GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. ____

Project No. G33-J05 DATE 4-20-82

Project Director: Dr James C. Powers School/Co Chem

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

Type Agreement: Grant No. 5-R01-GM25181-05

Award Period: From 4-1-82 To ~~3-31-83~~ (Performance) ~~6-30-83~~ (Reports)

Sponsor Amount: $96,720 11-30-83 2-28-84 Contracted through:

Cost Sharing: $5091 (G33-371) ~~GTRI~~ GIT

Title: Tritiation of Proteins and Other Biomolecules

ADMINISTRATIVE DATA — OCA Contact Don Hastey

1) Sponsor Technical Contact:

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Defense Priority Rating: N/A

2) Sponsor Admin/Contractual Matters:

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Security Classification: N/A

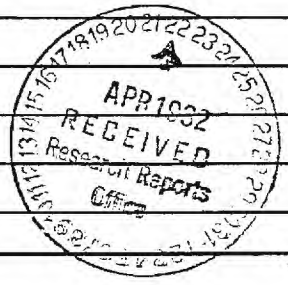
RESTRICTIONS

See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval — Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with N/A - None Proposed

COMMENTS: Follow on To G33-J04



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Other ____

FORM OCA 4-781

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date January 30, 1984

Project No. G-33-J05 School ~~Chem~~ Chemistry

Includes Subproject No.(s)

Project Director(s) Dr. James C. Powers ~~GTRI~~ / GIT

Sponsor

Title Tritiation of Proteins and Other Biomolecules

Effective Completion Date: 11/30/83 (Performance) 2/28/84 (Reports)

Grant/Contract Closeout Actions Remaining:

- [ ] None
- [x] Final Invoice or Final Fiscal Report Financial Status Report (ROE)
- [ ] Closing Documents
- [ ] Final Report of Inventions
- [x] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other

Continues Project No. G-33-J04 Continued by Project No.

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Form OCA 60:1028

G-33-J05

TRITIATION OF PROTEINS AND OTHER BIOMOLECULES

NATIONAL INSTITUTES OF HEALTH GRANT
5 R01 GM 25181-05

G-33-J05

FINAL PROGRESS REPORT

by

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January 1984

Period Covered

4/1/82 through 11/30/83

Publications Since Last Progress Report

None

Publications in Preparation

"Ion Beam Tritiation of Elastin, Collagen, Fibrinogen, Fibronectin and Actin, and A Study of Their Susceptibility to Degradation by Human Leukocyte Elastase, Cathepsin G, Collagenase and Thrombin. Development of New Assays for Proteases Involved in Connective Tissue Destruction," Fukunaga, Y., Teshima, T., Bush, G.A., Moran, T.F., and Powers, J. C.

"Tritiation of p-Amidinophenylmethanesulfonyl Fluoride and its Use in the Active Site Titration of Trypsin-like Serine Proteases," Bush, G.A., Moran, T. F., Powers, J. C., Laura, R. and Bing, P. H.

"Tritiation of Biological Molecules with $^{3}H_3^+$ Ion Beams," Richardson, B.C., Kam, M., Powers, J.C., and Moran, T.F.

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: During the final year of this project we have developed a small mass spectrometer system to deliver an intense controlled energy beam of T_3^+ ions for the tritiation of proteins and other biomolecules.

The experimental apparatus developed for this investigation consisted of an electron impact ion source, a magnetic mass sector, and ion-sample interaction region. A schematic of the ion beam apparatus is shown in Figure 1.

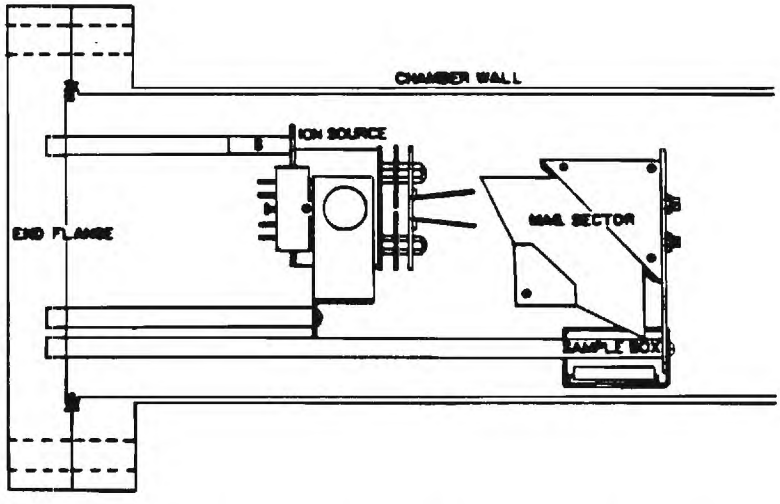

Fig. 1. Schematic drawing of the ion beam apparatus mounted on the end flange of the tritiation chamber.

Reactant ions were produced by controlled electron impact ionization of tritium gas. Electrons emitted by a directly heated filament were accelerated into the source chamber by a voltage variable from 0 to 60 volts. Two circular samarium-cobalt permanent magnets of 1.27 cm diameter were used to collimate the electron beam into a tight spiral as it passed through the ion source. Ions formed by electron impact were gently pushed through a relatively long (1 cm) distance to the ion source exit slit. As the ions traveled from their point of formation in the electron beam to the exit slit, the following ion-molecule reactions

$$T_2^+ + T_2 \quad T_3^+ + T$$

occurred to form T_3^+ ions. Both reactant T_3^+ and T_2^+ ions were accelerated to terminal velocities by voltages placed on plates L2 and L3. The relative positions of the various elements of this source are illustrated in figure 2.

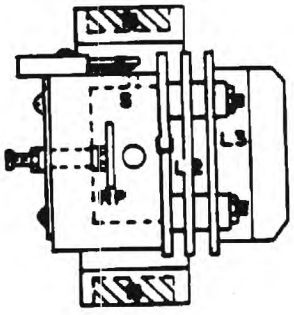

Fig. 2

The electron impact ion source. Labeled are the filament F, the source chamber S, the repeller plate RP, the electron collimating magnets M, and the beam focusing and acceleration plates L1, L2, and L3.

After acceleration the ions drift into a 90° inflection field magnetic sector with a 3.61 cm radius of curvature. This magnetic sector was constructed of high field samarium-cobalt. The magnets were machined from 5.1 cm x 5.1 cm x 1.3 cm stock and them magnetized. A field strength on the order of 2000 gauss was obtained for a 2.5 cm gap a between the pole faces.

Momentum analyzed ions were focused on the sample. The sample, in a cm^2 tray, was electrically insulated from the sample holder box which allows only focused ions from the magnetic sector to strike the sample. The tray was connected to ground through an ammeter which allowed measurement of ion current at the sample.

We have thus been able to measure the reactivity of T_3^+ as compared with T_2^+ and find that T_3^+ is 40 times more reactive than T_2^+. Using pure T_3^+ ion beams we have been able to tritiate leupeptin to a level higher than previously obtained using un-mass analyzed ion beams.

Biochemical Studies: The crude product of ^3H-leupeptin had a specific activity of 5 Ci/mole for T_2^+ tritiation, and 230 Ci/mole for T_3^+ tritiation. After tritiation, leupeptin was worked up by lyophilization for three or four times and ion exchange chromatograph (Bio-Rex 70 column) to remove exchangeable tritium. The purity of the reaction products was checked by thin layer chromatography and autoradiography. We also measured trypsin inhibitory activity of leupeptin before and after tritiation. The concentration of leupeptin in solution was determined by Sakaguchi colorimetric method. After purification, the

leupeptin obtained from T_3^+ tritiation had a specific activity of 87 Ci/mole. This is about four times higher than our previous best tritiation of leupeptin using the un-mass analyzed ion-beam labeling method. Samples with less than 8 mg gave slightly better results. The tritiated leupeptin was indistinguishable from pure native leupeptin.

Experimental Problems: Relatively few tritiation experiments were carried out during this grant period since we were preoccupied with modifications on the apparatus itself. We had hoped to tritiate molecules such as 2-iodobenzoic acid and cinnamic acid by the ion beam labeling method to see if the tritium beam would replace the iodine atom or would add to the double bond of cinnamic acid. However, the tritiation apparatus is beginning to feel its age and we experienced numerous difficulties. One major problem was a continuing loss of the tritium into the traps probably due to seals in the valves which are beginning to fail. Thus we were able to carry out only a few experiments with leupeptin.

Future Experiments: Even though the grant period has terminated, we plan a few last experiments with the tritiation apparatus before it is mothballed. Since we can no longer meter tritium accurately into the reaction chamber due to the valve problems, we are going to do up to seven tritiations using the full 5 Ci available in a vial of tritium gas. We are going to pack the reaction chamber with sample holders holding a total of 50-100 mg of sample. After introductionof the full 5 Ci of tritium into the reaction chamber, we will ion beam label one sample for 1 hr and then seal the chamber and allow gas labeling to take place on all the samples for a period of 1-2 weeks. One such experiment was started in the middle of December and was allowed to remain in the tritiation apparatus during the holidays. The sample is an experimental drug and the tritium labeled version will be utilized for metabolic studies. We expect to label up to six additional samples before our tritium supply is exhausted.

Perspectives and Conclusions: The ion beam tritiation method has proved to be a convenient method to label any organic compound. After removing the readily exchangeable tritium, we have obtained specific activities of 300-900 Ci/mole with proteins and 20-90 Ci/mole with small molecules. With proteins, this method along with all other general tritium labeling techniques, suffers a major limitation. It is nearly impossible to remove all the exchangeable tritium which continues to leak from the protein whenever it is used. So far the only use that we have made of tritiated proteins has been as protease substrates where leakage of tritium is beneficial for assay purposes. In contrast, tritiation of small organicmolecules have proved to be much more useful. Although there are many methods for producing tritium labeled organic compounds, most require considerable effort and purification. The ion beam method is simple, convenient, and gives products which require very little purification. The specific activities obtained with the ion beam method are not as high as those obtained by direct chemical synthesis, but are quite reasonable for most biochemical studies. Thus, most of the investigators who approached us with samples for tritiation have given us small organic molecules which are sensitive and quite difficult to tritiate by any other method. We have tritiated 1-phenyl 1-aminomethylethene (S. May, Georgia Tech) for use in receptor binding studies and mevinolin (R. Abeles, Brandeis) which is a transition state inhibitor for HMG-CoA reductase. We have also supplied several investigators with samples of leupeptin. The samples which we plan to tritiate in the future are all small molecule drug candidates which are needed for metabolic studies. This is the area where the ion beam tritiation method has the most potential usefulness. In the future, if this technique is to going to be used

more widely, it will be necessary to redesign the tritiation apparatus in order to simplify it. Our current apparatus was designed as a research tool and as such is very versatile, but contains too much plumbing to maintain.