THE SYNTHESIS OF N-[^{3}H] ACETYL MURAMYL DIPEPTIDES OF HIGH SPECIFIC RADIOACTIVITY

G. BASCHANG[+], D.E. BRUNDISH*,
A. HARTMANN[+], J. STANEK[+] and R. WADE*

*Research Centre, CIBA-GEIGY Pharmaceuticals Division,
Horsham, West Sussex, RH12 4AB, England and
[+]Research Department, CIBA-GEIGY Ltd.,
CH-4002, Basel, Switzerland

Abstract: N-[^{3}H]-Acetyl-muramyl-L-alanyl-D-isoglutamine has been synthesised by catalysed halogen/tritium exchange.

1. Introduction

N-Acetyl-muramyl-L-alanyl-D-isoglutamine (MDP) has been shown to be the smallest active molecule derived from mycobacterial peptidoglycan that can substitute for mycobacterial cells in Freund's complete adjuvant [1].

MDP has been synthesised labelled with ^{14}C at C-1 of the lactyl unit [2] and by using [U-^{14}C]-alanine [3] to give products of specific activities 42 and 164 mCi mmol^{-1} respectively and [6-^{3}H]-N-acetyl-muramyl dipeptide was prepared at a specific activity of 601 mCi mmol^{-1} from a photolytically-produced aldehyde by reduction with NaB^{3}H$_4$ [4].

Although it has been possible [2] to obtain metabolic and distribution data with these materials, we wished to prepare more highly radioactive molecules to allow investigations to be carried out at lower levels of MDP dosage and for more extended times. Using muramyl-L-alanyl-D-isoglutamine [5,6] and the

0362-4803/83/060691-06$01.00 Received December 14, 1982

corresponding compound in the nor-muramyl series [6], we were able to prepare materials with specific activities of 2-3 Ci $mmol^{-1}$ using [^{3}H]-acetic anhydride and with specific activities of 15 Ci $mmol^{-1}$ by iodoacetylation followed by catalytic dehalogenation using $^3H_2$ gas.

2. Materials and Methods

[^{3}H]-Acetic anhydride in toluene and $^3H_2$ gas were bought from Amersham International plc , Amersham, Bucks.

Compounds were examined by thin layer chromatography (t.l.c.) on precoated plates of silica gel (Merck F254) using the following solvent systems: A, acetonitrile:water = 3:1 (by vol.); B, ethyl acetate: n-butanol: pyridine: acetic acid: water = 42:21:21:6:10 (by vol.) and were then analysed with a Panax E.0111/XPD-05 scanner system. ^{3}H-FTNMR was performed on a Bruker WH90 96 MHz machine by Dr. J.R. Jones of the University of Surrey.

3. Experimental

3.1. Acetylation with [^{3}H]-acetic anhydride

Muramyl-L-alanyl-D-isoglutamine (12.0 mg) [6] was dissolved in water (1 ml and ethanol (5 ml) and 5% aq. $NaHCO_3$ (60µl) were added followed by [^{3}H]-Ac_2O (100 mCi, 5.5 Ci $mmol^{-1}$) in toluene (1 ml). The solution was kept at room temperature for 30 mins., N-HCl (40 µl) was added and the solution was evaporated to dryness. The residue was dissolved in water (0.5 ml) and applied to a column (50 x 0.7 cm) of Nucleosil $10C_{18}$ which was eluted at a flow rate of 5 ml min^{-1} with water: methanol: acetic acid (970:30:0.05 by vol.). The eluate was monitored at 210 nm and fractions (2.5 ml) were collected automatically. Fractions 6-9 and 17-23 representing the two anomers were combined and

evaporated to give 9.34 µmol (51%) of [^{3}H]-MDP with a specific activity of 2.32 Ci mmol^{-1} and radiochemical purity estimated by t.l.c. as 100.0 ± 0.1% (Solvent A) and 99.75 ± 0.1% (Solvent B).

The corresponding [^{3}H]-nor-MDP was prepared similarly in 58% yield with a similar specific activity (dependent upon the individual sample of anhydride used) with radiochemical purity estimated at 99.5 ± 0.1% (solvents A and B).

3.2. Preparation of N-iodoacetyl-MDP and -nor-MDP

The N-iodoacetyl compounds were prepared as in section 3.1. but using iodoacetic acid N-hydroxysuccinimide ester [7] in chloroform solution in place of the [^{3}H]-Ac_2O in toluene. Semi-preparative h.p.l.c. using water: methanol: acetic acid (930:70: 0.05 by vol.) gave the target compounds in 30-35% yield.

3.3. Tritiation using $^3H_2$ gas

N-Iodoacetyl-muramyl-L-alanyl-D-isoglutamine (3.16 µmol.) in water (0.5 ml) containing 5% aq. $NaHCO_3$ (60 µl) was stirred under $^3H_2$ gas (3.2 ml, 8 Ci) in the presence of 10% Pd/C (13 mg) for 40 mins. at room temperature. Catalyst was removed by filtration through a cellulose pad and N-HCl (40 µl) was added to the filtrate. The solution was evaporated to dryness, the residue redissolved in water (0.5 ml) and the product was purified by semi-preparative h.p.l.c. as described in section 3.1. [^{3}H]-MDP of specific activity 15.7 Ci mmol^{-1} was obtained in 48% yield with a radiochemical purity estimated as 99.5 ± 0.2% (Solvent A) and 99.0 ± 0.6% (Solvent B).

The corresponding [^{3}H]-nor-MDP was prepared analogously in 50% yield with a similar specific activity and radiochemical purity estimated at 99.3 ± 0.2% (Solvent A) and 99.4 ± 0.1% (Solvent B).

3.4. Exchange labelling

Synthetic N-acetyl-muramyl dipeptide was stirred with catalyst and $^{3}H_2$ gas under the conditions detailed in section 3.3. for a period of 40 mins. at room temperature. The material was purified as described previously and found to have a specific activity of 0.6 Ci $mmol^{-1}$. ^{3}H-FTNMR analysis indicated that 64% of the label was at the N-acetyl and hemi-acetal positions of the sugar.

4. Discussion

N-Acetyl-muramyl dipeptide and the corresponding nor-muramyl analogue have been synthesised labelled with ^{3}H using the known unacetylated muramyl dipeptide [5] and its nor-muramyl analogue [6] as starting materials. Products with specific activities of 2-3 Ci $mmol^{-1}$, higher than any previously published, could be prepared using [^{3}H]-acetic anhydride. These molecules have been used for studies of metabolism and distribution [8].

By iodoacetylation of the bases followed by catalytic tritiation, products with specific activities of 15 Ci $mmol^{-1}$ were prepared. It was inferred from a catalytic exchange labelled experiment followed by a ^{3}H-FTNMR analysis that the products prepared via deiodination probably contain 96.6% of the ^{3}H in the N-acetyl group, 1.9% at the hemi-acetal position and 1.5% in many unspecified locations. Labelling at the hemi-acetal position by exchange has been reported for simple sugars [9], the label is known to be stable at neutral pH [10]. We confirm that our products contain no ^{3}H which is labile at physiological pH, indicating that the label located in the acetyl group is also stable under these conditions.

All the labelled products described were better than 99% radiochemically pure. On storage at -196° in water at a concentration 5 mCi ml^{-1}, decomposition was approx. 10% in a year, as judged by t.l.c. and use of the Panax scanner system.

Acknowledgement

We thank Mr. B.E. Evans for amino acid analyses.

REFERENCES

1. Ellouz, F., Adam, A., Ciobaru, R. and Lederer, E. - Biochem. Biophys. Res. Commun. 59: 1317-1325 (1974).

2. Parant, M., Parant, F., Chedid, L., Yapo, A., Petit, J.F. and Lederer, E. - Int. J. Immunopharmac. 1: 35-41 (1979).

3. Kusumoto, S., Ikenaka, K. and Shiba, T. - Tetrahedron Lett., 4055-4058 (1977).

4. Durette, P.L., Rosegay, A., Walsh, M.A.R. and Shen, T.Y. - Tetrahedron Lett., 291-294 (1979).

5. Kiso, M., Kaneda, Y., Okumura, H., Hasegawa, A., Azuma, I. and Yamamura, Y. - Carbohydrate Res. 79: C17-C19 (1980).

6. Baschang, G. and Stanek, J. (unpublished).

7. Slotin, L.A. and Hampton, A. - Nucleic Acid Chem. 2: 843-846 (1978).

8. Ambler, L., Hudson, A.M. and McMartin, C. - Int. J. Immunopharmac. 2: 211 (1980).

9. Evans, E.A., Sheppard, H.C., Turner, J.C. and Warrell, D.C. - J. Labelled Compounds 10: 567-586 (1974).

10. Isbell, H.S., Frush, H.L. and Holt, N.B. - Anal. Chem. 33: 225-226 (1961).