[Chem. Pharm. Bull. 27(12)3199—3201(1979)]

UDC 547.466.1.04.09 : 615.225.2.011.5.015.4

Synthesis of an Octacosapeptide Amide corresponding to the Entire Amino Acid Sequence of Chicken Vasoactive Intestinal Polypeptide (VIP)

The octacosapeptide amide corresponding to the entire amino acid sequence of chicken vasoactive intestinal polypeptide (VIP) was synthesized using a new arginine derivative, N^G-mesitylene-2-sulfonylarginine. A great tendency of ring closure in the Asp–Asn sequence during the acidolytic deprotection was noted.

Keywords——synthesis of chicken vasoactive intestinal polypeptide; N^G-mesitylene-2-sulfonylarginine; reduction of Met(O) with thiophenol; aminosuccinimide formation of Asp–Asn by methanesulfonic acid or HBr; less tendency of aminosuccinimide formation of Asp–Asn by HF or trifluoroacetic acid; vasodilator action of synthetic chicken VIP

The structure of chicken vasoactive intestinal polypeptide (VIP) was determined by Nilsson[2] in 1975. We have synthesized the octacosapeptide amide corresponding to the entire amino acid sequence of this avian intestinal hormone by an approach (Chart) different from those employed for "*in situ*" synthesis of chicken VIP[3] and for the synthesis of structurally related porcine VIP by Bodanszky *et al.*[4] and Sakagami *et al.*[5]

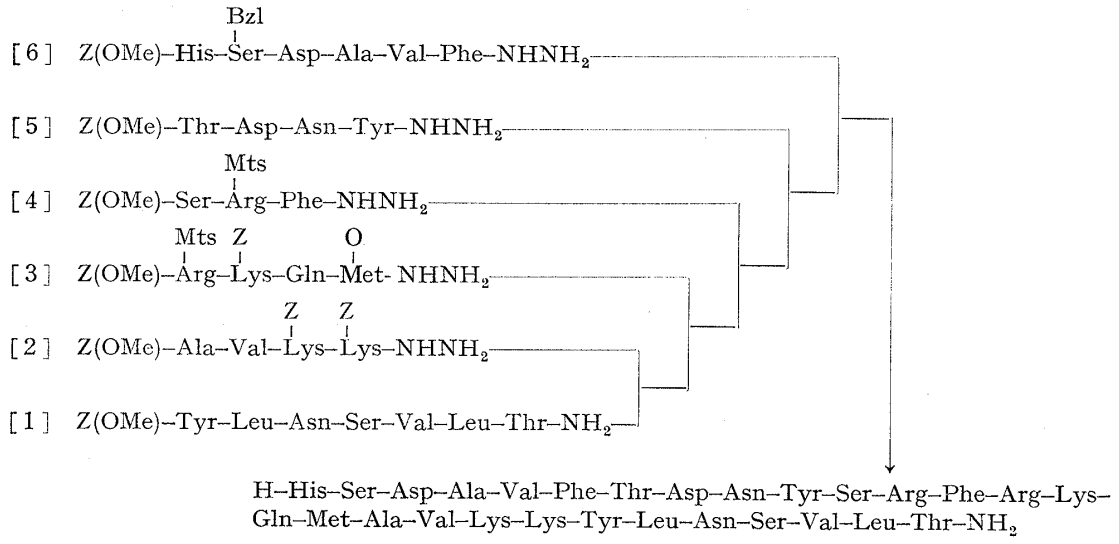

Chart 1. Synthetic Route to Chicken Vasoactive Intestinal Polypeptide

Besides Lys(Z), a new arginine derivative, Arg(Mts)[6] bearing an acidolytically removable protecting group, was employed. The Met residue was protected as the (±) sulfoxide[7]

1) Amino acids, peptides and their derivatives are of the L-configuration. The following abbreviations were used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Mts=mesitylene-2-sulfonyl, TFA=trifluoroacetic acid, DCC=dicyclohexylcarbodiimide, HOBT=N-hydroxybenzotriazole, DMF=dimethylformamide.
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7) B. Iselin, *Helv. Chim. Acta*, **44**, 61 (1961).

by oxidation with sodium metaperiodate.[8] The TFA labile Z(OMe) group[9] served as the temporary N^{α}-protection of six peptide fragments, which were selected as building blocks. These fragments were synthesized in a conventional manner by known amide forming reaction. Z(OMe)–Arg(Mts)–OH was incorporated smoothly into two fragments, [3] and [4], by DCC in the presence of HOBT.[10] For preparation of the homogeneous fragment [5] and [6], it was necessary to remove the β-benzyl ester protecting group from the two Asp residues linked to Asn and Ala respectively by hydrogenolysis, after their incorporation. Otherwise succinimide derivatives formed during the next coupling reactions.[11] The hydroxyl group of the Ser residue (position 2) located between His and Asp was protected by the benzyl group. Otherwise, it was difficult to achieve satisfactory incorporation of the fragment [6].

The fragments thus obtained were then successively assembled by the Honzl and Rudinger azide procedure.[12] Synthesis of the Boc-derivative of the c-terminal undecapeptide amide was already reported by Bodanszky *et al.*[13] All intermediates were purified by precipitation from DMF with methanol and the final protected octacosapeptide amide (mp 290° dec., $[\alpha]_D^{27}$ +2.6° in DMF, amino acid ratios in acid hydrolysate: Asp 3.85, Thr 1.75, Ser 2.31, Glu 1.10, Ala 1.97, Val 3.00, Met+Met(O) 0.89, Leu 2.00, Tyr 1.79, Phe 1.77, Lys 3.11, His 0.93, Arg 1.84. *Anal.* Calcd. for $C_{206}H_{285}N_{43}O_{56}S_3 \cdot 8\,H_2O$: C, 54.98; H, 6.74; N, 13.39. Found: C, 54.96; H, 6.60; N, 13.61) by gel-filtration on Sephadex LH-60 with DMF as eluent. Homogeneity of all protected peptides were assessed by tlc, elemental analysis and amino acid analysis after acid hydrolysis (with phenol).[14]

Our preliminary tests indicated that deprotection of fragment [5] with methanesulfonic acid (MSA)[15] gave a product with low recovery of Asp after aminopeptidase digestion. The possibility of succinimide formation even in Asp residues with free β-carboxyl group was previously predicted.[16] Recovery of Asp adjacent to Asn was found to depend on deprotecting reagents employed. Hydrogen bromide deprotection exhibited a tendency similar to that of MSA, but deprotection with HF[17] or TFA gave a product with a better recovery of Asp (in 94 or 100% respectively). Therefore, in the present synthesis, we decided to select HF as a deprotecting reagent. Because of the instability of the structurally related secretin in solution,[18] the Met(O) residue of the protected octacosapeptide amide was reduced with thiophenol[19] prior to deprotection. The reduced product was then exposed to HF in an ice-bath for 60 minutes to remove all protecting groups. *m*-Cresol was employed as cation scavenger, since this reagent was found more efficient than anisole to suppress a side reaction at the Tyr residue, *i.e.*, O-mesitylene-2-sulfonation.[6]

The deprotected peptide was precipitated with ether, converted to the corresponding acetate by Amberlite CG-4B and treated with 0.5 N ammonia in an ice-bath for 30 minutes to reverse the N→O shift at the Ser and Thr residues,[20] in case such side reaction took place. The product was purified by gel-filtration on Sephadex G-25 with 0.6% acetic acid as eluent

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followed by column chromatography on CM-cellulose with gradient elution of 0.1 M ammonium bicarbonate buffer (pH 8.0) according to the procedure used for the purification of natural porcine VIP.[21] The product was further purified by isolectric focusing using Ampholine pH 9—11 (LKB). The synthetic chicken VIP thus purified exhibited a single spot on tlc and behaved as a single component on disc electrophoresis at pH 2.3 [*Rf* 0.22 in *n*-BuOH–AcOH–pyridine–H_2O=4: 1: 1: 2, identical with that of natural chicken VIP supplied by Professor V. Mutt. $[\alpha]_D^{27}$ −40.1° in 0.1 N AcOH. Amino acid ratios in 6 N HCl hydrolysate and AP–M (Merck, Lot No. 9652457) digest (numbers in parentheses): Asp 4.00 (1.96), Thr 1.84, Ser 2.44, Glu 1.05, Ala 1.95 (2.12), Val 3.00 (3.06), Met 0.92 (0.99), Leu 2.00 (2.00), Tyr 1.93 (1.84), Phe 1.97 (1.98), Lys 3.02 (3.32), His 0.95 (0.97), Arg 2.01 (1.99), (Gln+Thr)(Asn+Ser) not determined). *Anal.* Calcd. for $C_{148}H_{233}N_{43}O_{42}S \cdot 5\ CH_3COOH \cdot 14H_2O$: C, 49.02; H, 7.32; N, 15.56. Found: C, 49.30; H, 7.32; N, 15.27].

When vasodilator action in dogs was examined, our synthetic peptide exhibited essentially the same potency as natural chicken VIP and a sample of synthetic porcine VIP (Beckmann, Lot 000046).

Acknowledgement This investigation was supported in part by a grant from the Ministry of Education, Science and Culture (No. 477927). The authors express their sincere appreciations to Professor Viktor Mutt, Karolinska Institute, Stockholm, Sweden, for his generous gift of natural chicken VIP.

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Received October 11, 1979

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