ENZYMATIC PREPARATION OF ^{3}H-LABELED ß-UREIDOISOBUTYRIC ACID AND ß-AMINOISOBUTYRIC ACID FROM ^{3}H-THYMIDINE.

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SUMMARY

We have synthesized ß-aminoisobutyric acid (3-amino-2-[^{3}H]methyl-propanoic acid) and ß-ureidoisobutyric acid (3-[(aminocarbonyl)amino]-2-[^{3}H]methyl-propanoic acid) starting with [^{3}H]thymidine (1-(2-deoxy-ß-D-ribo-furanosyl)-5-[^{3}H]methyluracil). We have developed a simplified enzymatic preparation using a dialyzed supernatant of homogenized dog liver. The homogenate was incubated with [^{3}H]thymidine (41 Ci/mmol) in the presence of NADPH, and ß-ureidopropionic acid. After extraction the supernatant was purified by HPLC.

Key Words: ß-aminoisobutyric acid, ß-ureidoisobutyric acid, thymidine

INTRODUCTION

Thymidine labeled with ^{3}H or with ^{14}C has found extensive use in biologic research for the measurement of DNA synthesis and cell growth. More recently [^{11}C]-labeled thymidine has been developed for use in positron emission tomography (PET), allowing one to obtain non-invasive measurements of thymidine uptake and retention in tissues (1,2). Since PET imaging measures all of the labeled chemical species present in a tissue, it is important to understand the distribution of labeled metabolites such as ß-aminoisobutyric acid and ß-ureidoisobutyric acid (3). The preparation of ^{14}C labeled ß-ureidoisobutyric and ß-aminoisobutyric acids from thymine using purified enzymes has been described (4). With the goal of developing a simplified method of preparation from thymidine, without the need for enzyme purification, we used a crude dog liver homogenate. This tissue homogenate was then incubated with [^{3}H]thymidine in the presence of NADPH, and ß-ureidopropionic acid to yield the desired products. The addition of the ß-ureidopropionic acid acts as a competitive enzymatic inhibitor increasing the yield of ß-ureidoisobutyric acid relative to ß-aminoisobutyric acid (4). The desired compounds were isolated by HPLC.

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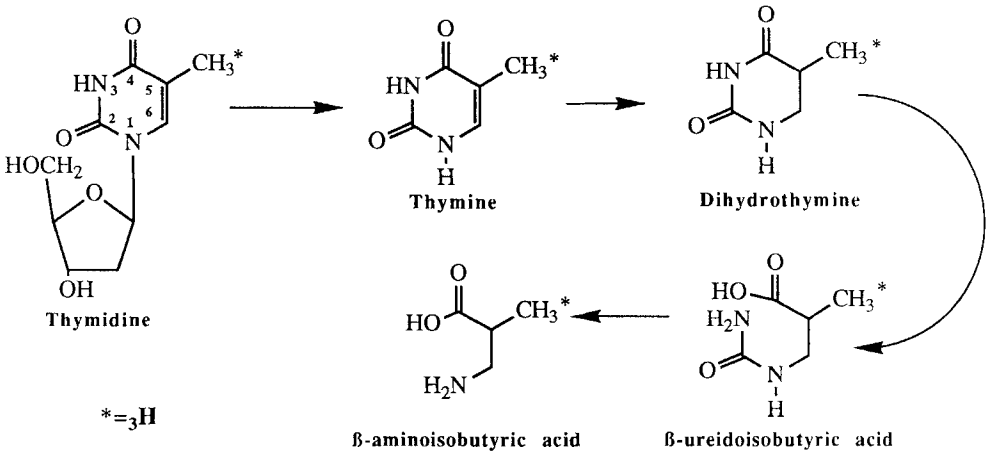

EXPERIMENTAL

Dog liver (1 gm) was homogenized in 1 mL 200 mM Tris (pH 7.6). The homogenate was then centrifuged at 2200 rpm (500 x g) for 5 minutes, and 400 µL of supernatant was then dialyzed twice against 4 L of phosphate buffered saline, 2 mM $CaCl_2$, 2 mM $MgCl_2$ over 48 hours at 4°C. The dialysis was done to remove unlabeled compounds in the tissue homogenate.

100 µCi [^{3}H]thymidine with a specific activity of 41 Ci/mmol (Research Products International, Mount Prospect, IL) was added to 200 µl of dialyzed supernatant and brought to 2.3 mMolar NADPH (ß-nicotinamide adenine dinucleotide phosphate reduced form), and 10 mMolar ß-ureidopropionic acid (2-[(aminocarbonyl)amino]-propanoic acid) (Sigma; St. Louis, MO), resulting in a final volume of 400 µl. The mixture was incubated in a water bath at 37°C for 75 min followed by protein precipitation using 1.2 mL of 3:1 methanol/acetonitrile. After centrifugation of the mixture in a microfuge at 14,000 rpm for 2 minutes, the (clear) supernatant was drawn off. The supernatant was dried and the residue was reconstituted in 800 µl of deionized water, and the solution passed through a 2 µM filter.

The preparation was purified by HPLC using a reverse phase column (Ultracarb 5 ODS(20) 5 µ, Phenomenex, Rancho Palos Verde, CA) with a mobile phase of 20 mM Tris (pH 5.3) for 5 minutes, followed by a 10 minute linear gradient to 20% methanol, and flow rate of 1 ml/minute. Fractions of 0.25 mL were collected and aliquots counted by liquid scintillation spectroscopy. The fractions containing ß-aminoisobutyric acid and ß-ureidoisobutyric acid were then pooled, dried down, dissolved in water and repurified by HPLC as above with a pH of 3.3. Fractions of 0.25 mL were collected and aliquots counted in 10 mL of Scint A (Packard, Downer's Grover, IL). The retention volumes at pH 5.3 were 3.5 mL for ß-ureidopropionic acid, 6 mL for ß-aminoisobutyric acid, 4.4 and 10 mL (as two peaks) for ß-ureidoisobutyric acid, 14 mL for thymine, and 17.5 mL for thymidine. The retention volumes at pH 3.3 were the same except that ß-ureidoisobutyric acid eluted as a single peak at 10 mL, and ß-ureidopropionic acid eluted at 5.4 mL. A standard of unlabeled ß-ureidoisobutyric acid was produced from dihydrothymine (5).

From 100 µCi of [^{3}H]thymidine we obtained 18.8 µCi ß-ureidoisobutyric acid and 59.3 µCi ß-aminoisobutyric acid, with the remainder as mostly thymine. The radiochemical purity was determined by analysis on HPLC and found to be >94% for each compound. These results were comparable to those obtained using TLC on microcrystalline cellulose plates (Bakerflex; J.T. Baker, Philipsburg, NJ)

developed with a 4:4:1 mixture of t-butyl alcohol: methylethylketone:water containing 0.26% formic acid(6). The identity of the compounds was also supported by performing the synthesis using thymidine labeled with ^{14}C in the ring-2 position. As expected we isolated labeled ß-ureidoisobutyric acid, but no labeled ß-aminoisobutyric acid. In the synthesis of ß-aminoisobutyric acid the ^{14}C is lost as carbon dioxide.

RESULTS AND DISCUSSION

The incorporation of labeled thymidine into DNA has been extensively used in the evaluation of cell growth. The ability to non-invasively measure [^{11}C]thymidine retention has led us to re-evaluate the metabolism of thymidine and the importance of its degradation products in interpreting images obtain with PET (3). The ready synthesis of labeled metabolites will allow us to conduct biodistribution studies in animals.

Labeled thymidine is commercially available with ^{14}C, as well as ^{3}H, in various positions, therefore one can readily produce alternate labeled forms of ß-ureidoisobutyric acid and ß-aminoisobutyric acid.

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