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Design and Synthesis of Cyclic Fibronectin Related Peptides, RGDSPASS Containing Cystine Peptides[1)]

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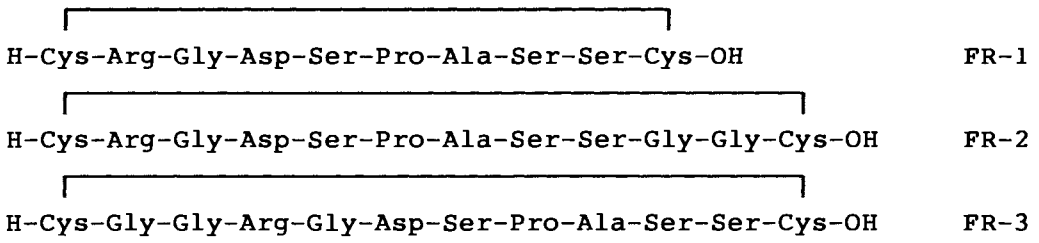
Arg-Gly-Asp-Ser (RGDS) is an active site in the cell binding domain of fibronectin (FN). Three types of RGDSPASS containing cystine peptides were designed on the basis of the position of Pro in β turn and synthesized.

Arg-Gly-Asp-Ser (RGDS) sequence is an active site in the cell binding domain of fibronectin (FN), cell adhesion protein,[2)] and the RGD sequence also exists in some bioactive proteins and is predicted as an important sequence for biological activity.[3)]

Previously, we reported that the binding of exogenous FN to the primary mesenchyme cells (PMCs) of sea urchin was inhibited by synthetic peptide, Pro-Ala-Ser-Ser (PASS), but not by RGDS. FN-promoted PMC migration was also inhibited by RGDS and PASS.[4)] These results suggest that the PASS sequence is a novel PMC surface interaction site in FN. Circumference of FN active site has been expected to form β turn by the method of Chou and Fassman.[2,5)]



We were interested in the secondary structure of the RGDSPASS sequence in FN and designed 3 types of peptides (FR-1, FR-2, and FR-3) on the basis

of the position of Pro, i+1, i+2 or i+3 in the β turn. These peptides were synthesized by the solution method. The Boc group and DCC-HOBt procedure[6] were used for the synthesis of protected peptide fragments which have Cys, Gly, or Pro at the C-terminal. In the case of Asp(OcHex) containing fragments, the carboxyl group of Pro at the C-terminal was protected by trichloroethyl group. The protective group of the side chain of each amino acid was shown in the sequences of peptides 1, 2, and 3.[6]

Boc-Cys(Acm)-Arg(Tos)-Gly-Asp(OcHex)-Ser(Bzl)-Pro-Ala-Ser(Bzl)-Ser(Bzl)-Cys(Acm)-OBzl 1

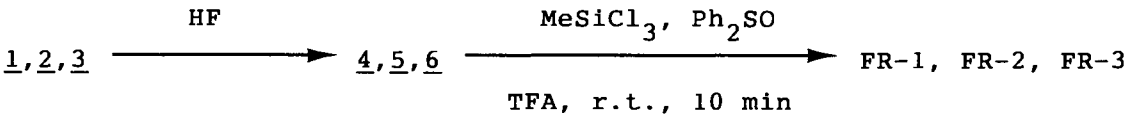
Boc-Cys(Acm)-Arg(Tos)-Gly-Asp(OcHex)-Ser(Bzl)-Pro-Ala-Ser(Bzl)-Ser(Bzl)-Gly-Gly-Cys(Acm)-OBzl 2

Boc-Cys(Acm)-Gly-Gly-Arg(Tos)-Gly-Asp(OcHex)-Ser(Bzl)-Pro-Ala-Ser(Bzl)-Ser(Bzl)-Cys(Acm)-OBzl 3

H-Cys(Acm)-Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Cys(Acm)-OH 4

H-Cys(Acm)-Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Gly-Gly-Cys(Acm)-OH 5

H-Cys(Acm)-Gly-Gly-Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Cys(Acm)-OH 6



Scheme 1.

Peptide 1 was synthesized from Boc-Cys(Acm)-Arg(Tos)-Gly-Asp(OcHex)-Ser(Bzl)-Pro-OH and H-Ala-Ser(Bzl)-Ser(Bzl)-Cys(Acm)-OBzl by the fragment condensation with EDC-HOBt.[6,7] Peptides 2 and 3 were also synthesized by similar procedures.[7] These protected deca- and dodecapeptides 1, 2, and 3 were treated with liq. HF at 0 °C for 1 h and all protective groups except the Acm groups were removed (Scheme 1). The structures of peptides 4, 5, and 6 were confirmed by FABMS and elemental analysis.[8] Whereas I_2 oxidation of these peptides did not attain the formation of intramolecular disulfide bond, this bond formation was achieved by the silyl chloride-sulfoxide method.[9] The cyclic peptides[10] were purified by HPLC (Biofine RPC-SC18 column, 10 mm x 250 mm, JASCO Co.) and confirmed by FABMS and NMR (COSY and ROESY) spectra.

Table 1 shows the biological activities of 4, 5, 6, FR-1, FR-2, and FR-3.[11] Under the same conditions as with the compounds in Table 1, RGDS (1 mM) inhibited only about 10% of the platelet aggregation. On the other hand, the cyclic peptides exhibit high activity as platelet aggregation inhibitors. These results suggest that the secondary structure of RGDSPASS in FR-1 is appropriate for binding to fibrinogen receptor on the platelet surface and the structure corresponds to that of the active site of FN which inhibits platelet aggregation.[12]

Table 1. Inhibition of platelet aggregation in rabbit platelet rich plasma[a)]

Compound	$IC_{50}/\mu M$
4	740
5	760
6	510
FR-1	67
FR-2	280
FR-3	135

a) Platelet aggregation was induced by collagen($10\mu g/cm^3$).

NMR study on these cyclic peptides is currently in progress.

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6) Boc, t-butyloxycarbonyl; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; Acm, acetoamidomethyl; cHex, cyclohexyl; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.
7) Peptide 1: FABMS m/z 1820(MH^+); $[\alpha]_D^{25}$ -28.2° (c 1.00, DMF); Found: C, 56.41; H,6.38; N,11.19%. Calcd for $C_{87}H_{117}N_{15}O_{22}S_3 \cdot 1.5H_2O$: C,56.54; H, 6.54; N,11.37%.
Peptide 2: FABMS m/z 1934(MH^+); $[\alpha]_D^{24}$ -26.3° (c 0.99, DMF); Found: C, 54.28; H,6.79; N,11.48%. Calcd for $C_{91}H_{123}N_{17}O_{24}S_3 \cdot 4H_2O$: C,54.45; H, 6.58; N,11.86%.
Peptide 3: FABMS m/z 1934(MH^+); $[\alpha]_D^{25}$ -32.6° (c 1.06, DMF);

Found: C,56.19; H,6.61; N,11.58%. Calcd for $C_{91}H_{123}N_{17}O_{24}S_3 \cdot 1.5H_2O$: C,55.70; H, 6.47; N,12.13%.

8) Peptide 4: FABMS m/z 1124(MH^+); Found: C,37.57; H,5.08; N,14.22%. Calcd for $C_{41}H_{69}N_{15}O_{18}S_2 \cdot 3TFA \cdot 1.5H_2O$: C,37.80; H,5.06; N,14.07%. Peptide 5: FABMS m/z 1238(MH^+); Found: C,35.87; H,4.46; N,13.13%. Calcd for $C_{45}H_{75}N_{17}O_{20}S_2 \cdot 4TFA \cdot 4H_2O$: C,36.04; H,4.96; N,13.48%. Peptide 6: FABMS m/z 1238(MH^+); Found: C,35.60; H,4.91; N,13.84%. Calcd for $C_{45}H_{75}N_{17}O_{20}S_2 \cdot 4TFA \cdot 4.5H_2O$: C,35.85; H,5.00; N,13.41%.

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10) FR-1: Yield,18%(from 1); HPLC: t_R=20.16 min (0.1%TFA/4%CH_3CNaq., Biofine RPC-SC18, 4.6 mm x 250 mm); HRMS: Found 980.3568, Calcd for $C_{35}H_{57}N_{13}O_{16}S_2$: 980.3580; Amino acid analysis(theoretical): Asp 0.92(1), Ser 2.65(3), Gly 1.07(1), Ala 1.00(1), $(Cys)_2$ 0.46(1), Arg 0.88(1), Pro 0.90(1); Found: C,34.39; H,5.11; N,12.31%. Calcd for $C_{35}H_{57}N_{13}O_{16}S_2 \cdot 3TFA \cdot 6.5H_2O$: C,34.22; H,5.11; N,12.65%.
FR-2: Yield,17%(from 2); HPLC: t_R=18.84 min (under the conditions described above); HRMS: Found 1094.3990, Calcd for $C_{39}H_{63}N_{15}O_{18}S_2$: 1094.3995; Amino acid analysis(theoretical): Asp 0.92(1), Ser 2.66(3), Gly 2.83(3), Ala 1.00(1), $(Cys)_2$ 0.57(1), Arg 0.94(1), Pro 0.97(1); Found: C,34.97; H,4.93; N,14.22%. Calcd for $C_{39}H_{63}N_{15}O_{18}S_2 \cdot 3TFA \cdot 5H_2O$: C,35.41; H,5.02; N,13.77%.
FR-3: Yield,20%(from 3); HPLC: t_R=27.72 min (under the conditions described above); HRMS: Found 1094.4000, Calcd for $C_{39}H_{63}N_{15}O_{18}S_2$: 1094.3995; Amino acid analysis(theoretical): Asp 1.03(1), Ser 2.69(3), Gly 2.91(3), Ala 1.00(1), $(Cys)_2$ 0.47(1), Arg 0.99(1), Pro 1.01(1); Found: C,34.70; H,5.36; N,13.08%. Calcd for $C_{39}H_{63}N_{15}O_{18}S_2 \cdot 3TFA \cdot 7.5H_2O$: C,34.40; H,5.20; N,13.37%.

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