SPECIFIC TRITIATION OF OXYTOCIN BY CATALYTIC DEIODINATION

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The availability of isotopically labeled polypeptide hormones would be of obvious value for many biochemical and metabolic studies. Because of its complexity, de novo synthesis of a polypeptide with a skeleton-labeled amino acid, (du Vigneaud, et al., 1962) has limited application at the present time. Tritium labeling by the Wilzbach method has yielded randomly labeled polypeptides of low specific activity, (du Vigneaud, et al., 1962: Von Holt, Voelker and Von Holt, 1960) and current catalytic exchange methods, which require elevated temperature for efficient tritiation, are deleterious to the heat-labile polypeptides. We have recently investigated an old technique for catalytic deiodination of insulin (Harrington and Neuberger, 1936) using tritium in place of hydrogen for tritiation of several tyrosine-containing polypeptides (Dingman, et al., 1963). This report summarizes our experience with tritiation of oxytocin (OT) by a modification of this method.

Twenty mg. (20 micromoles) synthetic OT (430 units/mg.) in 0.25% aqueous acetic acid (HAc) were iodinated (Lee and Wiseman, 1959) with 20 micromoles ^{127}I containing tracer quantities of ^{131}I. After extraction of the solution with 5 x 2 vol. cyclohexane, the I-OT was found to contain 1.0 micromole I/micromole hormone, representing quantitative incorporation of the iodide. To 16 mg. I-OT in 4 ml. dilute HAc was added 16 mg. palladium black

and the solution was shaken for 16 hrs. at room temperature with 5 curies ^{3}H gas (performed by the New England Nuclear Corp.). The evacuated and centrifuged solution was transferred to an IRC 50-XE 64 cation (H^{+} form) exchange resin column 0.7 x 30 cm., the column washed with 50 ml. distilled water, and the cationic ^{3}H-OT eluted with 40 ml. 50% HAc. The eluate contained 10.8 mg. peptide (Folin-Lowry) and 1.84 millicuries ^{3}H/mg.

This material showed one tritiated peptide with the cathodic mobility of the OT standard on paper electrophoresis in pyridine acetate buffer pH 6.5 (400 v., 6 hrs.). However, descending chromatography on Whatman 1 paper in n-butanol-acetic acid-water (4-1-5) showed a radioactive peptide at the R_f of OT and several faster running compounds.

The entire sample was then chromatographed on a Sephadex A 25 column 0.7 x 120 cm., using 1.0 M pyridine acetate, pH 7.0 for elution. Two tritiated peptides (I and II, Fig. 1) were eluted within the inner volume of the gel, whereas the original oxytocin prior to tritiation showed only one peptide peak with the mobility of Fr. II. Fr. I was found to be biologically inactive (avian depressor and rat uterus assays) and Fr. II had a bioactivity of 475 units/mg. These findings suggested that Fr. II represented tritiated oxytocin monomer(s) and Fr. I a biologically inactive, larger molecular moiety probably composed of oxytocin polymer(s) formed during tritiation.

Treatment of Fr. I with sodium thioglycolate to disrupt disulfide bonds resulted in slowing of the mobility of this peptide on Sephadex to the position of Fr. II. This finding suggested that polymer formation may have been caused by a reductive action of tritium gas on the disulfide of the oxytocin monomer, the cysteinyl derivatives so formed subsequently polymerizing in the concentrated solution by a process of intermolecular disulfide bonding (Boissonnas, 1960).

Paper electrophoresis of Fr. II in pyridine acetate pH 6.5 revealed one tritiated peptide with the mobility of OT (Fig. 1A) but 2 tritiated

compounds were apparent on paper chromatography, one of which had the R_f of OT (Fig. 1B).

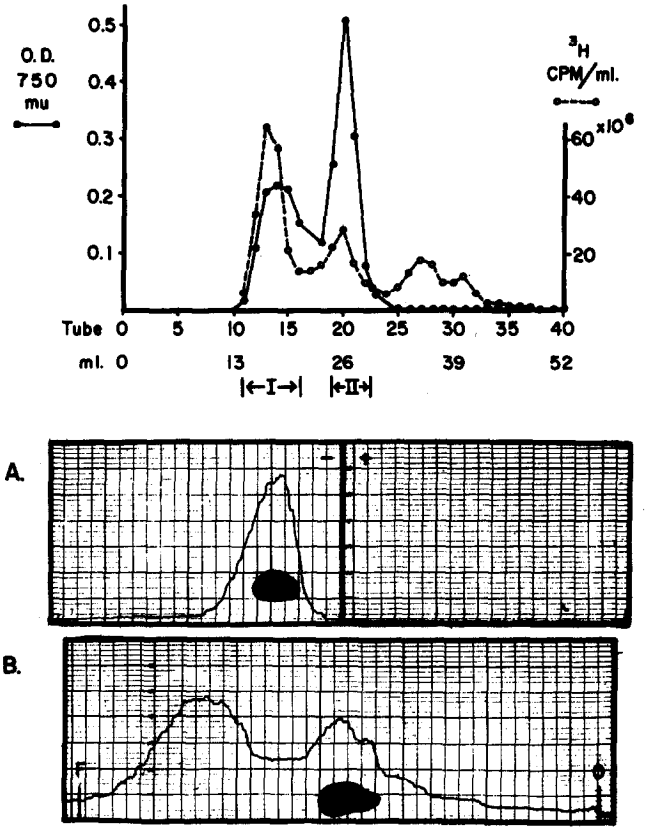

Fig. 1. Gel Filtration of Tritiated Oxytocin. DEAE Sephadex (A25 Fine) column eluted with 1.0 M pyridine acetate buffer pH 7.0 Volumes of 0.05 ml. for Folin-Lowry color, 750 mu (●——●); 0.01 ml. for tritium assay (·----·). A = Paper Electrophoresis of Fraction II, pyridine acetate pH 6.5, 400 V, 6 hrs.
B = Descending paper chromatography of Fraction II.
The position of Folin positive peptide is superimposed on the radiochromatograms.

Fr. II was then chromatographed on a carboxymethylcellulose column (Light, Acher and du Vigneaud, 1957). Two tritiated peptides were eluted from the CMC column (Fig. 2) but paper chromatography showed that peptide A had the R_f of the faster running component in Fr. II (Fig.1B). This

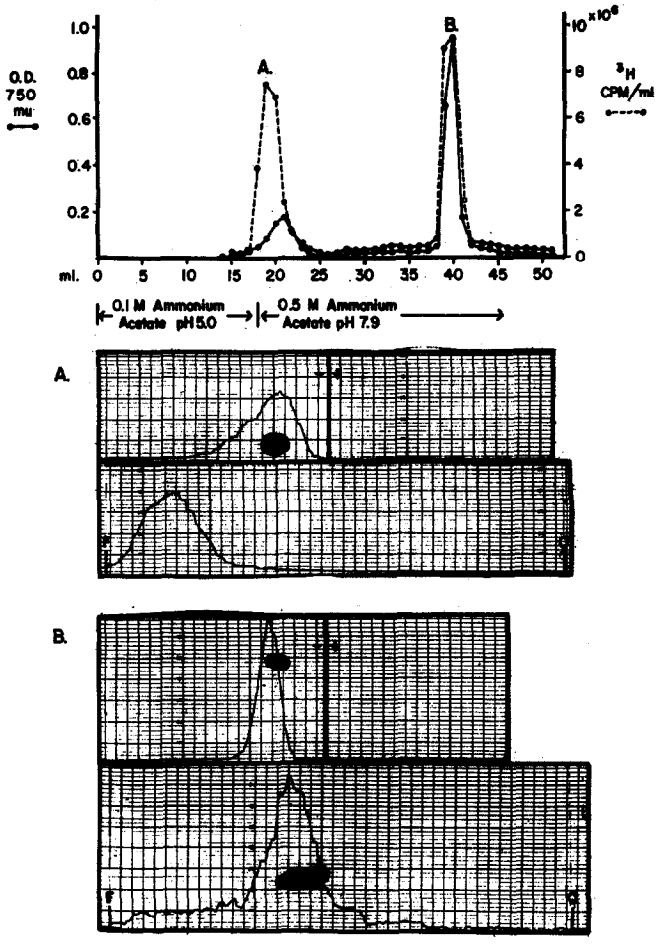

Fig. 2. Chromatography of Fraction II on Carboxymethylcellulose. 0.7 x 60 cm. column equilibrated and eluted with 0.1 M ammonium acetate buffer pH 5.0. Gradient elution with 0.5 M ammonium acetate pH 7.9 begun at 16 ml. elution volume. Optical density at 275 mu. (•——•), tritium counts {•----•} on 0.01 ml. The lower portion of the figure shows the electrophoretograms above and the paper chromatograms below for peptides A and B.

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compound was found to be biologically inactive. Paper chromatography of peptide B showed one tritiated peptide with the R_f of OT (Fig. 2B). This fraction contained 2.36 mg. peptide, which had a bioactivity of 520 units/mg. and a specific radioactivity of 228 microcuries/mg. After one month of storage in 0.25% aqueous HAc, re-chromatography of peptide B on Sephadex A25

(see Fig. 1) showed a small tritium peak (about 5%) devoid of peptide with the elution volume of Fr. I and the bulk of the biologically intact tritiated peptide appearing in the position of Fr. II.

Iodination of a sample of peptide B resulted in the removal of 92% of the tritium from the hormone. Ascending paper chromatography in n-butanol-acetic acid-water (4-1-5) of acid hydrolysates (6N HCl, 110° C, 72 hrs.) of peptide B and the ^{3}H-OT "polymer" (Fr. I, Fig. 1) showed tyrosine to be the only tritiated amino acid present. On several occasions, multiple lyophilizations from dilute HAc of aliquots of peptide B failed to reveal the formation of labile tritium even after 2 months of storage. These studies provided evidence for the specific tritiation of tyrosine both in the oxytocin monomer as well as in the polymerized material. The C-^{3}H bond appeared to be stable in dilute acid. This tritiation technique was reproducible in a second experiment, with essentially similiar results being obtained. Attempts are now being made to increase specific activity by modification of the basic method.

The fact that this radiochromatographically homogeneous ^{3}H-OT had a bioactivity as high as du Vigneaud's synthetic ^{3}H-OT after 1000 tube countercurrent distribution attests to the precision of these standard purification techniques. Despite the use of 12 curies ^{3}H leucine, the ^{3}H-OT synthesized by du Vigneaud had a specific radioactivity of only 130 uc/mg. which is much too low for human use, and limited to biochemical and pharmacological (Aroskar, et al., 1964) studies. This simple and specific external exchange method may make possible the preparation of tritiated polypeptides of high specific activity for biochemical and metabolic studies within the physiological range.

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