Production of Radioisotopes for Radiology Applications

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Two radioisotope production projects are under way. Medical researchers in the MSU Department of Radiology have been interested in acquiring a positron imaging system for clinical use. Since the radionuclide ^{11}C(half life 20.4 min.) is most favored as a label for this purpose, we have made plans to produce it when needed, using the technique developed by Wolf and ollaborators[1] at Brookhaven National Laboratory. A pressurized gas target containing nitrogen and hydrogen yields curie amounts of carrier-free ^{11}C as HCN, when bombarded with a proton beam from a cyclotron. The carbon produced by the $^{14}N\ (p,\alpha)^{11}C$ reaction forms $^{11}CH_4$ which is then converted completely to $H^{11}CN$ by radiolysis due to the presence of the intense proton beam. As a first step we are duplicating the Brookhaven target for testing.

The other isotope production project is to supply researchers at the Department of Radiology of Harvard University with source of ^{178}W (half life 21.5 days) for their work aimed at developing a generator for radiopharmacuticals based on ^{178}Ta (half life 9.3 min) and also experimenting with a gamma ray imaging system using the 93.2 keV gamma ray emitted by this nuclide. The desired activity is produced by the $^{181}Ta(p,4n)^{178}W$ reaction. The first target bombarded was a stack of 5 tantalum sheets, each about 1.7 MeV thick to the incident 40 MeV beam. The activities were measured by the strength of the 93 keV gamma ray using a Ge(Li) detector, and indicated that a target 1MeV thick at 35 MeV proton energy will yield 70 μCi/μA-hr. The only significant contaminant observed was the 113 keV line from ^{177}Hf, due to the (p,5n) reaction which seems to be negligible below 35 MeV beam energy. A production rate of 1mCi/hr is adequate for the present purpose and requires less than 3 μA on a target 6 MeV thick.

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Parasitic Production of ^{13}N in Water Faraday Cups

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With the development of techniques for the production and analysis of ^{13}N-labeled substances (see discussion elsewhere in this report) it appears likely that use of these substances will expand. While efficient production of labeled nitrogen gas uses ^{12}C or ^{13}C targets[1] and apparently requires a dedicated beam, labeled ammonia ($^{13}NH_3$) and nitrate ($^{13}NO_3$) can be readily made in water in 30-50 mCi amounts. Since we already use water for stopping the beam in the Faraday cups of the Enge spectrograph and the beam swinger, it appears that the short (10-20 minute) bombardments necessary for production of $^{13}NH_3$ and $^{13}NO_3$ could be done in these Faraday cups with little interruption of other experiments.

Initial experiments have been aimed at investigating the radiochemical purity of the bombarded water. We find:

1) In the freshly bombarded water (^{13}N presumably in $^{13}NO_3$) Main impurity is ^{18}F ($\tau_{1/2}$=110 min).

$$\left.\frac{\text{Count rate from } ^{13}N}{\text{Count rate from } ^{18}F}\right|_{\text{Beam Off}} \simeq 10^3$$

2) In water subjected to an acid digestion and distillation. (^{13}N presumably as $^{13}NH_3$), Little ^{18}F. Main inpurity is an unknown with $\tau_{1/2}$ > 160 min.

$$\left.\frac{\text{Count rate from } ^{13}N}{\text{Count rate from unknown}}\right|_{\text{Beam Off}} \simeq 3\times10^4$$

These impurity levels are already sufficient for many experiments, especially those involving $^{13}NO_3$ which are often of short duration. Improved distillation procedures,[2] or alternate Faraday cup metals, may further improve the purity of the $^{13}NH_3$.

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Metabolism of ^{13}N-Labeled Inorganic Nitrogen by Blue-Green Algae

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In the past few years we have developed methods both for the production of intense sources of ^{13}N-labeled radioactive gases (^{13}N ^{14}N and $^{13}NH_3$)[1-3] and for the indentification[4-5] (on a time scale consistent with the 10 min. half life of ^{13}N) of the compounds in which ^{13}N is later incorporated by biological activity. Recently, we have been applying these methods to study the biochemical pathways for nitrogen metabolism in the blue-green algae.

Previously such studies have used nitrogen compounds labeled with ^{15}N. With this technique, however, as increasingly better time resolution is employed (shorter and short labeling intervals) ever smaller amounts of ^{15}N must be detected and quantified. These techniques are limited by air leaks (containing ^{14}N) and by the practical sensitivities of mass or emission spectrometers, to fixation times of over a minute. The use of ^{13}N labeled gases circumvents these difficulties, permitting a decrease in labeling time of about two orders of magnitude or the study of extremely small (∿0.5%) branches in the metabolic sequence.

The technique is as follows. Labeled nitrogen gas and ammonia are formed from ^{13}N generated in the $^{13}C(p,n)^{13}N$ reaction[1]. After fixation by whole or segmented blue-green algae for periods of from 1 sec. to 15 min. the products of fixation are extracted from the biological sample with 80% methanol. The methanolic extracts are concentrated under vacuum and spotted in a strip (2 x 10 mm) on a glass plate coated with a thin (0.1 mm) layer of cellulose. The ^{13}N-labeled products are separated by electrophoresis at a potential difference of about 150 V/cm, and are then quantified with a radiochromatogram scanner, or by electron scintillation spectrometry. Processing times of 1 to 1.5 h are possible after fixation of ^{13}N ^{14}N and of up to 2.5 h after fixation of $^{13}NH_3$. Several experiments have been performed:

a) Fixation of labeled nitrogen gas (^{13}N ^{14}N) by whole blue-green algae. These experiments yielded the first direct determination of the metabolic sequence in the alga *Anabaena cylindrica* (grown on N_2 and capable of fixing N_2). After brief fixation of ^{13}N ^{14}N the first product formed is $^{13}NH_3$, identified by its distillation properties (see Fig. 1). The second product formed is the amide nitrogen of the amino acid glutamine [$H_2NCH\ (COOH)\ CH_2\ CH_2CO^{13}NH_2$]; this product predominates for fixation times between about 2 and 90 sec. It was identified by its distillation properties, its electrophoretic migration at pH 9.2 and 2.0, and its rate of chromatographic movement in an organic solvent system. Also when the enzyme glutamine synthetase is inhibited by methionine sulfoximine, $^{13}NH_3$ accumulates and ^{13}N-labeled glutamine is not formed. After 90 sec, the amino acid glutamic acid [$H_2{}^{13}NCH(COOH)\ CH_2CH_2COOH$] predominates. This substance was identified by is electrophoretic and chromatographic properties. The enzyme glutamate synthase, which catalyzes the transfer of nitrogen from the amide group of glutamine to a nitrogen acceptor to form glutamate, is inhibited by azeserine, a structural analog of glutamine. In the presence of azaserine, ^{13}N-labeled glutamine accumulates, and glutamic acid remains unlabeled. If, following 15 sec of labeling, ^{13}N ^{14}N is removed and replaced with stable N_2, the radioactivity of glutamine decreases, while that of glutamate increases.

The above observations demonstrate unequivocally that N_2-derived ammonia is metabolized by *Anabaena cylindrica* intially by the glutamine synthetase/glutamate synthase pathway.

b) Fixation of ^{13}N ^{14}N and $^{13}NH_3$ by heterocysts of blue-green alage[2]. Much, perhaps all, of the nitrogen is fixed in specialized cells called heterocysts, which comprise from 5-7 percent of the cells in the algal filaments. Using heterocysts isolated by a new technique we could show that they can transform much of the fixed N_2 into glutamine. However, even after 15 min., at most two percent of the radioactivity in glutamine was transferred to glutamic acid. Enzymatic experiments corroborated the interpretation that heterocysts have very low activity of glutamate synthase. It therefore appears that glutamine newly synthesized in heterocysts is transported to the other cells (vegetative cells) of the algal filament where glutamate is synthesized.

3) Fixation of $^{13}NH_3$ by blue-green algae grown on $^{14}NH_3$. In addition, we have begun a study of the pathway of assimilation of $^{13}NH_3$ by algae grown in the presence of $^{14}NH_3$, a condition in which few or no heterocysts are formed and the capacity to fix N_2 is suppressed. It is clear that glutamine synthetase is again the prinicpal initial enzyme metabolizing the $^{13}NH_3$. However, whether the amide group of glutamine is then transferred principally to form glutamic acid is as yet uncertain. In contrast to the studies with N_2 grown algae, glutamic acid never predominates among the labeled products; after about two minutes of labeling more ^{13}N-aspartic acid [$H_2{}^{13}NCH(COOH)\ CH_2COOH$] than ^{13}N-glutamic acid is present. In the presence of methionine sulfoximine, glutamic acid and another amino acid, alanine, are formed at a slow rate. These results show clearly that the enzymes glutamic acid dehydrogenese and alanine dehydrogenase participate,

but only to a limited extent, in the assimilation of NH_3 by Anabaena cylindrica.

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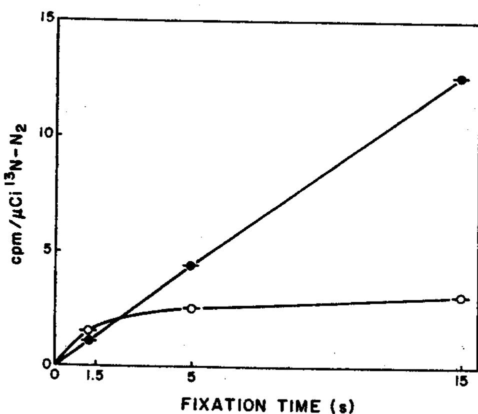

Fig. 1. Time course of incorporation of ^{13}N into pools of NH_3 and amide nitrogen. After fixation of ^{13}N-^{14}N for 15, 5, and (approximately) 1.25 s, the suspension of algae was extracted with 80% methanol, and the extract subjected first to vacuum distillation at pH 10, to determine $^{13}NH_3$ (-o-), and then to steam distillation in the presence of 40% NaOH, to determine amide-^{13}N (-●-).