NOTE

SYNTHESIS OF TRITIUM-LABELLED PHOSPHONIUM CHOLINE

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SUMMARY

Tritium-labelled phosphonium choline chloride ([2-hydroxyethyl]trimethyl phosphonium chloride) has been synthesised using base-catalysed hydrogen exchange of the hydrogen atoms attached to the carbon adjacent to the phosphonium phosphorus. Using $^{3}H_2O$ diluted in $^{2}H_2O$, during the exchange reaction, it is shown by proton-NMR that substitution of the methylene protons is almost complete when only 25% of the methyl protons have exchanged. Comparison of the measured specific activity (16.12mCi/mmol) with that calculated from the total proton exchange values shows that there is a large isotope effect favouring exchange to deuterium rather than tritium.

Key words :- tritiation, deuteration, hydrogen-exchange, quaternary phosphonium, choline, isotope effect.

INTRODUCTION

Using [1,2-^{14}C] phosphonium choline chloride ([2-hydroxyethyl] trimethyl phosphonium [1,2-^{14}C]choline chloride), it has been shown that the choline analogue is incorporated into phosphatidylcholine in cultured cells (1), rat tissues (2,3) and bacterial membranes (4). There are no apparent toxic effects and the choline analogue protects against the lesions of a choline-deficient diet in laboratory animals. The phosphonium analogue of phosphatidyl choline is a useful biosynthetically-incorporated ^{31}P - NMR probe of membrane phosphatidylcholine (3,5). However phosphonium choline is not readily incorporated into sphingomyelin (3). The tritiated compound has been prepared in order to continue investigation of the metabolism of phosphonium choline.

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Since quaternary phosphonium compounds undergo exchange of the α-hydrogen atoms in the presence of base (6), phosphonium choline should participate in both reactions (A) and (B).

(A) $(CH_3)_3P^+ CH_2CH_2OH + B^- \rightleftharpoons (CH_3)_3 P CHCH_2OH + HB$

(B) $(CH_3)_3 P^+ CH_2CH_2OH + B^- \rightleftharpoons (CH_3)_2CH_2 P CH_2CH_2OH + HB$

The tritiation of phosphonium choline chloride was carried out in ^3H_2O diluted in ^2H_2O to allow the course of the reaction to be followed by proton NMR and also to distinguish between exchange into the α-methylene (A) and α-methyl (B) positions.

EXPERIMENTAL

Tritiation of phosphonium choline chloride Phosphonium choline chloride (0.56 mmol), synthesised as described previously (2), in 0.1ml ^3H_2O (approx. 500 mCi) (Amersham International plc, Amersham, U.K.) was diluted with ^2H_2O (Ryvan Chemical Co., Southampton, U.K.) (0.9ml) containing sodium metal (1.1μmg/ml). The proton NMR spectrum of the reaction mixture was obtained immediately on mixing. The spectrum (Fig.1a) shows a pair of triplets (τ=6) from $(CH_3)_3P^+CH_2\underline{CH_2}OH$ protons and a pair of overlapping triplets (τ=7.5) from $(CH_3)_3P^+\underline{CH_2}CH_2OH$ protons. The doublet from the methyl protons is centred at τ=8.2. The proton NMR spectrum of the reaction mixture was recorded periodically. After 23 hours at 37°C, the spectrum showed a pair of triplets at τ=6, a slight signal at τ=7.5 and a pair of doublets at τ=8.2 (Fig.1b), which showed that the exchange reaction was almost complete for the methylene protons, and the reaction was stopped by acidification with 0.1M HCl (20ml). The product was freeze-dried and resuspended in 20ml of 0.1M HCl and freeze-dried again to remove any labile tritium.

Determination of radiochemical purity Two separation methods were used :-

a) paper chromatography with ethanol/ 1M ammonium acetate pH 5.0, 7/3 (v/v) as solvent system. Phosphonium choline chloride standard has an Rf of 0.73 in this solvent.

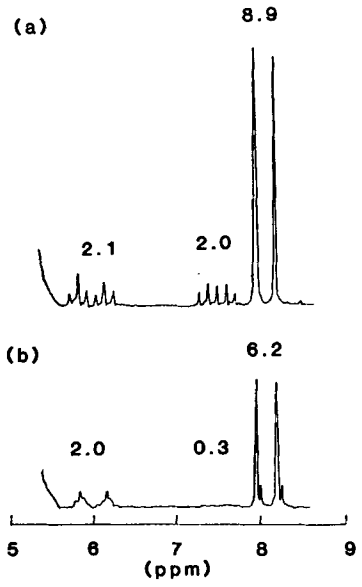

Fig.1. ^{1}H-NMR Spectra of Phosphonium choline chloride

a) at start of reaction and

b) after 23 hours at 37°C with ^3H_2O, ^2H_2O and sodium metal. Relative intensities of the peaks, obtained from electronic integration are shown above their respective resonances. The τ value is shown in ppm relative to Tetramethyl silane (τ =10).

b) paper ionophoresis with 2% (w/v) ammonium carbonate pH 8.9 as buffer. Under the conditions used (2000v, 45 min) standard phosphonium choline chloride migrates 17 cm towards the cathode.

In both methods, phosphonium choline chloride was detected as a brown spot on exposure to I_2 vapour. Radioactivity was detected by cutting 1 cm strips and eluting each 3 times with 0.5 ml of H_2O as follows :- Each strip was rolled into a 5 ml plastic tube with a pin hole at the bottom, supported in a 15 ml constricted centrifuge tube. The eluates were collected after centrifugation (2000g, 10 min) and samples were dried and measured in an LKB Wallac liquid scintillation counter, as previously described (1). In both systems, at least 97% of the radioactivity migrated with authentic phosphonium choline chloride.

Determination of the molar specific radioactivity of phosphonium choline chloride Incubation of phosphonium choline with perchloric acid for 1 hour at 210°C oxidises the phosphonium group such that it can be detected quantitatively by measuring the phosphorus content (Table 1).

Table 1. Determination of phosphonium choline chloride after oxidation

	μg phosphorus (calculated)	μg phosphorus (measured)
Expt. 1	2.04	1.95; 2.11
Expt. 2	4.08	3.80; 4.02

Dry samples of phosphonium choline chloride were incubated at 210°C with 1 ml of 72% perchloric acid behind a safety shield. The phosphorus content was then determined by the method of Bartlett (7).

Using this method, recovery of phosphorus in the tritiated product was determined as 0.5 mmol (89%) containing 8.06 mCi which corresponds to a molar specific radioactivity of 16.12 mCi/mmol.

The amount of incorporation is also calculated from the decrease in the total integration area of the proton NMR spectrum. The total integration area has decreased from 13.0 to 8.5. Thus 4.5 moles of hydrogen have exchanged per mole of phosphonium choline. If ^{2}H and ^{3}H exchange equally well, this would correspond to 0.45 moles of ^{3}H per mole of phosphonium choline chloride. With a recovery of 89% of phosphonium choline, the incorporation of radioactivity calculated in this way is 12.4 mCi, which represents 154% of the observed incorporation. Thus it appears that there is a considerable isotope effect in the exchange of the α protons. Although the observed isotope effect is very large, it is well-established that reaction rates are slower with the heavier isotopes of hydrogen (8,9).

DISCUSSION

Proton NMR spectra of isotopically labelled and parent compounds. After 23 hours of reaction with base, the proton signal from the methylene protons (reaction scheme (A) was reduced by over 85% (Fig.1a) and the signal of protons of the methyl groups (reaction scheme (B)) was reduced in intensity by 25% (Fig.1b). There are other differences in the proton NMR spectra of the starting material and the product.

1) In the product, there is a smaller coupling constant within the triplets of the signal from the β protons (τ=7.5). This is due to the weaker H-D spin-spin coupling constant ($J_{H,D}$) compared with the H-H spin-spin coupling constant ($J_{H,H}$). $J_{H,H}$ is a factor of 6.5 greater than $J_{H,D}$ (10).

2) In the tritiated/deuterated compound, the spectrum of the methyl protons has changed from a doublet to a pair of doublets and it is well-documented (11) that partial deuteration causes increased chemical shielding and can result in an upfield shift in the resonance position of the remaining protons.

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REFERENCES

1. Sim,E. & Pasternak,C.A. - Biochem. J. 154: 105 (1976).
2. Edwards,R.G. & Hands A.R. - Biochim. Biophys. Acta 431: 303 (1976).
3. Sim,E. & Cullis,P.R. - FEBS Lett. 79: 340 (1977).
4. Sim,E.-unpublished work.
5. Sim,E., Cullis,P.R. & Richards,R.E. - Biochem, J. 151: 555 (1975).

6. Beck,P. - Organic Phosphorus Compounds (Kosolapoff, G.M. & Maier, L. eds) Vol.2. Chap.4. Wiley Interscience (1972).

7. Bartlett,G.R. - J. Biol. Chem. 234: 466 (1959).

8. King,E.L. - Ann. Review Phys. Chem. 8: 273 (1957).

9. Wang,J.H., Robinson,C.V. & Edelman,I.S. - J.A.C.S. 75: 466 (1953).

10. Jackman,L.M. & Sternhell,S. - Applications of Nuclear Magnetic Resonance in Organic Chemistry. 2nd Ed. Pergamon Press, Oxford (1969).

11. Bovey,F.A. - Nuclear Magnetic Resonance Spectroscopy. Academic Press (1954).