Solid-Phase Synthesis of C-Terminal Hexapeptide Analogs of Substance P[1)]

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Synopsis. C-Terminal hexapeptide analogs of substance P (SP) were synthesized by a solid-phase method. These analogs, [Dab^6]-SP(6-11), [Orn^6]-SP(6-11), and [Lys^6]-SP(6-11), showed a contractile activity on an isolated guinea-pig ileum.

Substance P (SP) is a smooth muscle-contracting peptide having a sequence H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2. In studies of the relationships between chain length and activity of SP,[2,3)] it was found that the potency of the C-terminal hexapeptide of SP was similar to that of SP. We reported the relative potencies of C-terminal hexapeptide analogs of SP each having either Gly, γAbu (4-aminobutyric acid), δAva (5-aminovaleric acid), or εAcp (6-aminocaproic acid) at the N-terminus.[4)] In this paper,[5)] we would like to report the synthesis and structure-activity relationships of the other C-terminal hexapeptide analogs, **1**, **2**, and **3**, N-terminus of which is Dab, Orn,[6)] or Lys, respectively. These analogs were synthesized by a solid-phase method according to Merrifield's procedure.[7)]

H-Dab-Phe-Phe-Gly-Leu-Met-NH_2 **1**
H-Orn-Phe-Phe-Gly-Leu-Met-NH_2 **2**
H-Lys-Phe-Phe-Gly-Leu-Met-NH_2 **3**

For the synthesis, one of the present authors[8)] designed a manually operated apparatus with a Diflon vessel. The coupling was performed on a 2% cross-linked benzhydrylamine (BHA) resin support[9)] using Boc-amino acids. First of all, Boc-Met-OH was coupled with the BHA group on the resin by DCC. The resulting resin was treated with Ac_2O and Et_3N in CH_2Cl_2 in order to mask the residual free BHA groups. Then, the Boc-group was removed with 30% TFA/CH_2Cl_2 containing anisole as a scavenger. Boc-Leu-OH, Boc-Gly-OH, Boc-Phe-OH, and Boc-Phe-OH were coupled to the N-terminus on the resin, successively. In each case, for the first coupling a 2.5-fold excess of Boc-amino acid was used and for the second a 1.25-fold excess. The Boc-Phe-Phe-Gly-Leu-Met-BHA resin was divided into several parts. After deprotection of the N-terminus of the peptide-resin, Boc-Dab(Boc)-OH, Boc-Orn(Boc)-OH, and Boc-Lys(Boc)-OH were coupled with each. The peptide-resin bond of H-Dab-Phe-Phe-Gly-Leu-Met-BHA resin derived from Boc-Dab(Boc)-derivative by treatment with TFA was cleaved with liquid HF containing anisole and methionine.[10)] Column chromatography on Bio-Gel P-2 was used to separate the methionine from analog **1**. For further purification, partition chromatography was performed by using the upper phase of the mixture of *n*-BuOH and 0.1% AcOH (1 : 1) as an eluent on a column of Sephadex G-25. The other two analogs, **2** and **3**, were prepared from Boc-Orn(Boc)-Phe-Phe-Gly-Leu-Met-BHA resin and Boc-Lys(Boc)-Phe-Phe-Gly-Leu-Met-BHA resin by the same procedure respectively. The homogeneities of the analogs (**1**, **2**, and **3**) were confirmed by HPLC on an ODS column. The analogs were highly soluble in water and showed that the CD spectra had one peak at 220 nm and one trough at 235 nm. Their molar ellipticity values are summarized in Table 1. These CD spectra are referred to as an "unordered structure" by Mehlis et al.[11)] Their contractile activities, as measured on an isolated guinea-pig ileum, showed the relative potencies compared with the authentic SP in Table 2. Among them, [Orn^6]-SP(6-11) had the highest potency, though it was still less than that of SP. These results suggest that Dab, Orn, and Lys at the N-terminus of these analogs have a capability to increase the activity of H-Phe-Phe-Gly-Leu-Met-NH_2;[2)] and that the potency of each depends upon the length of the side chain of each basic amino acid.

Table 1. Circular Dichroism of SP(6–11) Analogs

Compound	[θ] (10^3 deg cm^2/dmol)[a)]	
	Peak (220 nm)	Trough (235 nm)
[Dab^6]-SP$^{6-11}$ (**1**)	+10.8	−1.4
[Orn^6]-SP$^{6-11}$ (**2**)	+10.8	−1.4
[Lys^6]-SP$^{6-11}$ (**3**)	+15.4	−1.5

a) Molar ellipticity value in water at room temp.

Table 2. Contractile Activities of SP and SP(6–11) Analogs on an Isolated Guinea-Pig Ileum

Compound	EC_{50}(M)[a)]	Relative Potencies
SP	4.4×10^{-9}	100
1	2.0×10^{-8}	22
2	9.7×10^{-9}	45
3	1.1×10^{-8}	40

a) EC_{50}: effective concentration (half maximal contraction).

Experimental

All coupling steps were carried out with a manual peptide synthesizer (TSP-D type, Taiatsu Scientific Glass Co., Ltd.). The cleavage of the peptide-resin was achieved with a HF reaction apparatus (Type II, Peptide Institute, Inc. and

Toho Kasei Co., Ltd.). The melting points were determined on a Mel-Temp apparatus and are uncorrected. The homogenity of the synthetic peptides was confirmed by TLC on Merck silica gel F_{254} plates and HPLC. HPLC was performed with 4.6 mm×25 cm Finepak SIL ODS column with UV detection at 215 nm. The following solvent system was used for HPLC: *n*-PrOH–10% $NaClO_4$aq (1:4) at a flow rate of 1.5 ml min^{-1}. TLC was developed in the following solvent system: *n*-BuOH–AcOH–pyridine–water (4:1:1:2). Ninhydrin and Rydon[12] reagents were used for detection on TLC plates. Amino acid analyses were performed with a JEOL automatic amino acid analyzer, after the samples had been hydrolyzed with constant-boiling HCl in evacuated sealed ampoules for 20 h at 110 °C. The CD spectrum was measured with a JASCO model J-20 spectrometer.

Preparation of Boc-Met-BHA Resin. BHA resin hydrochloride[13] (5 g, 0.4 mequiv of NH_2 group/g) was placed in the Daiflon vessel of a peptide synthesizer, and operations were carried out as shown in Table 3.

Preparation of Boc-Phe-Phe-Gly-Leu-Met-BHA Resin. According to the series of operations outlined in Table 4, the above Boc-Met-BHA resin was treated in the vessel succesively. The pentapeptide resin, thus derived, was dried in a vacuum desiccator; it weighed 6.45 g.

[Dab^6]-SP(6-11). Boc-Phe-Phe-Gly-Leu-Met-BHA resin (1.63 g, 0.5 mequiv) was placed in the vessel, and the coupling of Boc-Dab(Boc)-OH (1.25 mmol)[14] was carried out, as shown in Table 4. After the second coupling (steps 12—15 in Table 4) used 0.625 mmol of Boc-Dab(Boc)-OH, the Boc groups on Dab were removed by steps 1—3. H-Dab-Phe-Phe-Gly-Leu-Met-BHA resin was treated with anhydrous liquid HF (ca. 15 ml) containing anisole (2 ml) and D,L-methionine (171 mg) at 0 °C for 1 h. After evaporation of HF in vacuo, the mixture of peptide and

Table 3. Program for Preparation of Boc-Met-BHA-resin

Step	Reagents[a]	Vol/ml	Mixing time (min)×repeated times
1	CH_2Cl_2	50	5×2
2	0.5 M Et_3N/CH_2Cl_2	20	15×2
3	CH_2Cl_2	30	5×3
4	Boc-Met-OH/CH_2Cl_2	4 mmol/40	15 h
	0.5 M DCC/CH_2Cl_2	8	
5	CH_2Cl_2	30	5×3
6	DMF	30	5×2
7	CH_2Cl_2	30	5×2
8	0.5 M Et_3N/CH_2Cl_2	10	10×2
9	CH_2Cl_2	30	5×2
10	Boc-Met-OH/CH_2Cl_2	2 mmol/20	15 h
	0.5 M DCC/CH_2Cl_2	4	
11	CH_2Cl_2	30	5×2
12	DMF	30	15×2
13	CH_2Cl_2	30	5×2
14	25% Ac_2O/CH_2Cl_2	10	2 h
	0.5 M Et_3N/CH_2Cl_2	50	
15	CH_2Cl_2	30	5×2

a) 1 M=1 mol dm^{-3}.

Table 4. Program for Coupling of Boc-Amino Acid

Step	Reagents	Vol/ml	Mixing time (min)×repeated times
1	30% TFA/CH_2Cl_2	40	3
	anisole	a few drops	
2	30% TFA/CH_2Cl_2	40	1 h
	anisole	a few drops	
3	CH_2Cl_2	30	5×3
4	0.5 M Et_3N/CH_2Cl_2	30	5×2
5	CH_2Cl_2	30	5×2
6	Boc-amino acid/CH_2Cl_2	5 mmol/40	5—20 h[a]
	0.5 M DCC/CH_2Cl_2	10	
7	CH_2Cl_2	30	5×2
8	DMF	30	5×2
9	CH_2Cl_2	30	5×2
10	0.5 M Et_3N/CH_2Cl_2	30	5×2
11	CH_2Cl_2	30	5×2
12	Boc-amino acid/CH_2Cl_2	2.5 mmol/20	5—20 h[a]
	0.5 M DCC/CH_2Cl_2	5	
13	CH_2Cl_2	30	5×2
14	DMF	30	5×2
15	CH_2Cl_2	30	5×2

a) Boc-Leu-OH 15 h, Boc-Gly-OH 5 h, Boc-Phe-OH 10 h; Boc-Dab(Boc)-OH, Boc-Lys(Boc)-OH, and Boc-Orn(Boc)-OH 20 h.

resin was washed with ethyl ether. The peptide was extracted with 30% AcOH and the extract was concentrated by lyophilization. The crude peptide was charged on a column (2×100 cm) of Bio-Gel P-2 and eluted with 2% AcOH. The main fractions (132 mg) were further chromatographed on a column (2×35 cm) of Sephadex G-25 eluted with the upper phase of the system 0.5% AcOH–*n*-BuOH (1:1). The fractions collected were evaporated in vacuo. The aqueous solution of the residue was lyophilized and a white crystalline peptide was obtained. Yield, 67 mg (15%); mp 177—178 °C; amino acid ratio: Dab 1.06, Phe 1.93, Gly 0.97, Leu 1.01, Met 1.03; TLC: R_f 0.69; HPLC: t_R(min) 11.4.

Found: C, 53.01; H, 6.95; N, 12.50%. Calcd for $C_{35}H_{52}N_8SO_6 \cdot 2AcOH \cdot 3H_2O$: C, 52.81; H, 7.50; N, 12.63%.

[Lys^6]-SP(6-11). This peptide was synthesized by the same procedure as described for the preparation of [Dab^6]-SP(6-11). Boc-Phe-Phe-Gly-Leu-Met-BHA resin (1.63 g, 0.5 mequiv) and Boc-Lys(Boc)-OH (1.25 mmol for the first coupling and 0.625 mmol for the second) were used and the yield was 18% (93 mg). Mp 188—191 °C; amino acid ratio: Lys 0.99, Phe 1.82, Gly 1.06, Leu 1.08, Met 1.05; TLC: R_f 0.63; HPLC: t_R(min) 9.8.

Found: C, 53.37; H, 7.05; N, 11.19%. Calcd for $C_{37}H_{56}N_8SO_6 \cdot 4AcOH \cdot 1.5H_2O$: C, 53.61; H, 7.50; N, 11.11%.

[Orn^6]-SP(6-11). Another Boc-Phe-Phe-Gly-Leu-Met-BHA resin (1.3 g, 0.61 mequiv) was used for the coupling with Boc-Orn(Boc)-OH (the first, 1.83 mmol and the second, 0.92 mmol). The procedure used was the same as that for the cases of the above two analogs. The purified analog weighed 38 mg (7%) and the mp was 168—171 °C. Amino acid ratio: Orn 0.96, Phe 1.98, Gly 1.01, Leu 1.01, Met 1.04; TLC: R_f 0.66; HPLC: t_R(min) 10.4.

Found: C, 54.55; H, 7.17; N, 12.41%. Calcd for $C_{36}H_{54}N_8SO_6 \cdot 2AcOH \cdot 2H_2O$: C, 54.41; H, 7.53; N, 12.69%.

Bioassay. Isolated ileum preparations from male guinea pigs (430—480 g) were suspended in a bath containing Tyrode's solution, bubbled with air and maintained at 37 °C. Contractions were recorded isotonically. The relative potencies of the test peptides were calculated from the EC_{50}(M) values.

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5) Amino acids used are of the L-configuration. Abbreviations for amino acids and peptides follow the IUPAC-IUB nomenclature described in *Eur. J. Biochem.*, **138**, 9 (1984). Abbreviations used are: Dab, 2,4-diaminobutyric acid; Boc, *t*-butoxycarbonyl; DCC, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; DMF, dimethylformamide.

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