activity. Simple tritium hydrogen exchange accounts for at least 82% of the reaction pathway with proteins and for 100% of the reaction with the peptide aldehydes. Thus the ion beam method is a mild procedure for general tritium labeling of fragile protein macromolecules and other sensitive biological molecules.

In the last year, most of our effort has been directed toward the tritiation of fibrous and connective tissue proteins and the study of their degradation by proteases. We have tritiated elastin, collagen, fibronectin, myosin, actin, and fibrinogen. Elastin, collagen and fibrinogen have been purified. The others have proven to be difficult to purify and the conditions utilized to date have resulted in major losses due to denaturation. We expect to be eventually successful with fibronectin and actin, but may not succeed with myosin. The rates of degradation of the tritiated proteins by bacterial collagenase, human thrombin, human cathepsin G and human leukocyte elastase has been studied. We hope that these studies will lead to new protease assays utilizing natural protein substrates that have been tritiated by the ion beam tritiation method.

Specific Objectives for the Coming Year.

1. Complete the construction and testing of our new high performance ion beam tritiation system.

2. Utilize this system to increase the specific activity of proteins and other biomolecules which are tritiated by our method. We would like to see a 10-100 fold increase in specific activity to the range of 1,000-10,000 Ci/mol. Our eventual goal is 10,000-30,000 Ci/mol. which is the level of materials produced by chemical synthesis.

3. Characterize the purity and biological activity of the materials produced by the new tritiation apparatus. In particular, we plan to investigate racemization of amino acid residues in the protein. Racemization is one reaction in which tritium for hydrogen exchange could take place and yet have only a subtle effect on the structure and conformation of a tritiated protein. We do not believe this reaction is taking place to any significant extent during our tritiation reaction. However we plan to investigate this possibility since one investigator has suggested that our method will not be completely accepted by some of the scientific community until we prove that racemization is not a significant side reaction.

4. Complete our studies on the degradation of fibrous and connective tissue proteins by proteases and publish the results.