RA

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: *April 4, 1979*

Project Title: *Tritiation of Proteins and Other Biomolecules*

Project No: *G-33-J02* Green Card

Project Director: *Dr. James C. Powers*

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

Agreement Period: From *4/1/79* Until *3/31/80 (02 year)*

Type Agreement: *Grant No. 5 R01 GM25181-02*

Amount: *$102,643 New PHS Funds (G-33-J02)*
9,013 GIT Contribution (G-33-337)
$111,656 Total

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

Sponsor Contact Person (s):

Technical Matters

Dr. Marvin Cassman
Program Administrator
National Institute of General Medical Sciences
Bethesda, MD 20014

Phone: (301) 496-7463

Contractual Matters
(thru OCA)

Ms. Ruth C. Monaghan
Grants Management Specialist
Office of Associate Director for Program Activities
National Institute of General Medical Sciences
Bethesda, MD 20014

Phone: (301) 496-7746

NOTE: FOLLOW-ON PROJECT TO G-33-J01 (01 YEAR).

Defense Priority Rating: *none*

Assigned to: *Chemistry* (School/Laboratory)

COPIES TO:

Project Director
Division Chief (EES)
School/Laboratory Director
Dean/Director—EES
Accounting Office
Procurement Office
Security Coordinator (OCA)
Reports Coordinator (OCA)

Library, Technical Reports Section
EES Information Office
EES Reports & Procedures
Project File (OCA)
Project Code (GTRI)
Other_____

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: May 30, 1980

Project Title: Tritiation of Proteins and Other Biomolecules

Project No: G-33-J02

Project Director: Dr. James C. Powers

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

Effective Termination Date: March 31, 1980 (02 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
x Other Annual Report of Expenditures (02 year) due by June 30, 1980

NOTE: Follow-on project (03 year) is G-33-J03.

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

Project Director
Division Chief (EES)
School/Laboratory Director
Dean/Director–EES
Accounting Office
Procurement Office
Security Coordinator (OCA)
Reports Coordinator (OCA)
Research Property Coordinator (OCA)
Library, Technical Reports Section
EES Information Office
Project File (OCA)
Project Code (GTRI)
Other

CA-4 (1/79)

G-33-502

SECTION IV

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →	GRANT NUMBER
SECTION IV—SUMMARY PROGRESS REPORT	GM 25181-03
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT
Powers, James C.	FROM: 12/1/1978 — THROUGH: 11/11/1980
NAME OF ORGANIZATION: Georgia Institute of Technology	

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

Tritiation of Proteins and Other Biomolecules

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1a. "Low Pressure Tritiation of Proteins," M. O. Lively, T. F. Moran and J. C. Powers, J. Biol. Chem. 254, 262-264 (1979).

1b. "Tritium Labeling of Thermolysin, Elastase and Ribonuclease by Exposure to Tritium Gas at Low Pressure", M. O. Lively, G. A. Bush, B. P. Mathur, T. F. Moran and J. C. Powers, Submitted to Arch. Biochem. Biophys.

2. Dr. Norio Yoshida (postdoctoral associate) will be leaving the project in March. We are currently trying to find a research scientist to replace him. There are no changes in effort.

3. Progress Report - Tritium Labelling Using Ion Beam Techniques. A clean and fast method of incorporating tritium into molecules using ion beams has been further developed and tested during the past year. This ion beam method is a very general and non-destructive approach to the production of tritiated molecules. The apparatus consists of a high vacuum stainless steel system composed of a main reaction chamber, T_2 storage vessels T_2 recovery system, miniature quadrupole mass spectrometer for situ gas analysis, electron beam production device, ion beam acceleration and focusing system, sample holder and irradiation system, pressure measurement devices (Barytron Manometric, ion gauge and thermocouple), liquid nitrogen traps, fast diffusion pump and roughing pumps. Various parts of this apparatus and methods have been improved during this time so as to hold to stringent quality control requirements. The small mass spectrometer has enabled us to quantitatively sample the T_2 reactant gas for purity as well as sample the residual background gases during the pump down time and during the irradiation process. With this spectrometer we have been able to eliminate all variables due to gas evolution from chamber walls, water and gas desorption from lyophilized samples under high vacuum conditions and thus improve the efficiency of the tritiation processes.

Tritium ions are produced by electron impact ionization of T_2 gas which is leaked into the ion source from a T_2 storage vessel using a Granville-Phillips precision needle valve. The path of the electrons has been carefully controlled so that electrons do not interact with the solid sample to be tritiated and the electrons cannot damage the sample. The ions are drawn into a grid assembly for focussing and further acceleration. The kinetic energies of the reactant tritium ions are controlled by voltages on the various grids. These fast reactant ions are focussed on the entire surface area of the sample. By analyzing the tritiated sample, section by section, we find that the samples are uniformly tritiated over the entire area exposed to the reactant ion beam. The incorporation has been examined as a function of sample thickness and sample weight. We find that provided the ion beam kinetic energy is sufficiently high to penetrate the sample, the exchange process occurs uniformly throughout the macroscopic sample. The time required for achieving given sample radioactivities has been shortened during the past year. For example, the initial requirement of 62 hours exposure time has been cut to 30 minutes and sample activity levels have been significantly increased.

The table listed below outlines the compounds which have been tritiated to date and the specific radioactivities which have been obtained.

Small Molecules

desmosine - an amino acid found in elastin	2.4 Ci/mole
nicotine sulfate	2.2
benzyl hydroxylamine tosylate salt	67
octanoic acid sodium salt	0.8
phenyl propylamine hydrochloride salt	0.5
leupeptin, a protease inhibitor isolated from actinomycetes (acetyl-leucyl-leucyl-argininal)	27

Proteins

thermolysin	ca300
elastase, porcine	280
ribonuclease, bovine	53
soybean trypsin inhibitor	859
Trasyol (basic pancreatic trypsin inhibitor)	50
α_1-protease inhibitor (α_1-antitrypsin)	415

In no case has any decomposition of the sample been observed.

After tritiation, the small molecules were worked up by dissolving the sample in water and then removing the water by evaporation or lyophilization. Repetition of this process for four or five times, completely removed exchanged tritium. The specific activities which we have obtained thus far with small molecules are low, but in the useful range for many types of experiments. In each case, the purity of the products were checked by thin layer or high pressure liquid chromatography. No decomposition products were detected. We are currently carrying out radioautography experiments to check for the presence of low amounts of radioactive byproducts.

With proteins, much higher specific activities have been obtained. However, the removal of exchangable tritium is more difficult. A representative workup procedure is given below for soybean trypsin inhibitor.

	Specific Activity Ci/mole	Trypsin Inhibitory activity
Before tritiation	—	100%
After tritiation	6833	73
After 1st dialysis	6644	80
After 2nd dialysis	3364	-
DEAE -cellulose column	937	91
Sephadex G-100 column	857	100
After lypholization	859	99

In all cases studied thus far, one or two column steps have been required to remove most of the readily exchangable tritium. The recoveries of protein are typically in the range of 70-80%. Losses seem to be due to some denaturation during handling. In all cases the protein retains 100% biological activity and shows only one band on gel electrophoresis.

Research Goals. We are making steady progress in achieving higher tritium levels in various types of molecules and we plan to continue exploring all routes to efficient tritium labelling using our method. The influence of such factors as ion kinetic energy neutralization of ion space charge on solid samples, and reactant ion internal energy will be examined in detail with a view to maximizing tritium incorporation. Thus far we have utilized only small amounts of tritium (300 m Ci) in our experiments. We intend to increase the amount of tritium utilized and the extent of exposure to the beam until a point is reached where sample decomposition begins to occur. Furthermore, we are giving to begin to locate the sites at which tritium exchange occurs in order to understand the mechanism of the exchange process.

Significance. The potential usefulness of our tritiation procedure is almost limitless. We have thus far been able to tritiate every molecule that we have investigated. These include proteins from human sources which are difficult or impossible to label otherwise. Minimal decomposition has been observed and the products are biologically active. Tritiated molecules can be used in numerous ways in research and therapy. We have already tritiated molecules for four other investigators and we believe the demand for tritiated molecules is going to be quite high once the scientific community becomes aware of our method.

The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

Jan 12, 1980
Date

James C. Powers
Principal Investigator or
Program Director

DEPARTMENT OF HEALTH AND HUMAN SERVICES

(Instructions are on reverse)

NAME AND ADDRESS OF GRANTEE INSTITUTION	TRANSACTION NO.	Grant No. 5 R01 GM25181-02
Georgia Institute of Technology Atlanta, Georgia 30332	(08)RIGM25181A	DATE OF THIS REPORTING PERIOD FROM 4/1/79 TO 3/31/80
	INSTITUTIONAL ID NO. G-33-J02	PROJECT PERIOD FROM 4/1/78 TO 3/31/81
		☐ CHECK IF FINAL REPORT

1. Expenditures of DHHS Funds for this Reporting Period

a. Personnel	$	h. Alterations and renovations	
b. Consultant services		i. Other	
c. Equipment			
d. Supplies		j. Total direct costs	65,642.02
e. Travel, domestic		k. Indirect costs: Rate 76 % ☒ S&W ☐ TDC	
f. Travel, foreign		Base $ 45,860.91	34,854.28
g. Patient care costs		l. TOTAL	$ 100,496.30

2. Expenditures from Prior Periods (previously reported)	90,309.00
3. Cumulative Expenditures	190,805.30
4. Total Amount Awarded — Cumulatively	192,952.00
5. Unexpended Balance (Item 4 less Item 3)	2,146.70
6. Unliquidated Obligations	
7. Unobligated Balance (Item 5 less Item 6)	2,146.70
8.a. Cost Sharing Information — Grantee Contribution This Period	9,045.74
b. % of Total Project Costs (Item 8a divided by total of Items 1 and 8a)	% 8.25
9.a. Interest/Income (enclose check)	-0-
b. Other Refundable Income (enclose check)	-0-

10. Remarks

I hereby certify that this report is true and correct to the best of my knowledge, and that all expenditures reported herein have been made in accordance with appropriate grant policies and for the purposes set forth in the application and award documents.

Dr. S. C. Powers — Professor — Sept 8, 1981

SIGNATURE OF INSTITUTION OFFICER — DATE

David V. Welch, Manager, Grants & Contracts Acctg.

404/894-4624

Formerly HEW-489

REPORT OF RESEARCH GRANT EXPENDITURES