THE SYNTHESIS OF TRITIUM LABELED DIMETHYLETHANOLAMINE AND MONOMETHYLETHANOLAMINE

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SUMMARY

A simple and efficient procedure has been developed for the synthesis of [^{3}H]monomethylethanolamine and [^{3}H]dimethylethanolamine. Schiff base formation between formaldehyde and either ethanolamine or monomethylethanolamine was followed by Na B[^{3}H]$_4$ reduction. The reaction products were obtained from preparative thin layer chromatography plates.

Key Words: [^{3}H]monomethylethanolamine, [^{3}H]dimethylethanolamine, Schiff base formation, Na B[^{3}H]$_4$ reduction

INTRODUCTION

Interest in the sequential methylation of membrane bound phospholipids by phospholipid N-methyltransferases (PMT) was stimulated by the proposal that these activities were involved in message transmission across biological membranes. (1) It has been suggested that phosphatidylcholine produced by the PMT pathway in nervous tissue may represent a pool of choline precursor supporting acetyl choline formation. (2) This series of reactions would be expected to produce phosphatidyldimethylethanolamine (PDME) and phosphatidylmonomethylethanolamine (PMME) as intermediates between phosphatidylethanolamine and phosphatidylcholine. Alternatively, appreciable amounts of these two intermediates can be generated in membranes by growth of cells in

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culture medium containing the corresponding free bases dimethylethanolamine (DME) and monomethylethanolamine (MME) respectively (3,4,5,6).

It became desirable to have radioactive DME and MME available in order to study their metabolism in relationship to their corresponding phospholipids. These labeled compounds are not routinely available from commercial suppliers. Chemical N-methylation of partially demethylated phosphatidylcholine with [^{14}C]methyl iodide has been employed to synthesize labeled lecithin (7). [^{14}C]Methyl iodide has been employed to produce labeled dimethylphenylethylamine from monomethylphenylethylamine (8). Labeled MME, DME and choline were produced from [^{14}C]ethanolamine in the presence of methyl iodide (9).

Shiff base formation between [^{14}C]acetaldehyde and phosphatidylethanolamine (10) or either L-serine or ethanolamine (11) followed with borohydride reduction produced the corresponding labeled N-ethyl derivatives.

This report describes the synthesis of [^{3}H]DME and [^{3}H]MME from a non radioactive base in a coupled reaction employing formaldehyde and Na B[^{3}H]$_4$.

MATERIALS

Sodium borohydride [^{3}H]$_4$ with a specific activity of 528 mCi/mole was purchased from N.E.N. (Boston, MA). Ethanolamine, dimethylethanolamine and monomethylethanolamine were purchased from Aldrich Chemical Co. (Milwaukee, WI). Non radioactive sodium borohydride and a 37% aqueous solution of formaldehyde were from Fisher Scientific (Fair Lawn, N.J.). Silica gel G 60 thin layer chromatography plates were from E. Merck (Darmstadt, Germany).

METHODS

Procedure for N methylation of ethanolamine or monomethylethanolamine

A 5 microliter sample of either ethanolamine (82 μmoles) or monomethylethanolamine (66 μmoles) was carefully placed on the bottom of a 10 x 75 mm test tube and 5 μ of a 37% aqueous formaldehyde (62 μmoles) was layered on the drop of the base, after gently mixing the tube was allowed to remain at ambient temperature for 1 hour. A 20 to 25 μl aliquot of a solution containing 25 mCi Na B[^{3}H]$_4$ dissolved in 0.01N NaOH was added to the mixture. The reaction vessel and contents remained in a vacuum hood for 30' at which

time an additional 20 to 25 µl of the $NaB[^3H]_4$ solution added. After 30 minutes 50 µl of a solution containing 36 mg of non radioactive sodium borohydride dissolved in 0.2 mls of 0.10 N NaOH was added and the tube remained in the hood for an additional 30 minutes. Sufficient 6N HCl, usually 50 µl to 75 µl, was then added to acidify the reaction mixture. This was determined either by pH measurements, complete gas evolution or precipitate formation. To the reaction mixture 1.5 mls of methanol was added and the contents mixed until a solution was obtained. Solvent was removed with a fine stream of nitrogen gas while the tube was placed in a water bath at 50°C. This methanol treatment was repeated 3 to 4 times assuring completely dissolving of the residues followed by a single cycle with absolute ethanol. The insoluble material was extracted by vigorous mixing with 0.7 mls of absolute ethanol and the tube centrifuged. The supernate was carefully removed. The insoluble material was extracted twice more and the ethanol supernates pooled and brought to dryness with nitrogen flow and warm water heating. The residue was dissolved in a known quantity of ethanol and aliquots removed for radioactivity determination.

Radioactive product(s) isolation

A silica gel TLC plate was prewashed overnight with a solvent composed of n-butanol-methanol-conc. HCl-water (50:50:10:10 v/v) and allowed to air dry in a hood. The ethanolic solution containing the radioactive products plus any non-radioactive precursors was applied as a single streak to the prewashed plate and appropriate standards applied. The solvent of n-butanol-methanol-con HCl-water (50:50:5:5 w/v) (12) was allowed to migrate to within 2 cm from the top of this plate. The plate was dried and the radioactive bands located with a thin layer chromatography radioscanner (Berthold Industries, Germany) and standards revealed by exposure to iodine vapor. The silica gel corresponding to the separate band(s) of radioactivity were removed, placed in 10 ml beakers and 5 mls. of a solution composed of methanol - glacial acetic acid - water (39:1:10) was added. The beaker and contents was placed on a magnetic mixer stirred for 30 minutes, and filtered through a medium scentered glass funnel. The silica gel was reextracted for

the same period of time with the same solution and the filtrate collected separately. Aliquots were removed for radioactivity determination and the remainder of the sample taken to dryness with nitrogen and warm water. The final residue was dissolved in a small amount of water, aliquots employed for TLC and the remainder stored at -20°C.

RESULTS

MME methylation

There was only a single radioactive product formed in the condensation between MME and formaldehyde with subsequent reduction employing sodium borotritide. This corresponded to DME and there was no choline produced. A typical yield was approximately 4 to 5 mCi of tritium introduced contained in approximately 33 μmoles of DME.

Ethanolamine methylation

There were two radioactive products formed in the condensation between Ea and formaldehyde with subsequent sodium borotritide reduction. These corresponded to MME and DME without any labeled choline being produced. A typical yield was 0.75 mCi of DME and 0.3 mCi of MME corresponding to approximately 33 μmoles and 11 μmoles respectively. A radioautogram of a thin layer chromatogram (Figure 1) shows that there was only a single radioactive material in each purified sample applied to the TLC plate.

Properties

There was no effect on product yield if ethanolamine hydrochloide was replaced the free base. Increasing the formaldehyde or ethanolamine concentration four fold the other remaining constant did not affect product yield.

DISCUSSION

A relatively simple and inexpensive procedure has been developed for the synthesis of tritium labeled DME and MME in reasonable yield. A major advantage is that under these conditions there is no choline produced which is in contrast to results employing methyl iodide (9). Therefore, starting with MME there is only a single product, DME, obtained and with ethanolamine both MME and DME are obtained.

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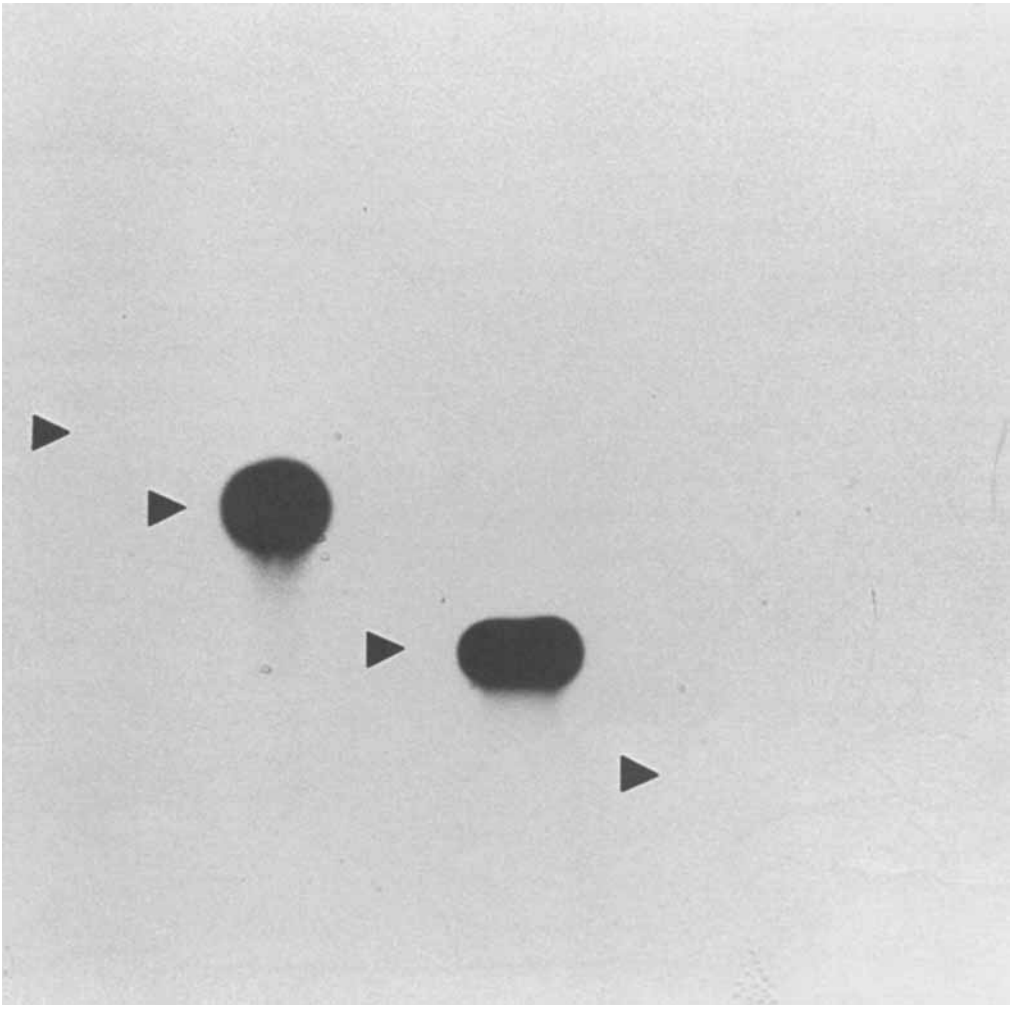

Fig. 1

Radioautogram of a TLC of purified products obtained by formaldehyde and sodium borotritide reaction with ethanolamine. Arrows indicate standard. Lanes from left to right ethanolamine standard, MME standard, [^{3}H]MME, DME Standard, [^{3}H]DME, choline standard.

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