Synthesis of Cyclic Oligopeptides Containing an Arg–Gly–Asp (RGD) Sequence

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Received June 11, 1999; accepted September 6, 1999

The synthesis of cyclic RGD peptides with 8—10 residues cyclized by an amide bond and the relationship between their structure and activity (i.e. circular dichroism spectrum and inhibition of platelet aggregation) are reported. The linear peptides were synthesized by the solution method and their cyclization was performed in high dilution using DPPA.

Key words Arg–Gly–Asp (RGD) sequence; platelet aggregation inhibitor; cyclic peptide; circular dichroism spectrum

We have studied the differentiation of sea urchin embryo using synthetic peptides related to the partial structure of the cell adhesion domain of fibronectin. In order to obtain a specific conformation of the RGDSP ASS peptide, we designed and synthesized a cyclic peptide, CRGDSP ASSC, having a disulfide bond (FR-1). FR-1 exhibited activity as a platelet aggregation inhibitor and some FR-1 analogs were found to be more potent than FR-1 itself.

In the present investigation, we synthesized RGD peptides (1—5) cyclized by an amide bond, and studied the relationship between their structure and activity, in particular, their size and the side-chain effect of an amino acid residue located adjacent to the RGD sequence. We evaluated the circular dichroism (CD) spectra and the inhibition of platelet aggregation in human platelet-rich plasma.

cyclo(–Arg–Gly–Asp–Ser–Pro–Ala–Ser–Ser–) 1


cyclo(–Arg–Gly–Asp–Phe–Pro–Ala–Ser–Ser–) 4

cyclo(–Arg–Gly–Asp–Cha–Pro–Ala–Ser–Ser–) 5

Protected peptides 6—10 were synthesized by the solution method in the same manner as for FR-1. The synthetic route for 6 is shown in Chart 1 and the routes for 9 and 10 are the same as that for 6. Chart 2 shows the route for 8. Peptide 7 was synthesized in a similar manner to that of 8 using Gly–OTce in place of Lys(Z)–Pro–OTce.

Cyclization of 6—10 was performed in high dilution using DPPA after removal of the protective groups on the N- and C-terminals. Cyclic protected peptides 11—15 were treated with liquid HF and then subjected to HPLC purification to obtain the final cyclic peptides 1—5 (Chart 3).

Peptides 4 and 5 were the most potent inhibitors of human platelet aggregation among the five cyclic peptides (1—5) as shown in Table 1. CD spectra of these cyclic peptides, the linear peptide RGDSPASSKP and FR-1 are shown in Fig. 1. The CD spectrum of peptide 3 was characterized by double-minima at 200 and 220 nm (π–π* and n–π* transition bands, respectively). This spectrum is similar in shape to that of gramicidin S, which is a cyclic decapetide containing two prolyl residues and a fixed conformation. Peptides 1 and 2 also have similar CD spectra. On the other hand, peptides 4 and 5 were characterized by the increased ratio of the π–π*/n–π* bands in their CD spectra. These spectra are not consistent with the preferred β-turn structure, and show that these cyclic peptides have unordered structures caused by the steric effect of Phe or Cha, compared with peptide 1. Needless to say, their structures need to be confirmed by another method such as NMR. The results of biological assay suggest that peptides 4 and 5 have a relatively flexible cyclic struc-
Column chromatography was performed using Silica-gel 60 from Merck. Mass spectra were measured on a JEOL JMS-SX mass spectrometer operating under FAB conditions. HF treatment was performed in an HF-reaction mixture to afford an amorphous compound which was dried in vacuo. The compound (i.e. H-Ala–Ser(Bn)–Arg(Tos)–Gly–OTce–HCl, 0.89 mmol), Boc–Asp(OcHex)–Ser(Bn)–Pro–OH (0.80 g, 1.36 mmol), and HOBT−H2O (0.20 g, 1.31 mmol) were dissolved in 10 ml DMF, DIEA (0.23 ml, 1.35 mmol) and EDC·HCl (0.25 g, 1.30 mmol) were added to the solution chilled in an ice bath. The reaction mixture was stirred at room temperature overnight. Then methylene chloride was added to the mixture which was washed successively with 0.5M HCl, saturated NaHCO3aq. and saturated NaClaq. After filtration of the solution through anhydrous Na2SO4, the solvent was evaporated. The crude peptide was purified on a silica-gel column (CHCl3: MeOH 5:1) to afford 6 (0.75 g, 0.50 mmol) in 56% yield; FAB-MS m/z: 1871 (MH+).

**Fig. 1. CD Spectra of Peptides 1, 2, 3, 4, 5, RGDSPASSKP, and FR-1 in Water**

Table 1. Inhibition of Human Platelet Aggregation

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IC50 (μM)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>12.0</td>
</tr>
<tr>
<td>2</td>
<td>14.0</td>
</tr>
<tr>
<td>3</td>
<td>37.0</td>
</tr>
<tr>
<td>4</td>
<td>3.0</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
</tr>
<tr>
<td>RGDSPASSKP</td>
<td>22.0</td>
</tr>
<tr>
<td>FR-1</td>
<td>11.0</td>
</tr>
</tbody>
</table>

Peptide aggregation in human platelet-rich plasma was induced by ADP (2.3 μM).

**Experimental**

Column chromatography was performed using Silica-gel 60 from Merck. Mass spectra were measured on a JEOL JMS-SX mass spectrometer operating under FAB conditions. HF treatment was performed in an HF-reaction apparatus type II from Peptide Institute, Inc. The final products were isolated by HPLC on a Jasco Gulliver system equipped with a PU-980 UV/970 detector (210 nm) using a Megapak SIL C18 column, 10×250 mm. CD spectra of 0.015% peptide solution (ca. 1 mm) in water were recorded at room temperature and can change to a conformation favorable for the receptors on human platelets.

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Peptide 4: FAB-MS m/z: 818 (MH+). Anal. Caled for C₃₅H₅₁N₁₁O₁₂·3TFA·2.5H₂O: C, 40.86; H, 4.93; N, 12.78. Found: C, 41.40; H, 5.06; N, 13.03.

Peptide 5: FAB-MS m/z: 824 (MH+). Anal. Caled for C₃₅H₅₇N₁₁O₁₂·2TFA·3H₂O: C, 42.35; H, 5.92; N, 13.93%). Found: C, 42.63; H, 5.87; N, 13.67.

H–Arg–Gly–Asp–Ser–Pro–Ala–Ser–Ser–Lys–Pro–OH (RGDSPASSKP) Protected peptide 8 (2.63 g, 1.40 mmol) was treated with Zn/90% AcOH aq. at room temperature overnight and then liquid HF at 0 °C for 1 h, as described in reference 5. The yield of purified RGDSPASSKP was 75%. FAB-MS m/z: 1,001 (MH+). Anal. Caled for C₄₀H₆₈N₁₄O₁₆·4TFA·3H₂O: C, 38.15; H, 5.20; N, 12.97. Found: C, 37.68; H, 4.89; N, 13.12.

Acknowledgments We thank Mr. Tamotsu Yamamoto and staff members of the Institute for Life Science Research, Asahi Chemical Industry Co., Ltd. for measurement of mass spectra and elemental analysis. We also thank Dr. Isao Yanagisawa and Miss Yumiko Sakai of Yamanouchi Pharmaceutical Co., Ltd. for the biological assay.

References and Notes
1) The amino acids used had the L-configuration. Abbreviations used in this paper: Cha=cyclohexylalanine; Boc=tert-butoxycarbonyl; Bn=benzyl; cHex=cyclohexyl; Tce=2,2,2-trichloroethyl; Tos=p-toluene-sulfonyl; Z=benzylxycarbonyl; ADP=adenosine 5’-diphosphate; DCC=diisocyctheycarbodimide; DPPA=diphenylphosphorylazide; DIEA=diisopropylethylamine; DMF=N,N-dimethylformamide; EDC=1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt=1-hydroxybenzotriazole; NMM=N-methylmorpholine; TFA=trifluoroacetic acid.