SYNTHESIS OF [2-^{14}C(U)TYROSINE]ARGININE VASOTOCIN WITH HIGH SPECIFIC RADIO AND BIOLOGICAL ACTIVITIES

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SUMMARY

The synthesis of [2-^{14}C(U)tyrosine]arginine vasotocin at a specific activity of 102 mCi/mMole was performed using the solid-phase approach of Merrifield for peptide synthesis. Following purification by gel filtrations the labeled hormone was recovered at an average yield of 40% with an avian vasodepressor activity of 408 $\pm$ 24 U/mg.

Key Words: Arginine vasotocin, neurohypophyseal hormones, carbon-14, solid-phase peptide synthesis, peptide

INTRODUCTION

Synthetic vasopressin and oxytocin molecules with ^{14}C-labeled amino acid residues in specific loci (1,2) have played not only an important role in the discovery of sites in neurohypophyseal hormones susceptible to enzymatic attack (3-8) but also during the isolation as well and characterization of the proteases responsible (9-11).

It is now planned to extend these kind of studies to arginine vasotocin (AVT) which is found both in non-mammalian (12,13) and mammalian species (14-17). Therefore, the synthesis of AVT, specifically labeled with [^{14}C(U)]tyrosine in postion 2, was undertaken using the solid-phase approach of Merrifield (18).

EXPERIMENTAL AND RESULTS

Nomenclature is in accord with IUPAC-IUB rules on Biochemical Nomenclature, Biochem. J. 126, 773 (1972); and J. Biol. Chem. 242, 555 (1967). All optically active amino acids are of the L configuration. The following ab-

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breviations are used: AVT, arginine vasotocin; Boc, t-butyloxycarbonyl; DMF, dimethylformamide; tlc, thin layer chromatography. All tlc were run on Merck silca gel plates and the compounds were detected by radiochromatogram scanning, ninhydrin or the method of H. Zahn and E. Rexroth (19). Solvent systems used for tlc: A, ethanol:water (7:3, v/v); B, trifluoroethanol:water (7:3); C, n-butanol:acetic acid:water (4:1:1); D, n-butanol:pyridine:acetic acid:water (15:10:3:6); E, n-butanol:pyridine:water (20:10:11). The biological activity of AVT was measured against the U.S.P. Posterior Pituitary Reference Standard by a four-point design.

Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-Resin (1). Boc-Glycine was esterified (20) to chloromethylated (0.75 meq Cl/g resin) polystyrene resin, 1% cross-linked with divinylbenzene, with a substitution of 0.55 meq Gly/g of resin as determined by hydrolysis with 6N HCl in propionic acid (conc. hydrochloric acid:propionic acid, 1:1, v/v) (21) followed by amino acid analysis (22). An individual cycle for the incorporation of an amino acid was as follows: three rinses with methylene chloride (10 ml of solvent/g of resin for two min, except as noted) removal of the Boc group with trifluoroacetic acid (48% in 2% anisol and 50% methylene chloride) for 30 min; five rinses with methylene chloride; two washes with 7% diisopropylethylamine, the second being for 10 min; four rinses with methylene chloride; addition of the Boc-amino acid (2.5 equivalents) and 1-hydroxybenzotriazole (23) (5 equivalents) in 1/2 volume of DMF and dicyclohexylcarbodiimide (24) (2.5 equivalents) in 1/2 volume of methylene chloride for 2 h; three rinses with DMF and three rinses with ethanol. The resin was then checked for complete acylation with the respective amino acid by ninhydrin (25) and if warranted the coupling was repeated.

Boc-^{14}C(U)Tyr (2). ^{14}C(U)Tyrosine (50 mCi, 0.109 mmole, 460 mCi/mmole, Schwartz-Mann) was examined by tlc in three solvent systems (R_fA, 0.63; R_fB, 0.41 and R_fC, 0.37) and was found to be 98% radiochemically pure. The label-

ed amino acid was diluted to a specific radioactivity of 102 mCi/mmole with unlabeled tyrosine (69.2 mg). The primary amino group was protected with the Boc group using S-Boc-4,6-dimethyl-2-mercaptopyrimidin-2-yl thiol carbonate by the method of Nagasawa et al. (26). The reaction was followed by tlc and the product was compared with an authentic sample of Boc-Tyr, R_fA 0.81, R_fC 0.78. Based on radioactivity the yield of this reaction was 98%.

Cys(Bzl)-^{14}C(U)Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)Gly-NH_2 (3). Compound 2 was dissolved in 5 ml of DMF and coupled to 1 by the method described above, except that 1.5 rather than 2.5 equivalents of 2 were used. The resultant protected octapeptide was elongated to the protected nonapeptide which was then partially deprotected with trifluoroacetic acid. The protected peptide was removed from the resin by ammonolysis, extracted with DMF and precipitated with water. The precipitated peptide was dissolved in DMF to 3.64 mg/ml (as determined on the basis of specific radioactivity of the peptide).

[2-^{14}C(U)tyrosine]AVT (4). Prior to deprotection with sodium in liquid ammonia (27), 6 ml aliquots of peptide 3 solution were evaporated to dryness, the residue rinsed with ethyl acetate and ether, and dried in vacuo over NaOH for 48 h. After deprotection the ammonia was allowed to evaporate under a nitrogen stream and the residue was dissolved in 125 ml methanol:water (1:1 v/v, previously deaerated). Oxidation was performed with 1.1 equivalents of diiodoethane (28). The solution was made acidic with 1 ml glacial acetic acid and evaporated to a small volume. Purification was attained by two gel filtrations on columns (113 x 0.9 cm) of Sephadex G-15 (particle size 40-120μ). The first utilized 50% acetic acid at a flow rate of 1 ml/h, the second 0.2 M acetic acid at a flow rate of 2 ml/h (29). The hormone was detected by scintillation counting (Beckman LS-355 Liquid Scintillation Spectrometer, scintillation coctail "Dioxol" from Yorktown Research) and found in the eluates at 35% and 65% of the column volumes respectively. The appropriate

fractions were pooled and diluted to 100 ml. On the basis of radioactivity, the concentration of AVT was determined to be 0.05 mg/ml; calculated yield, 5 mg of purified hormone.

An aliquot was examined by tlc in 2 systems, R_fD 0.33, R_fE 0.15 and found to give a single spot by radiochromatogram scanning (Packard model 7201) that was identical with an authentic standard simultaneously chromatographed. Electrophoretic analyses were carried out at pH 3.5 and 6.5 in 1% pyridine-acetic acid buffers at 2500 V for 1.5 h. The ratios, expressed relative to the dye methylene green, were identical with those of a standard of unlabeled AVT: pH 3.5, migration of 109 mm, single spot, R_{dye} 0.66; pH 6.5, migration of 64 mm, single spot, R_{dye} 1.64. The solution was bioassayed for avian vasodepressor activity (30,31) and was found to be 20.4 $\pm$ 1.2 U/ml; thus the biological activity of AVT amounts to 408 $\pm$ 24 U/mg. After bioassay, the hormone was concentrated to 0.1 mg/ml in 0.2 M acetic acid (2.002 x 10^7 cpm/ml), and 4-chlorobutanol was added as a preservative. The solution was ampouled and stored at 4° C.

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REFERENCES

1. Hoffman P.L. and Walter R. - FEBS Lett. 66: 176 (1976)
2. Walter R. and Havran R.T. - Experientia 27: 645 (1971)
3. Walter R. and Hoffman P.L. - Biochim. Biophys. Acta 336: 294 (1974)
4. Walter R. and Bowman R.H. - Endocrinology 92: 189 (1973)
5. Walter R. - in: Peptides 1972; Proc. 12th Europ. Peptide Symp. pp. 363 (H. Hanson and H.D. Jakubke, eds.) Elsevier, Amsterdam, 1973
6. Marks N. Abrash L. and Walter R. - Proc. Soc. Exp. Biol. and Med. 142: 455 (1973)
7. Shlank H. and Walter R. - Proc. Soc. Exp. Biol. and Med. 141: 452 (1972)
8. Walter R. and Shlank H. - Endocrinology 89: 990 (1971)
9. Yoshimoto T. Orlowski R. and Walter R. - Biochemistry, in press (1977)
10. Koida M. and Walter R. - J. Biol. Chem. 251: 7593 (1976)
11. Walter R. - Biochim. Biophys. Acta 422: 138 (1976)
12. Acher R. - in: Polypeptide Hormones, Molecular and Cellular Aspects, Ciba Foundation symposium 41 (R. Porter and D.W. Fitzsimons, eds.) pp. 31, Elsevier, Amsterdam, 1976
13. Sawyer W.H. - in: Handbuch der Experimentellen Pharmakologie, (B. Berde, ed.) Vol. 23, pp. 717, Springer-Verlag, Berlin, 1968
14. Legros J.J. Louis F. Demoulin A. and Franchimont P. - J. Endocr. 69: 289 (1976)
15. Rosenbloom A.A. and Fisher D.A. - Endocrinology 96: 1038 (1975)
16. Cheesman D.W. and Farris B.L. - Proc. Soc. Exp. Biol. and Med. 133: 1254 (1970)
17. Milcu S.M. Pavel S. and Neacsu C. - Endocrinology 72: 563 (1963)
18. Merrifield R.B. - in: Rec. Prog. Horm. Res. 23: pp. 451 (G. Pincus, ed.) Academic Press, New York, 1967
19. Zahn H. and Rexroth E. - Z. Analyt. Chem. 148: 181 (1955)
20. Gisin B.F. - Helv. Chem. Acta 56: 1476 (1973)

21. Westall F. and Hesser H. - Anal. Biochem. 61: 610 (1974)

22. Spackman D.H. Stein W.H. and Moore S. - Anal. Chem. 30: 1190 (1958)

23. Konig W. and Geiger R. - Chem. Ber. 103: 788 (1970)

24. Sheehan J.C. Goodman M. and Hess G.P. - J. Am. Chem. Soc. 78: 1367 (1956)

25. Kaiser E. Colescott R.L. Bossinger C.D. and Cook P.I. - Anal. Biochem. 34: 595 (1970)

26. Nagasawa T. Kuroiwa K. Narita K. and Isowa Y. - Bull. Chem. Soc. Japan 46: 1269 (1973)

27. Sifferd R.A. and du Vigneaud V. - J. Biol. Chem. 180: 753 (1935)

28. Weygand F. and Zumach G. - Z. Naturforsch. B. 17: 807 (1962)

29. Manning M. Wuu T.C. and Baxter J.W.M. - J. Chromatogr. 38: 396 (1968)

30. Coon J.M. - Arch. Int. Pharmacodyn. 62: 77 (1939)

31. Munsick R.A. Sawyer W.H. and van Dyke H.B. - Endocrinology 66: 860 (1960)