Synthesis of Oxytocin: Suppression of Dimer Formation

Recent work in this laboratory on posterior pituitary hormones necessitated the synthesis and isolation of oxytocin. To maximize the yield of hormone we have modified the procedure of cyclization in such a way as to reduce the formation of dimers. The last step in the synthesis of oxytocin involves cyclization of oxyticeine by oxidation[1,2]. *Intra*molecular oxidation of the linear dithiol intermediate gives oxytocin, whereas *inter*molecular oxidation between 2 oxytoceine molecules leads to the formation of a mixture of approximately equal amounts of isomeric α- and β-dimers[3] (Figure). Both isomers exhibit a low oxytocic activity of the order of 1 unit/mg[3], compared with approximately 500 units/mg for oxytocin.

In previous experiments, N-benzyloxycarbonyl-S, S'-dibenzyl-oxytoceine, prepared by the stepwise *p*-nitrophenyl ester method[4], was reduced with sodium in liquid ammonia[5]. The ammonia was allowed to evaporate spontaneously and the dithiol was oxidized to the disulfide with a solution of potassium ferricyanide[6]. The amount of ferricyanide required to oxidize all of the thiol present was indicated by a bright yellow colour when a slight excess was present; the amount of ferricyanide consumed in previous experiments was always approximately 60–70% of the theory, based on the amount of thiol which could be formed from the protected nonapeptide intermediate. A considerable amount of aireal oxidation, presumably occurred before the titration with potassium ferricyanide was carried out. The *inter*molecular oxidation reaction is more likely to occur in solutions containing a high concentration of oxytoceine such as pertains in the residue after the removal of liquid ammonia. If dimer formation were to occur mainly in the time between the removal of ammonia from oxytoceine and the titration of its aqueous solution with potassium ferricyanide, exclusion of oxygen during that stage would be of primary importance in the prevention of undesirable side reactions. With this in mind, we modified the procedure for the cyclization of oxytoceine to oxytocin as follows.

Materials and methods. In a typical experiment the protected nonapeptide N-benzyloxycarbonyl-S, S'-dibenzyl-oxytoceine (1 g) was reduced with sodium (stick method[2]) in liquid ammonia (800 ml) at –33° until a blue colour persisted for 10 sec. Glacial acetic acid (2 ml) was added and the ammonia was evaporated as quickly as possible by immersing the flask in water at 30–40° and passing a rapid stream of dry, oxygen-free nitrogen through the flask. The solid, white residue was dissolved in freshly distilled, deaerated water (1.5 l; disulfydryl concentration 0.5 mg/ml) and the flow of nitrogen was maintained until the solution (pH 6.8) had been treated with 0.02 *M*-potassium ferricyanide (70 ml). Excess ferri- and ferrocyanide ions were removed on a column (2×7 cm) of Dowex 2×8 (200–400 mesh; Cl^--form), the effluent was concentrated to approximately 70 ml and subjected to countercurrent distribution (625 transfers) in the system *n*-butanol: *n*-propanol: 0.05% acetic acid (2:1:3) to separate oxytocin ($K = 0.36$) from the mixture of dimers ($K = 0.19$). Isolation by lyophilization of the material from the 2 peaks gave pure hormone (474 mg; 62%) and a mixture of dimers and sodium acetate (386 mg). The crude dimeric material was purified by gel filtration on a column (2×150 cm) of Sephadex G-25 in 2 *N* acetic acid at a flow rate of 25 ml/h. The peptide was detected in aliquots of column fractions by the Folin-Lowry procedure[7] and the selected fractions were lyophilized to give the salt-free mixture of dimers (43 mg). Bioassay[8,9] on the hormone revealed an oxytocic activity of 532 ± 20 ($n = 3$) IU/mg. The moisture content of the lyophilized powder was 4.2% (P_2O_5; 50°/2 mm). Found: C, 50.9; H, 6.9; N, 16.4; S. 6.2. Calc. for $C_{43}H_{68}N_{12}O_{12}S_2$: C, 51.2; H, 6.8; N, 16.7; S, 6.3%. Amino acid analysis of an acid hydrolisate gave the following molar ratios, with the value for proline taken as 1.0: ammonia, 3.0; aspartic acid, 1.1; glutamic acid, 1.0; proline, 1.0; glycine, 1.0; half-cystine, 2.0; isoleucine, 0.9; leucine, 1.1; and tyrosine, 0.9. An acid hydrolisate of the dimeric material had an amino acid composition identical with that of the hormone.

Results and discussion. In all about 95% of the thiol was accounted for by oxidation by ferricyanide, i.e., 25–35% more than in experiments where no precautions were taken to avoid oxidation by atmospheric oxygen. The weight ratio of oxytocin to the mixture of dimers was 11:1, compared with the results of previous experiments where the weight ratio of hormone to dimers was of the order of 4:1 (similar result obtained by Yamashiro, Hope, and du Vigneaud[3]), thus the formation of dimer was reduced 3-fold. This finding indicates that oxytoceine can be oxidized to monomeric oxytocin in high yields, providing that precautions are taken to avoid premature oxidation of the dithiol intermediate by atmospheric oxygen; we believe that this reaction is mainly responsible for the formation of oxytocin dimers during the synthesis of oxytocin.

Zusammenfassung. Die Bildung von Oxytocin-Dimer (α+β), einem Nebenprodukt der Oxytocin-Synthese, wird verringert durch Ausschluss atmosphärischen Sauerstoffes während der Zyklisation von Oxytocein zu Oxytocin. Das Gleichgewichtsverhältnis von Monomer zu Dimer wird durchschnittlich von 4:1 auf 11:1 verbessert.

M. Wälti and D. B. Hope

University Department of Pharmacology, South Parks Road, Oxford OX1 3QT (England),
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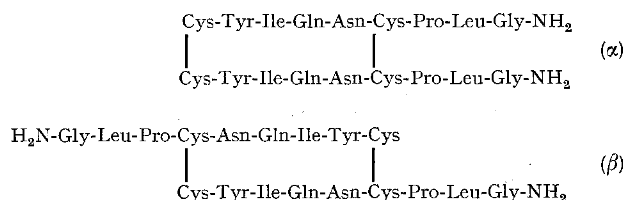

Parallel (α) and antiparallel (β) dimers of oxytocin.

1 V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis and S. Gordon, J. Am. chem. Soc. *75*, 4879 (1953).
2 V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts and P. G. Katsoyannis, J. Am. chem. Soc. *76*, 3115 (1954).
3 D. Yamashiro, D. B. Hope and V. du Vigneaud, J. Am. chem. Soc. *90*, 3857 (1968).
4 M. Bodanszky and V. du Vigneaud, J. Am. chem. Soc. *81*, 5688 (1959).
5 R. H. Sifferd and V. du Vigneaud, J. biol. Chem. *108*, 753 (1935).
6 D. B. Hope, V. V. S. Murti and V. du Vigneaud, J. biol. Chem. *237*, 1563 (1962).
7 O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).
8 P. Holton, Br. J. Pharmac. *3*, 328 (1948).
9 R. A. Munsick, Endocrinology *66*, 860 (1960).