Structure and Activity of Fibronectin-related Peptides. The Role of Amino Acid Residues of Positions 5 and 6 in RGDSPASS-containing Cyclic Decapeptide (FR-1)

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Side chains' repulsion between AA^5 and AA^6 in the cyclic decapeptide (FR-1) is important for binding to the fibrinogen receptor on the platelet surface. [Phe^5]-FR-1 exhibited higher activity as a platelet aggregation inhibitor than FR-1.

The Arg-Gly-Asp (RGD) sequence is an active site in the cell binding domain of fibronectin (FN), cell adhesion protein, and is predicted to be an important sequence for binding to the receptor.[1] We had found that Pro-Ala-Ser-Ser (PASS), located adjacent to the RGDS toward the C-terminal in FN, participated in cell binding and cell migration.[2] Our interest is focused on the secondary structure of the RGDSPASS sequence of FN. We had designed and synthesized RGDSPASS-containing cystine peptide, FR-1 which exhibited high activity as a platelet aggregation inhibitor.[3a,3b]

H-Cys[1]-Arg[2]-Gly[3]-Asp[4]-Ser[5]-Pro[6]-Ala[7]-Ser[8]-Ser[9]-Cys[10]-OH

FR-1

In order to investigate the relationship between the structure and the biological activity of FR-1, we designed four analogs of FR-1: [D-Ser^5]-FR-1, [Gly^5]-FR-1, [D-Pro^6]-FR-1, and [Gly^6]-FR-1.

Peptides **1**, **2**, **3**, and **4** were prepared by the solution method in the same manner as FR-1.[3b] The formation of intramolecular disulfide bond was achieved by I_2 oxidation in MeOH (room temperature, 10 min.) in the high dilution ($1x10^{-3}$ M) (Scheme 1). The yields of peptides **5**, **6**, **7**, and **8** were 87, 72, 85, and 76%, respectively. Protected cyclic peptides **5**, **6**, **7**, and **8** were treated with liq. HF for deprotection in the presence of anisole (Scheme 1). The final products[4] were purified by HPLC.

RGD-containing peptides are known to inhibit platelet aggregation.[5] Using human platelet rich plasma, the IC_{50} of FR-1, [D-Ser^5]-FR-1, [Gly^5]-FR-1, [D-Pro^6]-FR-1, and [Gly^6]-FR-1 were found to be 7.6, 257, 118, 251, and 162 μM, respectively. Platelet aggregation was induced by adenosine diphosphate.

NMR measurements were performed with a FT-NMR spectrometer, JEOL JNM GSX 400 (^1H, 400 MHz), in the same manner as FR-1.[3c] Two conformers (major:minor = 6:1 - 4:1) of each cyclic peptide were confirmed by NMR; in this study we discuss the major conformer. Chemical shift assignments were made by the combined use of COSY and NOESY (mixing time: 150, 250, and 400 ms) experiments.

The sequential NOEs between αH(i) and NH(i+1) were observed in all residues except for the Pro residues in four cyclic peptides. The NOEs indicate that these cyclic peptides take a sheet structure. Non-sequential (spatial) and NH(i)-NH(i+1) NOEs were observed as shown in Fig. 1. NMR results support that Asp-D-Ser-Pro-Ala in [D-Ser^5]-FR-1, Asp-Gly-Pro-Ala in [Gly^5]-FR-1, Asp-Ser-D-Pro-Ala in [D-Pro^6]-FR-1, and Asp-Ser-Gly-Ala in [Gly^6]-FR-1 form a turn, respectively.

In FR-1, spatial NOE was not observed[3c] but in [Gly^5]-FR-1, [D-Pro^6]-FR-1 and [Gly^6]-FR-1, spatial NOEs between NH of the side chain of Arg^2 and NH of Ser^9 were observed, respectively (Figs. 1 and 2). These NOEs indicate that the side chain of Arg^2 faces Ser^9 in each analog. We predicted that the decline in inhibitory activity was due to the direction of the side chain of the Arg residue. [D-Ser^5]-FR-1 also exhibited weak

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

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

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

Boc-Cys(Acm)-Arg(Tos)-Gly-Asp(OcHex)-Ser(Bzl)-Gly-Ala-Ser(Bzl)-Ser(Bzl)-Cys(Acm)-OBzl **4**

Boc-Cys-Arg(Tos)-Gly-Asp(OcHex)-D-Ser(Bzl)-Pro-Ala-Ser(Bzl)-Ser(Bzl)-Cys-OBzl **5**

Boc-Cys-Arg(Tos)-Gly-Asp(OcHex)-Gly-Pro-Ala-Ser(Bzl)-Ser(Bzl)-Cys-OBzl **6**

Boc-Cys-Arg(Tos)-Gly-Asp(OcHex)-Ser(Bzl)-D-Pro-Ala-Ser(Bzl)-Ser(Bzl)-Cys-OBzl **7**

Boc-Cys-Arg(Tos)-Gly-Asp(OcHex)-Ser(Bzl)-Gly-Ala-Ser(Bzl)-Ser(Bzl)-Cys-OBzl **8**

1, 2, 3, 4 —(1) I_2 / MeOH, r.t., 10 min; (2) HF-anisole, 0 °C, 1 h)→ [D-Ser^5]-FR-1 (46%), [Gly^5]-FR-1 (38%), [D-Pro^6]-FR-1 (33%), [Gly^6]-FR-1 (33%)

Scheme 1.

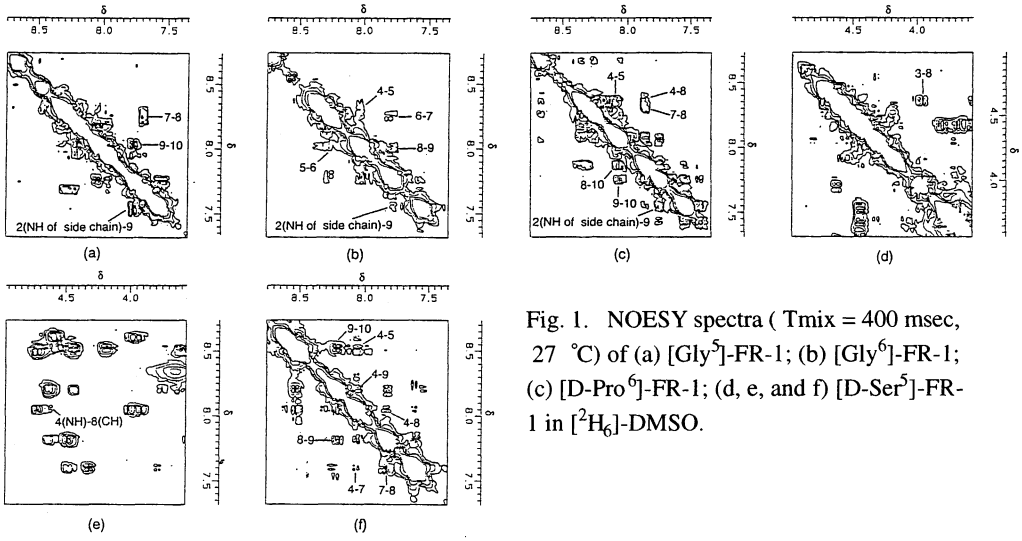

Fig. 1. NOESY spectra (Tmix = 400 msec, 27 °C) of (a) [Gly^5]-FR-1; (b) [Gly^6]-FR-1; (c) [D-Pro^6]-FR-1; (d, e, and f) [D-Ser^5]-FR-1 in [$^2H_6$]-DMSO.

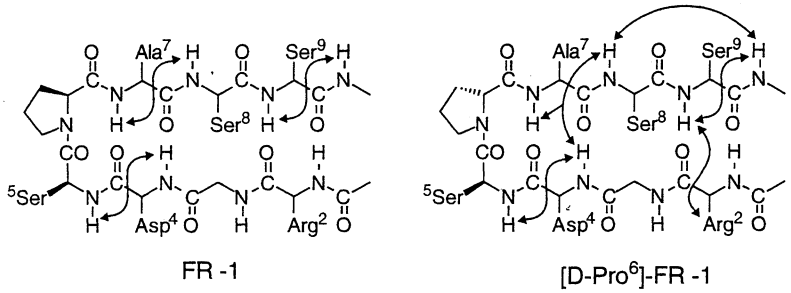

Fig. 2. Turn structures and NOEs of FR-1 and [D-Pro^6]-FR-1. Arrows indicate the protons giving NOEs by NOESY.

inhibitory activity. This result suggests that the L-configuration of AA^5 is essential for binding to the receptor.

From the NMR and biological results, we suppose that the side chains' repulsion between AA^5 and AA^6 provides outside the side chain of the Arg residue for binding to the fibrinorgen receptor; in addition, L-Pro (AA^6) has also an important role in fixing the turn structure and takes a part in the repulsion with L-AA^5 in the turn. As a demonstration of this hypothesis, we synthesized [Phe^5]-FR-1 substituted with Phe having a bulky side chain.[6] The IC_{50} of [Phe^5]-FR-1 was found to be 2.5 μM which was about 3 times higher than that of FR-1.

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References and Notes

1 M. D. Pierschbacher and E. Ruoslahti, *Pro. Natl. Acad. Sci. U.S.A.*, **81**, 5985 (1984).

2 H. Katow, S. Yazawa and S. Sofuku, *Exp. Cell Res.*, **190**, 17, (1990).

3 a) Y. Yamamoto, H. Katow, and S. Sofuku, *Chem. Lett.*, **1993**, 615; b)Y. Yamamoto and S.Sofuku, *Bull. Chem. Soc. Jpn.*, **67**, 2167 (1994); c) Y.Yamamoto and S.Sofuku, *J. Chem. Soc., Chem. Commun.*, **1993**, 1235.

4 **[D-Ser^5]-FR-1**: FABMS m/z 980 $(M+H)^+$; amino acid analysis (theoretical) : Asp 0.95(1), Ser 2.48(3), Gly 0.95(1), Ala 1.00 (1), $(Cys)_2$ 0.92 (1), Arg 0.91 (1), Pro 0.92 (1); Found: C, 35.51; H, 5.43; N, 12.80%. Calcd for $C_{35}H_{57}N_{13}O_{16}S_2 \cdot 3TFA \cdot 4.5H_2O$: C, 35.10; H, 4.96; N, 12.98%. **[Gly^5]-FR-1**: FABMS m/z 950 $(M+H)^+$; amino acid analysis(theoretical): Asp 0.95(1), Ser 1.78(2), Gly 1.86(2), Ala 1.00(1), $(Cys)_2$ 0.93(1), Arg 0.94(1), Pro 0.90(1); Found: C, 35.39; H, 4.89; N, 11.94%. Calcd for $C_{34}H_{55}N_{13}O_{15}S_2 \cdot 4TFA \cdot 2.5H_2O$: C, 34.73; H, 4.41; N, 12.54%. **[D-Pro^6]-FR-1**: FABMS m/z 980 $(M+H)^+$; amino acid analysis (theoretical): Asp 1.03(1), Ser 2.70(3), Gly 1.00(1), Ala 1.00 (1), $(Cys)_2$ 0.79(1), Arg 1.03(1), Pro 0.98(1); Found: C, 36.46; H, 4.57; N, 12.97%. Calcd for $C_{35}H_{57}N_{13}O_{16}S_2 \cdot 3TFA \cdot 3.5H_2O$: C, 35.97; H, 4.93; N, 13.30%. **[Gly^6]-FR-1**: FABMS m/z 940 $(M+H)^+$; amino acid analysis (theoretical): Asp 1.01(1), Ser 2.68(3), Gly 1.94(2), Ala 1.00(1), $(Cys)_2$ 0.56(1), Arg 0.97(1); Found: C, 30.20; H, 4.95; N, 11.44%. Calcd for $C_{32}H_{53}N_{13}O_{16}S_2 \cdot 4TFA \cdot 10H_2O$: C, 30.48; H, 4.92; N, 11.55%.

5 S. Cheng, W. S. Craig, D. Mullen, J. F. Tschopp, D. Dixon, and M.D.Pierschbacher, *J. Med. Chem.*, **37**, 1 (1994).

6 Y.Yamamoto and S.Sofuku, in preparation.