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The Solution Structure of 18 residue YH motif Peptide within the Second fas-1 domain of βig-h3

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Abstract **:** βig-h3 is an extracellular matrix protein that mediates cell adhesion through interaction with integrins. The 18 residue YH motifs within each fas-1 domain are known to be responsible for the interaction with the $\alpha_v\beta_5$ integrin, and the synthetic YH motif peptides are known to inhibit endothelial tube formation and reduces the number of blood vessels, and so expected to be an effective inhibitor of angiogenesis. In this study, we solved the 3D structure of the 18 residue YH motif peptide (EALRDLLNNHILKSAMCA; D2 peptide) within the second fas-1 domain of βig-h3 using NMR. The Peptide has α-helix structure at the C terminal region but the N terminal region is flexible. The present structural information may be helpful for developing more effective peptide drug candidate for the treatment of diseases dependent on angiogenesis.
Keywords : Structure, YH motif peptide, Second fas-1 domain of βig-h3

INTRODUCTION

The βig-h3 is an extracellular matrix protein and induced by transforming growth factor-β in several cell types.[1] It mediates the adhesion of several cell types through interaction with integrins.[2-4] In addition, βig-h3 has been reported to be involved in cell growth, cell differentiation,

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and wound healing.[1,5-7] The βig-h3 protein comprises 683 amino acids containing four homologous internal repeat domains. These domains are homologous to similar motifs in the Drosophila protein fasciclin-I and thus are denoted fas-1 domains.[8] Just the second and forth fas-1 domains of βig-h3 are involved in corneal epithelial cell adhesion via interacting with $\alpha_3\beta_1$ integrin,[2] whereas all four fas-1 domains of βig-h3 are involved in the adhesion of fibroblastic cell and endothelial cell, interacting with the $\alpha_v\beta_5$ and $\alpha_v\beta_3$ integrin, respectively.[3-4] The 18 residue YH motifs within each fas-1 domain are known to be responsible for the interaction with the $\alpha_v\beta_5$ and $\alpha_v\beta_3$ integrin.[3-4] In addition, the synthetic YH motif peptides inhibit endothelial tube formation and reduces the number of blood vessels in a Matrigel plug assay, so expected to be an effective inhibitor of angiogenesis and a drug candidate for the treatment of diseases dependent on angiogenesis.[4] In this study, we solved the 3D structure of the 18 residue YH motif peptide (EALRDLLNNHILKSAMCA; D2 peptide)) within the second fas-1 domain of βig-h3 using NMR in order to obtain the information about the structure-activity relationship and to conduct further peptide engineering for more effective peptide drug candidate.

MATERIAL AND METHODS

Samples for NMR measurements contained 3mM synthetic peptide (EALRDLLNNHILKSAMCA; D2 peptide) purchased from AnyGen Co. Ltd. (Kwangju, Korea) in a 50%TFE/H_2O solution containing 7% D_2O at pH 4.0. Conventional 2D DQF-COSY, TOCSY (60 ms mixing time), and NOESY (200 ms mixing times) spectra were acquired by using a Bruker AVANCE-600 spectrometer at 303 K. The Spectra were processed and analyzed using NMRPipe/NMRDraw[9] and NMRView.[10] The sequence-specific assignments of the proton resonances were achieved by spin system identification from the TOCSY and DQF-COSY spectra, followed by sequential assignments through the NOE connectivities. Distance restraints were obtained mainly by manual assignments of the NOE cross-peaks in the NOESY spectra. A total of 100 structures were calculated for each peptide by the simulated annealing and energy

minimization protocol in the program CNS 1.1.[11] Finally, eight structures with the lowest energ-

Table I. The chemical shifts of D2 peptide

Amino Acid		HN	Hα	Hβ	Hγ	Hδ	Hε	Etc
1	E	7.935	n.d.	1/962/2.097	2.718/2.467			
2	A	8.585	4.596	1.761				
3	L	8.587	4.425	1.945/1.945	1.893	1.223/1.166		
4	R	7.936	4.216	2.162/2.097	1.962	3.499		
5	D	7.977	4.414	3.117/2.987				
6	L	8.261	4.414	2.146	2.01	1.177		
7	L	8.593	4.218	2.074	1.905	1.155/1.102		
8	N	8.681	4.415	3.267/3.066				γNH_2 7.912, 6.695
9	N	8.372	4.416	3.246/3.099				γNH_2 7.653, 6.955
10	H	8.52	4.624	3.666/3.585				4H 7.251
11	I	8.752	3.907	2.281	2.089/1.950/1.42	1.175		γCH_3 1.428
12	L	8.363	4.367	2.059	1.951	1.173		
13	K	8.449	4.224	2.176/2.126	1.745	1.864	2.867	
14	S	8.205	4.278	4.086				
15	A	8.191	4.489	1.767				
16	M	8.528	4.612	2.490/2.384	2.948/2.865		2.291	
17	C	8.294	5.041	3.534/3.289				
18	A	7.64	4.385	1.71				

n.d. not detected

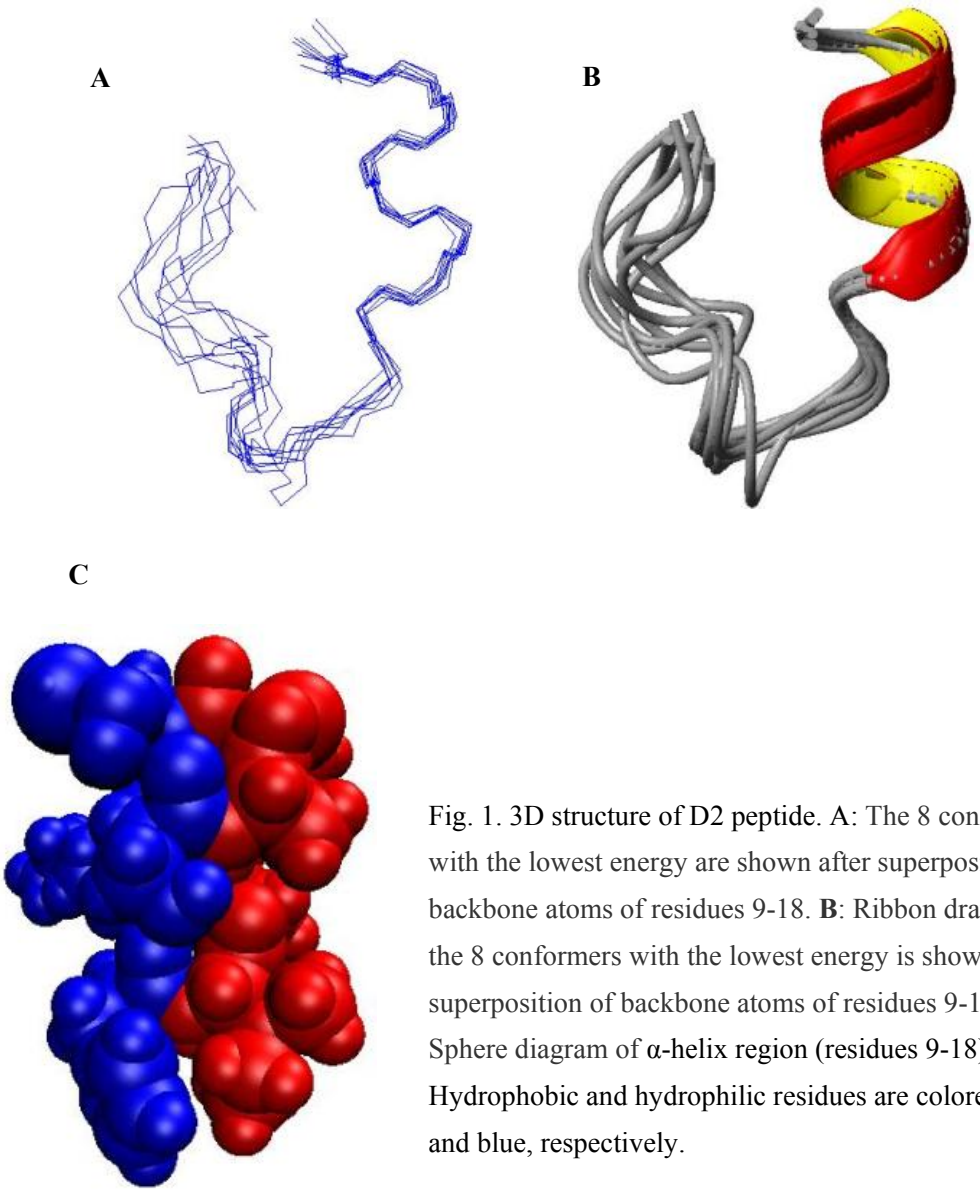

Fig. 1. 3D structure of D2 peptide. A: The 8 conformers with the lowest energy are shown after superposition of backbone atoms of residues 9-18. **B**: Ribbon drawing of the 8 conformers with the lowest energy is shown after superposition of backbone atoms of residues 9-18. **C**: Sphere diagram of α-helix region (residues 9-18). Hydrophobic and hydrophilic residues are colored in red and blue, respectively.

ies were accepted to represent ensemble structure and to obtain the energy-minimized average structure. Analyses of final structures were performed using the program PROCHECK-NMR[12] and MOLMOL.[13] The molecular graphics images were produced by the MOLMOL program.

RESULTS AND DISCUSSION

Table I shows the chemical shifts of the protons in the D2 peptide. Almost all proton resonances were successfully assigned. Fig. 1 shows 3D structure of the D2 peptide. The N terminal region of the D2 peptide is flexible, but the C terminal region (residues 9-18) has α-helix structure. This α-helix shows an amphipatic shape.

The present structural information of the 18 residue YH motif peptide within the second fas-1 domain of βig-h3 may be helpful for developing more effective peptide drug candidate for the treatment of diseases dependent on angiogenesis.

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