Structure and Activity of Fibronectin-related Peptides: RGDSPASS containing Cyclic Peptides

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The turn structures of cyclic fibronectin related RGDSPASS containing cystine peptides FR-1, FR-2 and FR-3 are determined by NMR studies.

The Arg-Gly-Asp-Ser(RGDS) sequence is an active site in the cell-binding domain of fibronectin (FN),[1] the cell-adhesion protein. It also exists in some bioactive proteins and is predicted to be an important sequence for biological activity.[2] Previously, we reported that Pro-Ala-Ser-Ser (PASS), located adjacent to the RGDS sequence toward the C-terminal in FN, participated in cell binding and cell migration.[3] We were interested in the secondary structure of the RGDSPASS sequence in FN. We designed three types of cyclic peptides on the basis of the position of Pro in the β-turn and synthesized RGDSPASS containing cyclic peptides, FR-1, FR-2 and FR-3.[4]

RGD peptides are known to inhibit FN from binding to platelet[5] and FN is also known to inhibit platelet aggregation.[6] Our cyclic peptides also exhibited high activity as a platelet-aggregation inhibitor. The assay was carried out using rabbit platelet rich plasma and the aggregation was induced by collagen. IC_{50} of FR-1, FR-2 and FR-3 were 67, 280 and 135 μmol dm^{-3}, respectively.[4,7] On the other hand, inhibitory activities of RGDS (1 mmol dm^{-3}) and PASS (1 mmol dm^{-3}) were less than 10% of the platelet aggregation under the same conditions.[4]

NMR measurements were performed with a FT-NMR spectrometer, JEOL-JNM GSX 400 (^1H, 400 MHz). The samples, (7–8 mmol dm^{-3}), contained *ca.* 6–8 mg of peptide dissolved in [$^2H_6$]DMSO (dimethyl sulfoxide) (0.6 ml). Assignments were made using COSY and ROESY[8] with mixing times of 150 and 250 ms, and using NOESY[9] with mixing times of 250 and 400 ms at 300 K. Conformational studies were made using NOESY[9] with mixing times of 250 and 400 ms, and the H–D exchange of the amide proton with CD_3OD (0.1 ml) at 300 K. Two conformers of each cyclic peptide were confirmed by NMR and HPLC; in this study we will discuss the major conformer. The sequential ROEs and NOEs obtained for nearly all connections between α-proton and amide proton gave the assignment of all amide proton in FR-1, FR-2 and FR-3 (Table 1). The H–D exchange rate of amide proton exhibited an obvious difference in each amino acid residue (Table 1). The slow H–D exchange rate indicates that the amide proton is shielded from the solvent. In FR-2, 8-Ser (NH) was shielded from solvent and may be involved in intramolecular hydrogen bonding. In FR-3, 6-Asp(NH) and 9-Ala(NH) were shielded from the solvent and may be involved in an intramolecular hydrogen bond. The spatial NOEs were observed between 5-Ser(NH) and 8-Ser(NH) of

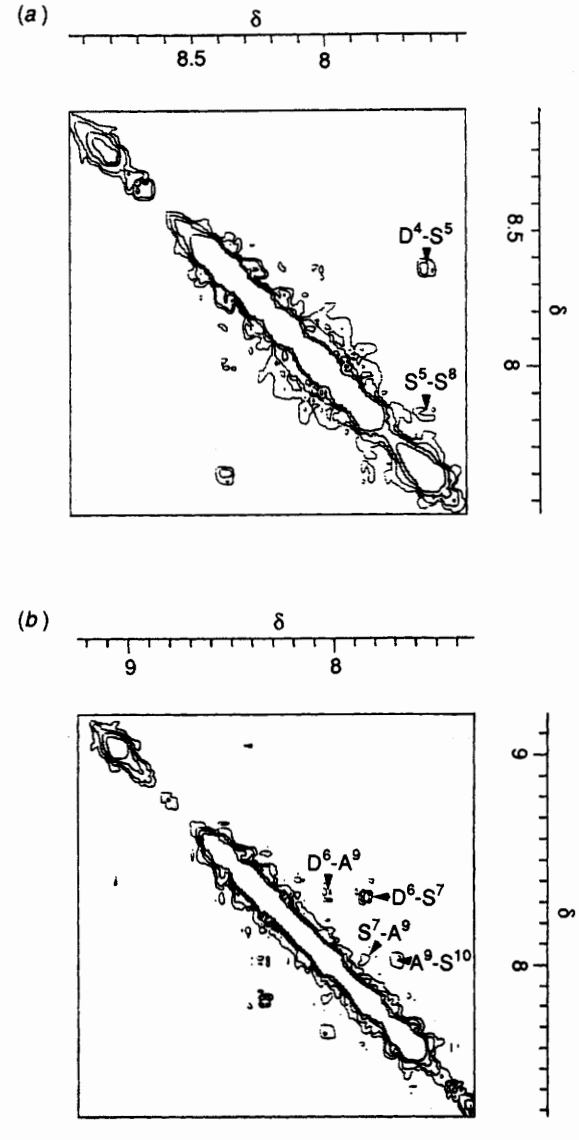

Fig. 1 NOESY spectra of (*a*) FR-2 and (*b*) FR-3 in the NH–NH cross peak region in [$^2H_6$]DMSO at 300 K; T_{mix} = 400 ms

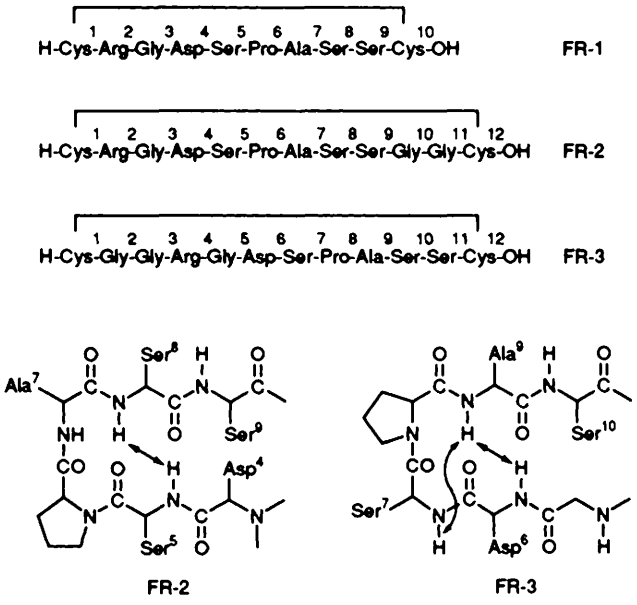

Fig. 2 Turn structures and NOEs of FR-2 and FR-3. Arrows indicate the protons giving NOEs by NOESY.

FR-2; 6-Asp(NH) and 9-Ala(NH), and 7-Ser(NH) and 9-Ala(NH) of FR-3, respectively (Fig. 1). Neither peptides showed a NOE between the α proton of the residue i + 1 and the NH of the residue i + 3 typical of type-I and type-II β turns.[10] However, it was predicted that in the former, 5-Ser(NH) was close to 8-Ser(NH), and in the latter, 6-Asp(NH) and 7-Ser(NH) were close to 9-Ala(NH).

These results indicate that these cyclic peptides do not form the typical β turn, but Ser-Pro-Ala-Ser in FR-2 and Asp-Ser-Pro-Ala in FR-3 form a turn, respectively. Fig. 2 shows the turn structures expected by spatial NOEs and the H–D exchange of the amide proton. Although FR-1 did not show any spatial NOEs, we assume that the turn structure of FR-1 is similar to that of FR-3 from its H–D exchange rate (Table 1). It should be noted that both RGDS and PASS have an antiparallel situation in cyclic peptides, FR-1 and FR-3, which exhibit a higher activity as a platelet-aggregation inhibitor. The fibrinogen receptor on the platelet surface is able to recognize the unique structure of RGDSPASS within this sequence containing peptides. The above results suggest that the structure of FR-1 is appropriate for binding it to the fibrinogen receptor, and the structure corresponds to that of the active site of FN.

NMR measurement in the aqueous solution of these cyclic peptides is currently in progress.

Table 1 Chemical shift and H–D exchange rate of amide proton, and coupling constant[a]

	FR-1			FR-2			FR-3		
	NH	D/H	J_{NH-CH}	NH	D/H	J_{NH-CH}	NH	D/H	J_{NH-CH}
Cys		—			—			—	
Gly							9.04	f	B
Gly							8.36	f	B
Arg	8.74	f	8.0	8.82	f	B	7.75	s	B
Gly	8.35	s	A	8.44	s	B	8.52	s	B
Asp	8.23	s	A	8.35	m	8.1	8.30	s	7.3
Ser	7.58	m	7.3	7.57	m	7.3	7.83	f	7.7
Pro		—			—			—	
Ala	7.88	s	7.0	8.01	m	A	8.02	s	7.0
Ser	7.63	m	7.0	7.80	s	7.3	7.65	m	7.3
Ser	7.75	m	7.7	7.91	s	7.0	8.06	m	A
Gly				8.13	m	B			
Gly				7.95	f	A			
Cys	8.16	f	8.1	8.22	f	7.2	8.41	f	7.4

[a] Measurements were carried out by ^1H 400 MHz NMR spectrometer in [$^2H_6$]DMSO at 300 K; NH chemical shift (δ); H–D exchange rate (D/H): f = fast, m = medium, s = slow; J_{NH-CH} coupling constant (Hz), A: overlapped with other peak, B: broad.

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