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The Solid-phase Synthesis of Ile^5-Angiotensin II to Demonstrate the Use of N^{im}-Tosyl-histidine*[1a,b]

Toshiyuki FUJII*[2] and Shumpei SAKAKIBARA*[3]

Peptide Center, Institute for Protein Research, Osaka University, Kita-ku, Osaka

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There are still several problems about the protection of imidazole groups during the incorporation of histidyl residues into peptides by the solid-phase method.[1] Recently, we proposed a new method for protecting the imidazole residue of histidine with a Tos-group, and several N^{α}-protected N^{im}-Tos-histidine derivatives (I) were synthesized.[2] The present study is concerned with the application of I in the solid-phase method.

There are several advantages in the use of the Aoc-, Boc-, Z(OMe)-, or Nps-derivatives of I in the solid-phase method. That is, these derivatives are easily synthesized by conventional methods, and they are soluble in CH_2Cl_2, which is known to be the most suitable solvent for coupling reactions with dicyclohexylcarbodiimide (DCC) in the solid-phase synthesis. Further, as was mentioned in the preceeding paper,[2] the Tos-group can be removed simultaneously with other protective groups in the final stage of the synthesis, when the free peptide is isolated from the peptide-resin with HF.[3] Therefore, many difficulties which are encountered during the incorporation of histidyl residues into peptide chains by the solid-phase procedure can be overcome by the use of I. Among these N^{α}-protected derivatives, Aoc-His(Tos)-OH (II)[2] seemed to be the most suitable for solid-phase synthesis because it is highly soluble in CH_2Cl_2. Some of the Tos-groups are known to be cleaved when the N^{α}-Aoc-groups are removed with anhydrous acids,[2] but partial cleavage is probably not a serious problem in solid-phase synthesis. In this connection, it may be recalled that Gutte and Merrifield tried to synthesize ribonuclease A with unprotected Boc-histidine.[4]

The synthesis of human angiotension II was attempted with Compound II. The standard procedures of Marshall and Merrifield were followed for the synthesis;[5] the Aoc group was used for the N^{α}-protection of the amino acids, and all the coupling procedures were carried out in CH_2Cl_2 with DCC. The tyrosyl and the arginyl residues were incorporated as Aoc-Tyr(Bzl)-OH[6] and Aoc-Arg(Tos)-OH (III) respectively. The final aspartyl residue was coupled with Z-Asp(OBzl)-OH.[7] All the coupling reactions proceeded smoothly in CH_2Cl_2; this was in contrast with reports that Boc-His(Bzl)-OH[5] and Boc-Arg(Tos)-OH[8] had to be coupled in DMF or in a mixture of DMF and CH_2Cl_2 (1 : 9) at the risk of acyl-urea formation.[9] The high solubility and reactivity of Aoc-Arg(Tos)-OH in CH_2Cl_2 should also be counted as favorable properties due to the Aoc-group.

Finally, the free peptide was taken out from the

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*1b The abbreviations used conform with those tentatively proposed by the IUPAC-IBC: *J. Biol. Chem.*, **241**, 2491 (1966). DMF–dimethylformamide. Aoc=*t*-amyloxycarbonyl. The amino-acid symbols denote the L-configuration.

*2 Present address: Tanabe Seiyaku Co., Ltd., Higashiyodogawa-ku, Osaka.

*3 To whom requests for reprints should be addressed.

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peptide-resin by the HF-procedure,[3] and the bound HF was removed by passing the crude product through a column of Dowex-1; this procedure was also effective in removing almost all side products. Thus, practically pure angiotensin II was obtained without the use of any other specific procedure.

Experimental

Materials. Chloromethylated polystyrene (divinylbenzene 2%; 100—200 mesh; Cl-content, 1 mmol/g) was obtained from the Protein Research Foundation; it was converted to an Aoc-L-phenylalanyl-resin (0.29 mmol/g) by the procedure of Marshall and Merrifield.[5] The Aoc-amino acids were synthesized as has been reported previously.[6,10]

Aoc-Arg(Tos)-OH (III). Arg(Tos)[11] (9.9 g, 0.03 mol) was allowed to react with Aoc-azide[6] (5 g, 0.032 mol) at 35.0°C for 48 hrs in a mixture of dioxane (30 m*l*), 1N NaOH (30 m*l*), and triethylamine (4.2 m*l*, 0.03 mol). The reaction mixture was treated as described previously,[6] and the crude product was extracted into AcOEt. The AcOEt solution was concentrated under reduced pressure at room temperature. The crystalline residue was recrystallized from AcOEt, and dried over P_2O_5 *in vacuo*; wt, 11.8 g (88.7%); mp 79.5—83°C, $[\alpha]_D^{25}$ −13° (*c* 1.9, pyridine).

Found: C, 50.74; H, 6.85; N, 11.96%. Calcd for $C_{19}H_{30}N_4O_6S \cdot 1/2\ H_2O$: C, 50.54; H, 6.92; N, 12.41%.

Z-Aps(OBzl)-Arg(Tos)-Val-Tyr(Bzl)-Ile-His(Tos)-Pro-Phe-resin (IV). Aoc-L-phenylalanyl-resin (2 g, 0.58 mmol) was placed in a manual solid-phase apparatus, and synthesis was started as has been described in the literature.[1,4] The cleavage of the Aoc-groups was carried out with 50% trifluoroacetic acid in CH_2Cl_2,[4] and the amino groups generated were neutralized with triethylamine in DMF. Each Aoc-amino acid (3 eq) was coupled with DCC (3 eq) in CH_2Cl_2 for 3 hrs at room temperature. The couplings of Aoc-Ile-OH and Compound III were ensured by repeating the coupling procedure twice for each compound.

Finally, the protected peptide-resin (IV) (2.7 g) was obtained and dried over P_2O_5 *in vacuo* at 50°C. The overall yield (about 90%) was estimated at this stage from the weight-increase.

Ile^5-Angiotensin II. The peptide resin (IV) (700 mg) was placed in an HF-reaction vessel and mixed with anisole (0.5 m*l*). Anhydrous HF (10 m*l*) was then introduced into the vessel, and the mixture was allowed to react at 0°C for an hour. After the excess HF had then been evaporated off *in vacuo* at 0°C, the generated free peptide was extracted with 1% acetic acid. The extract was washed with ether and lyophilized; wt, 131.4 mg (90%, calcd from the amount of phenylalanyl-resin).

Then, the crude product (100 mg) was applied to a column of Dowex-1 × 2 (AcO^- form, 0.85 × 50 cm). This was eluted with water, and the UV absorption of each fraction was measured at 280 mμ (see Fig. 1).

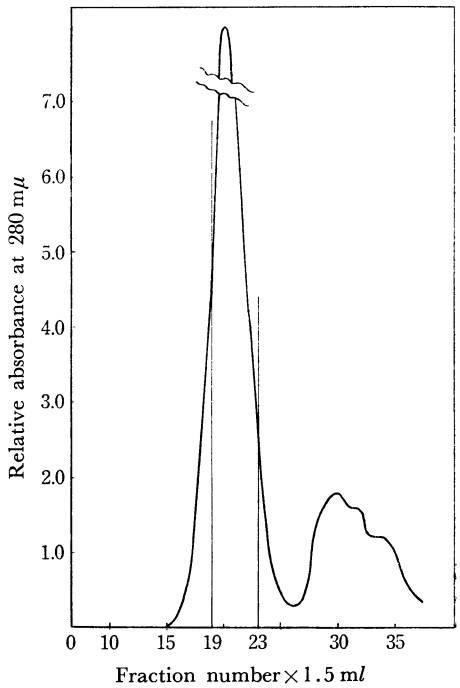

Fig. 1. Purification of synthetic angiotensin II by column chromatography.
Material was eluted from a column (0.85 × 50 cm) of Dowex 1×2 (Acetate form, 100—200 mesh) with water. 1.5 m*l* fractions were collected and their UV-absorption at 280 mμ was measured.

The major fractions (19—23) were combined and lyophilized to obtain the final product; wt, 45 mg (45% calcd from the crude product); $[\alpha]_D^{15}$ −67.8° (c 0.3, N HCl): reported $[\alpha]_D^{20}$ −66.98° (c, 0.4, N HCl),[12] $[\alpha]_D^{23}$ −67.3° (c 1.13, N AcOH),[13] $[\alpha]_D^{25}$ −66° (c 0.8, N HCl).[5]

Found: C, 54.40; H, 6.75; N, 15.35%. Calcd for $C_{50}H_{71}N_{13}O_{12} \cdot C_2H_4O_2 \cdot 3H_2O$: C, 53.83; H, 7.04; N, 15.69%.

This material showed a single spot (R_f 0.26) on paper chromatography with a solvent system *n*-butanol-acetic acid-water (4 : 1 : 1); reported R_f 0.29,[12] R_f 0.28.[5] Ratios of amino acids: Acid hydrolysate; $Asp_{1.02}Arg_{1.02}Val_{0.98}Tyr_{1.00}Ile_{0.93}His_{0.93}Pro_{1.05}Phe_{1.07}$. Amino-peptidase M digest;*4 $Asp_{1.00}Arg_{0.97}Val_{1.02}Tyr_{1.00}Ile_{1.09}His_{0.00}Pro_{0.00}Phe_{1.12}$.

This material showed full hypertensive activity when

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assayed on a rat carotid artery, using Hypertensin-Ciba as the standard.

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