Comparison of Tritiation Efficiency Using $^3H_3^+$ and $^3H_2^+$ Ion Beams

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SUMMARY

A new apparatus was constructed to tritiate biological molecules using mass analysed tritium ion beams. The magnetic sector of this device was constructed of high field Sm-Co permanent magnets. Mass analyzed ions of desired kinetic energy were impacted on an involatile solid and ^3H exchanged for H. The $^3H_3^+$ ions were found to be more reactive than $^3H_2^+$ ions. The sensitive peptide aldehyde leupeptin was utilized to test the efficacy of mass analyzed ion beam tritiation and leupeptin was not decomposed by this process.

Key Words: Tritium labeling, Ion beam tritiation

INTRODUCTION

Organic molecules have been tritiated in a number of ways[1] including direct chemical synthesis, catalytic hydrogen exchange, biosynthetic modifications and chemical modifications.[2] The Wilzbach[3] technique is a general labeling method but it leads to a high level of chemical decomposition in the sample.[4,5] Other general techniques such as electrical discharge.[6] the free radical interceptor[7] and microwave discharge[8] produce higher levels of radioactivity but for compounds of higher molecular weight, decomposition

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can be a problem.

A general method to label proteins, peptides and other nonvolatile organic compounds with a tritium ion beam was developed recently.[9] These samples were labeled in short times with no observable decomposition or loss of biological activity.

The present study was undertaken to: confirm the identity of the reactive species in the ion beam method, examine the reactivity of different tritium ion species thus maximizing tritium incorporation by this approach.

EXPERIMENTAL APPARATUS

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.

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

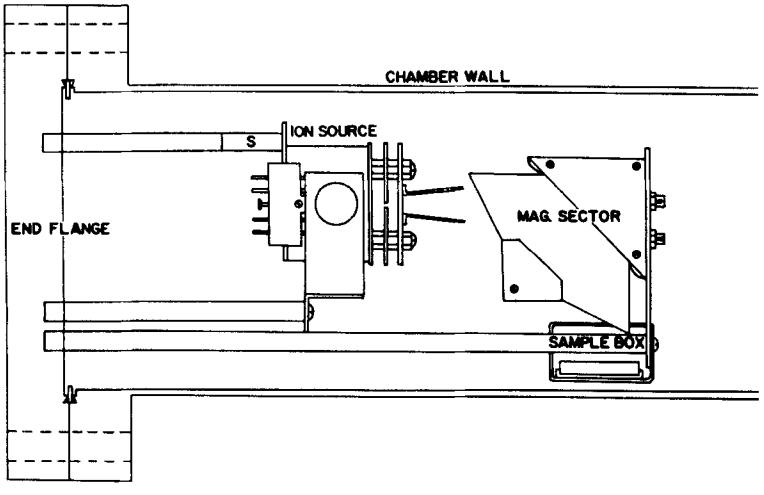

Fig. 1. Schematic drawing of the ion beam apparatus mounted on the end flange of the tritiation chamber.

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[10]

$$^3H_2^+ + {}^3H_2 \rightarrow {}^3H_3^+ + {}^3H$$

occurred to form $^3H_3^+$ ions. Both reactant $^3H_2^+$ and $^3H_3^+$ 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.

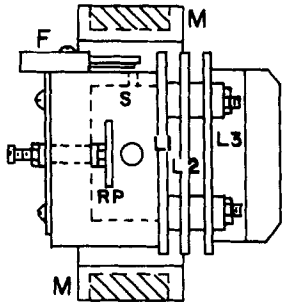

Fig. 2
The electron impact ion source. Labeled are the filament F, the source chamber S, the repeller plate RP, the electron collimating magnets M, and the beam focusing and acceleration plates L1, L2, and L3.

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 then magnetized. A field strength on the order of 2000 gauss was obtained for a 2.5 cm gap between the pole faces.

Momentum analyzed ions were focused on the sample. The sample, in a 1 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.

EXPERIMENTAL PROCEDURE

Samples were installed in the system by withdrawing the ion beam apparatus attached to the front flange of the vacuum chamber shown in figure 3. The sample, lyophilized onto the surface of a sample tray, was placed in the sample holder box. Although larger sample sizes can be tritiated, best results were obtained with a sample weight of 3-15 mg. The flange supporting the apparatus was then bolted to the end of the vacuum chamber and the system pumped to a pressure of 10^{-7} to 10^{-8} torr. Then the vacuum chamber was closed off from the pumping system and tritium was admitted into the chamber to a pressure of 2-5 mtorr.

With the ion acceleration voltage set to focus the reactant ion of interest onto the sample, a current of approximately 3 Amps was passed through the filament to produce an electron beam with a current of 200-400 μAmps. The electrons were accelerated to an energy of 50eV to ionize the tritium gas. Total exposure time of the solid leupeptin sample was 3-1/2 to 4 hrs. during which an average of 6×10^{-4} coulombs of charge strike the sample surface. Tritiated leupeptin was purified and characterized as previously described by Bush et al.[9]

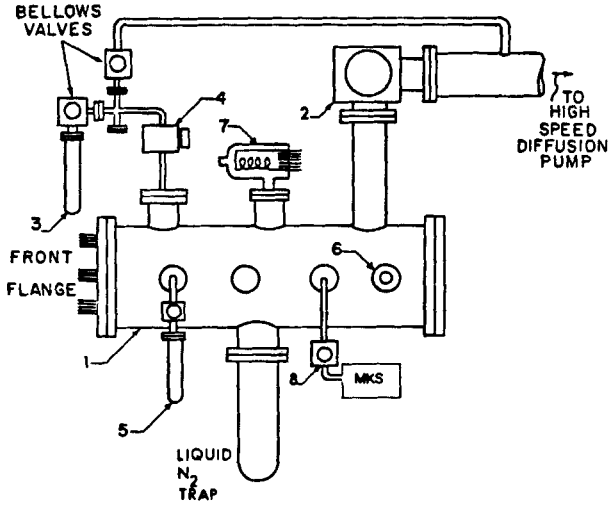

Fig. 3 The tritiation vacuum chamber, Labeled are; (1) the main chamber, (2) shut-off valve, (3) tritium gas reservoir, (4) needle valve, (5) Pd-C^3H_2 recovery system, and (6), (7), and (8) pressure gauges.

RESULTS AND DISCUSSION

Tritium ion beam labeling of a number of proteins and peptides has been reported by Bush et al.[9] who suggested that fast $^3H_3^+$ ions were the reactive species in this exchange process. However, these experiments lacked mass analysis and $^3H_2^+$ formed in electron impact ionization, as well as $^3H_3^+$ from ion-molecule reactions and fast $^3H_2$, from gas-phase charge transfer interactions, were considered as possible reactants. Subsequent ab initio self-consistent field calculations[11] showed a repulsive interaction potential between $^3H_2$ and the model system glycine. These calculations indicated an energetically favorable interaction exists between $^3H_3^+$ and glycine.

Previously, the sensitive peptide aldehyde leupeptin was tritiated to a level of 72 Ci/mol by Bush et al.[9] in experiments involving beams composed of a mixture of $^3H_3^+$ and $^3H_2^+$ reactant ions. Complete characterization of the product showed insignificant amounts of decomposition and demonstrated that all portions of the peptide were labelled. In order to compare the individual reactivities of $^3H_3^+$ and $^3H_2^+$ ions we have tritiated the same compound leupeptin using mass analyzed ion beams. Under optimum conditions a 9 mg sample of leupeptin was found to incorporate tritium to a level of 364 Ci/mol using 370eV $^3H_3^+$ ion beams. An average specific radioactivity value of 230 Ci/mol was obtained for samples in the 6-12 mg weight range when exposed to $^3H_3^+$ ions in the 300 to 400eV kinetic energy range. No decomposition of the tritiated leupeptin was observed. Approximately 2 out of every 100 incident $^3H_3^+$ particles bombarding the tray area results in substrate tritium incorporation. The use of $^3H_2^+$ as the bombarding species led to much lower levels of incorporation (5 Ci/mol) which indicate $^3H_2^+$ is an order of magnitude less effective tritiation agent than is $^3H_3^+$.

CONCLUSION

An ion beam tritiation apparatus that allows mass selection of the reactant ionic species was developed. Selectivity of the ionic species has

increased the level of ^{3}H incorporation and points to the importance of controlling conditions that favor $^{3}H_{3}^{+}$ as the reactant when using ion beam labeling techniques.

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