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Conformational analysis in solution of protein kinase C βII V5-1 peptide

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Abstract

One of protein kinase C (PKC) isozymes, PKC β binds to receptor for activated C kinase 1 (RACK1), and their complex is suggested to be translocated to melanosomes. The binding site of PKC β for RACK1 is considered one of its catalytic domains, V5 domain which consists of three motifs such as V5-1, V5-2, and V5-3. Among these, V5-1 region, extreme C-terminal residues of PKC β showed the highest RACK1-binding affinity. PKC β can be classified into PKC βI and PKC βII based on their different V5 domains. RACK1-binding affinity of PKC βII is five times greater than that of PKC βI. The structures of PKC βI, PKC βII, and RACK1 are not known. However, the conformational study on PKC βII V5-1 region showing high RACK1-binding selectivity may help us in understanding the interaction between RACK1 and PKC βII.

Keywords: NMR; Protein kinase C βII; Human; Peptide; Receptor for activated C kinase 1

Protein kinase C (PKC) is a subgroup of protein kinases and belongs to a phospholipid-dependent serine/threonine protein kinase [1]. PKC resides in the cytosol in its inactive state, but it translocates to the plasma membrane in its active state [2]. PKC consisting of at least 11 isozymes such as α, βI, βII, γ, δ, ε, ζ, η, θ, ι, and μ plays important roles in cellular proliferation and differentiation [3–5]. It can be classified by three groups based on their structure, cofactor, and calcium ion dependence [2]. While classical PKC (cPKC) including α, βI, βII, and γ needs diacylglycerol, phospholipids, and calcium ion for its activation, novel PKC (nPKC) including δ, ε, θ, η, and μ does diacylglycerol and phospholipids but not calcium ion. Atypical PKC (aPKC) represented by ζ and ι requires only phospholipid for an activation. PKC consists of several conserved regions (C1, C2, C3, and C4) which are separated by variable regions (V1–V5) [6]. Based on the structure, PKC is composed of two domains such as regulatory domain (V1–C1–V2–C2–V3) and catalytic domain (C3–V4–C4–V5). Combination of conserved and variable regions results in the formation of a regulatory domain and a catalytic domain. The regulatory domain is variable in three groups of PKC while the catalytic domain that has an arrangement of C3, V4, C4, and V5 is conserved. The structure of the whole sequence of PKC has not been solved yet. Only partial structures containing C2 domain are known [7–10].

Of PKC isozymes, PKC β binds to receptor for activated C kinase 1 (RACK1) [11]. It was suggested that the complex of PKC β and RACK1 translocates to melanosomes via interaction with the cytoplasmic tail of tyrosinase, where PKC β phosphorylates tyrosinase [11]. The binding site of PKC β with RACK1 is considered PKC β V5 region [12]. V5 domain consists of V5-1, V5-2, and V5-3. Of these three peptides, V5-1 region showed the highest RACK1-binding selectivity, which is composed of extreme C-terminal residues. PKC βI and βII consist of 671 and 673 residues, respectively. Fifty residues of the C-terminal in βI and 52 residues in βII belong to V5 domain. All residues, except V5 domain in βI and βII, are identical [13]. Comparing RACK1-binding selectivity of βI V5-1 with that of βII V5-1, the latter shows five times greater selectivity than βI V5-1, so that V5-1 region should cause the binding specificity. Therefore, the conformational studies on PKC βII V5-1 region may help us in understanding the interaction

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between RACK1 and PKC βII as well as its function. Authors report the solution structure of PKC βII V5-1 region obtained by NMR spectroscopy.

Materials and methods

Sample preparation. The PKC βII V5 peptide was synthesized by American Peptides (Sunnyvale, CA). Its primary sequence is Ser^{660}-Phe-Val-Asn-Ser-Glu-Phe-Leu-Lys-Pro-Glu-Val-Lys- Ser^{673} whose molecular weight is 1609.8 Da. The mass data obtained by ESI-MS (VG platform, VG BIOTECH, Manchester, UK) were *m/z* 1610.6 (MH^+) and the purity of the peptide determined by reversed-phase HPLC (Waters, Milford, MA) was 98.4%. For HPLC analysis, an injection volume of 20 μl, C18 column (4.6 mm × 250 mm), a mixture eluent of 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile, a flow rate of 1.5 ml/min, and a detection wavelength at 215 nm were used.

NMR spectroscopy. All NMR measurements were performed on a Bruker Avance 600 spectrometer system (14.1 T, Karlsruhe, Germany) with a cryoprobe at 298 K. The NMR spectra of ^1H NMR, correlated spectroscopy (COSY), double quantum filtered correlated spectroscopy (DQF-COSY), total correlated spectroscopy (TOCSY), and nuclear Overhauser and exchange spectroscopy (NOESY) were collected in 90% H_2O/10% D_2O. The concentration of the sample was approximately 1 mM. For ^1H NMR analysis, 16 transients were acquired with a 1 s relaxation delay using 32 K data points. The 90° pulse was 11.0 μs with a spectral width of 9615 Hz. Two-dimensional spectra were acquired with 2048 data points for t2 and 256 for t1 using time proportional phase increments, except the COSY experiment where magnitude mode was applied. The NOESY experiment was performed at the mixing time of 200 ms. The mixing time for TOCSY with MLEV17 spin-lock pulse program was 80 ms. In all experiments, water peaks were suppressed by presaturation. Prior to Fourier transformation, zero filling of 2 K and sine squared bell window function were applied using XWIN-NMR (Bruker, Karlsruhe, Germany) [14].

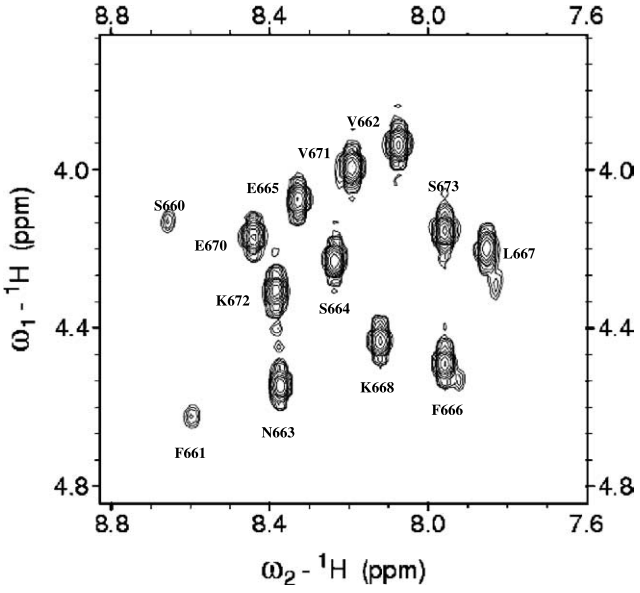

Fig. 1. The CαH–NH cross peaks observed in the COSY spectrum of βII V5-1 peptide.

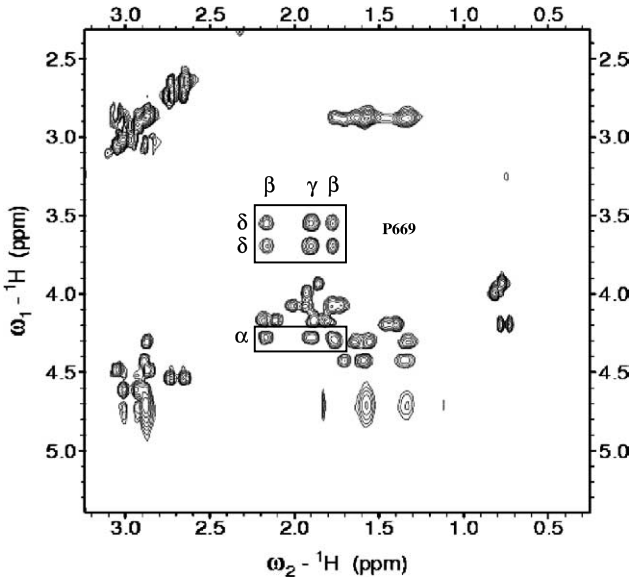

Fig. 2. The TOCSY spectrum of βII V5-1 peptide showing particular cross peak pattern of Pro^{669}.

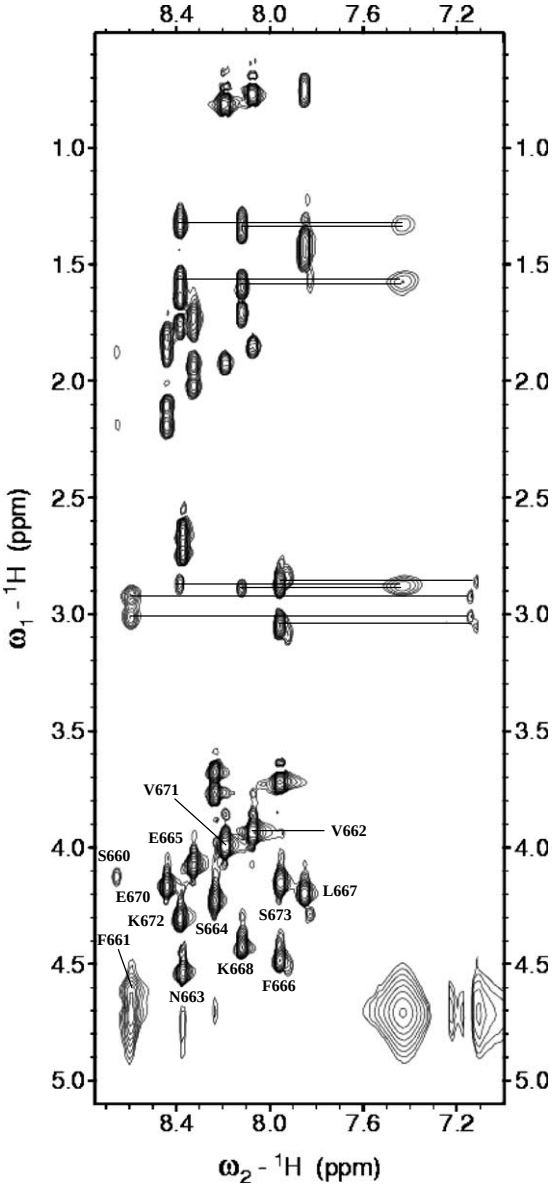

Fig. 3. The TOCSY spectrum of βII V5-1 peptide. The solid lines indicate the cross peaks of Phe and Lys ($\omega_1 = 0.4$–5.0 ppm, $\omega_2 = 7.0$–8.7 ppm).

Structure calculation. Data analysis was carried out using Sparky [15]. The molecular modeling calculations were carried out on an O2 R12,000 Silicon Graphics workstation [16]. The initial structure of the molecule was generated using InsightII/Bioplymer module (Accelrys, San Diego, CA). The constraints were given to the initial structure based on the distances obtained from the nOe data and the dihedral angles calculated from the ^{3}J coupling constants. The force field used for molecular dynamics (MD) and energy minimization was consistent valence force field (cvff) provided by Accelrys. The molecule was subjected to energy minimization by InsightII/Discover module. Steepest descents were carried out until maximum derivative of 0.1 kcal/molÅ, and conjugate gradients were followed until maximum derivative of 0.01 kcal/molÅ. After energy minimization, MD was performed from 300 to 1000 K with 100 K increment step. At the every step, MD was carried out during 2.5 ps. At 1000 K, MD was performed for 250 ps, and the output conformers were collected at every 2.5 ps and 100 conformers were saved in the history file. The conformer with the lowest total energy was chosen and used for further MD simulation from 1000 to 300 K with 100 K step. At 300 K, MD was carried out for 250 ps with 1 fs each step. The history file collected the out conformers at every 2.5 ps. The energy profile was analyzed using InsightII/Analysis module. Among 100 conformers, 10 conformers showing the low total energy were selected and they were superimposed. Of these, the conformer with the lowest energy was chosen and a statistical evaluation was performed using PROCHECK [17].

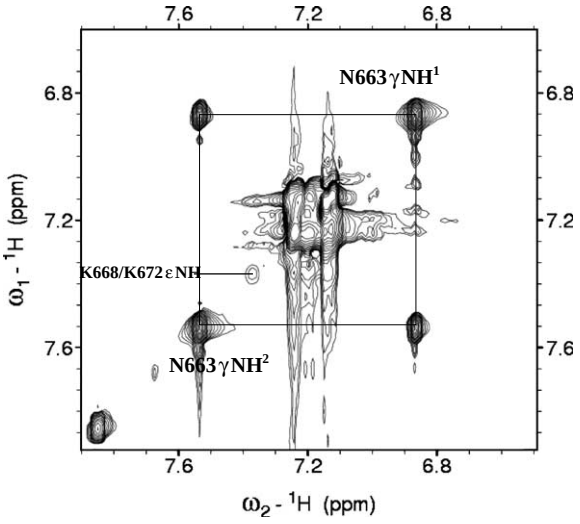

Fig. 4. The partial TOCSY spectrum defining the region of 6.8–7.6 ppm.

Distance constraints were calculated using the relationship $\eta_{ij}/\eta_{kl} = (r_{kl}/r_{ij})^6$, where η and r are the nOe intensity and distance for the hydrogen atom pairs i, j and k, l, respectively [18]. The amide protons of Asn^{663} were used as a reference, and the distance was 1.74 Å. We classified nOe intensity as strong, medium, and weak based on their distance ranges of 1.8–2.5, 2.5–3.5, and 3.5–5.0 Å, respectively.

Using the following Karplus type equation [19], the coupling constants were calculated

$$^3J_{NH\alpha} = 6.7\cos^2\phi - 1.3\cos\phi + 1.5,$$

where ϕ is the dihedral angle between the NH and CαH proton.

Results and discussion

The NMR data obtained in aqueous solution (90% H_2O/10% D_2O) were assigned based on the procedures as follows. Of 14 residues, 13 cross peaks between NH and CαH were observed in the COSY spectrum. The remained residue was Pro^{669}. As shown in Fig. 1, all cross peaks were dispersed between 7.85 and 8.66 ppm. Since Pro^{669} did not contain NH and did show a particular spin system, it could be assigned easily based on the interpretation of TOCSY (Fig. 2). The connectivities among NH, CαH, CβH, CγH, and so on were observed in the TOCSY spectrum (Fig. 3). Here, the intensities of the proton peaks connected with Ser^{660} NH were weaker than others because the labile proton of Ser^{660} could exchange fast with solvent molecules. In addition, the cross peaks between Phe^{661} CδH (7.14 ppm) and $C\beta H_2$ (2.92, 3.01 ppm), and those between Phe^{666} CδH (7.12 ppm) and $C\beta H_2$ (2.86, 3.05 ppm) were found in TOCSY (Fig. 3). Since the ^{1}H peak at 7.43 ppm showed the connectivities with protons of side chains of Lys^{668} and Lys^{672}, it should be assigned εNH_3^+ of Lys. However, two εNH_3^+ cannot be distinguished. Two peaks at 6.86 and 7.53 ppm must be Asn^{663} γNH_2 because of their large chemical shift difference (Fig. 4). The aromatic protons of Phe^{661} and Phe^{666} could be assigned based on the

Table 1
^{1}H chemical shifts (δ, ppm) of βII V5-1 peptide in water at 298 K

Residue	Chemical shift				$^3J_{NH\alpha}$ (Hz)
	NH	CαH	CβH	CγH and others	
Ser^{660}	8.66	4.13	2.19, 1.87	—	—[a]
Phe^{661}	8.59	4.62	3.01, 2.92	CδH 7.14; CεH 7.23; CζH 7.20	6.6
Val^{662}	8.07	3.93	1.85	0.77	7.8
Asn^{663}	8.37	4.54	2.74, 2.64	γNH_2 7.53, 6.86	6.5
Ser^{664}	8.23	4.23	3.77, 3.67	—	6.4
Glu^{665}	8.32	4.07	1.78, 1.73	2.02, 1.94	6.4
Phe^{666}	7.96	4.48	3.05, 2.86	CδH 7.12; CεH 7.24; CζH 7.17	7.5
Leu^{667}	7.85	4.20	1.45	1.40; CδH 0.75	7.2
Lys^{668}	8.12	4.43	1.70	1.34; CδH 1.59; CεH 2.89; εNH_3^+ 7.43	6.9
Pro^{669}	—	4.28	2.16, 1.77	1.90; CδH 3.69, 3.55	—
Glu^{670}	8.44	4.17	1.89, 1.81	2.19, 2.11	7.0
Val^{671}	8.19	3.99	1.93	0.81	7.9
Lys^{672}	8.38	4.30	1.76	1.32; CδH 1.64, 1.56; CεH 2.87; εNH_3^+ 7.43	7.2
Ser^{673}	7.96	4.15	3.72	—	7.5

[a] Not observed.

comparison of COSY with TOCSY. The ^{1}H chemical shifts of the βII V5 peptide are listed in Table 1.

The sequence of residues could be determined by the interpretation of the NOESY spectrum. The sequential nOes between $C\alpha H_i$ and NH_{i+1}, except Lys^{668}-Pro^{669}, were observed in NOESY (Fig. 5). The sequential nOes between NH_i and NH_{i+1} are shown in Fig. 6. Of 89 nOe cross peaks observed in NOESY, 16 peaks were caused by inter-residue nOe. The nOe cross peaks were investigated using Sparky program, and the distances between two protons were calculated based on the equation, $\eta_{ij}/\eta_{kl} = (r_{kl}/r_{ij})^6$. The nOe intensities are classified based on their distances such as strong, medium, and weak, which are listed in Table 2. According to the sequential nOes and the nOe intensities, the sequential map can be made and shown in Fig. 7. From the map, it can be confirmed that this peptide does not have a defined secondary structure. In order to clarify the information about the secondary structure obtained from the nOe sequential map, chemical shift index was investigated. As shown in the top of Fig. 7, four residues from Asn^{663} to Phe^{666} have a continuous negative index, so that the existence of a short coil can be considered.

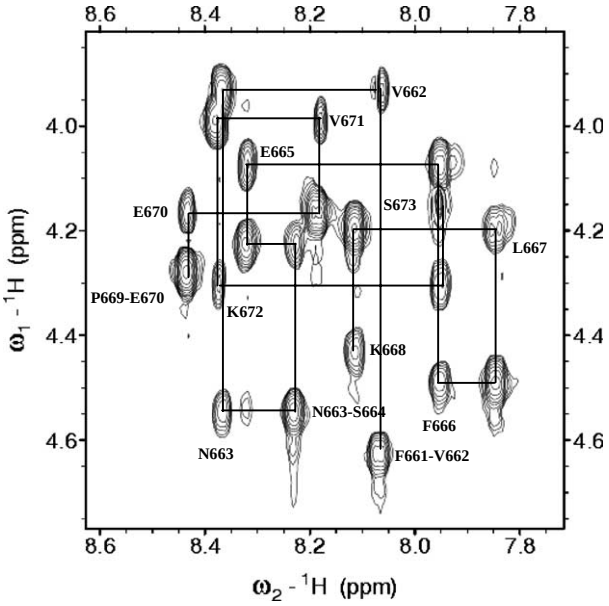

Fig. 5. The NOESY spectrum showing $C\alpha H_i$–NH_{i+1} cross peaks.

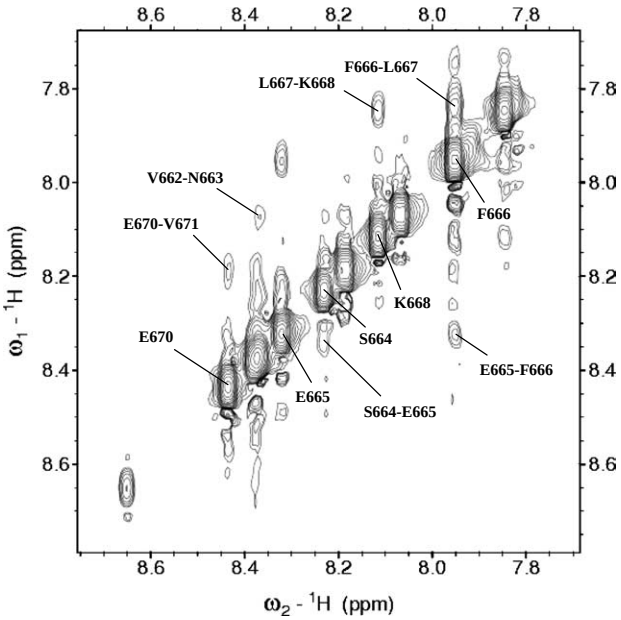

Fig. 6. The NOESY spectrum showing NH_i–NH_{i+1} region.

Table 2
The distances calculated from the nOe cross peaks

Proton 1	Proton 2	Distance (Å)	Intensity[a]
Asn^{663} NH	Val^{662} CγH	3.68	w
Asn^{663} NH	Ser^{660} CβH	3.02	m
Glu^{665} NH	Asn^{663} CαH	3.70	w
Glu^{665} NH	Ser^{664} CβH	3.81	w
Leu^{667} NH	Phe^{666} CβH	3.29	m
Lys^{672} NH	Leu^{667} CδH	3.44	m
Lys^{672} NH	Pro^{669} CδH	2.27	s
Lys^{672} NH	Leu^{667} CβH	2.34	s
Lys^{672} NH	Leu^{667} CγH	2.66	m
Lys^{672} CαH	Pro^{669} CδH	2.22	s
Lys^{672} CβH	Pro^{669} CδH	3.18	m
Glu^{670} NH	Pro^{669} CαH	2.01	s
Glu^{670} NH	Pro^{669} CδH	4.08	w
Glu^{670} NH	Pro^{669} CβH	3.14	m
Val^{671} NH	Pro^{669} CαH	3.80	w
Val^{671} CαH	Pro^{669} CγH	3.14	m

[a] w, weak; m, medium; s, strong.

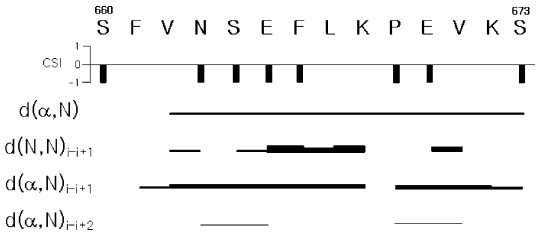

Fig. 7. The nOe sequential map and chemical shift indices (CSI) of βIIV5-1 peptide.

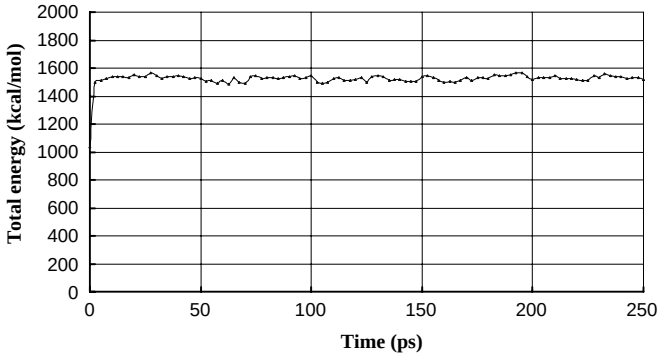

Fig. 8. A plot of total energy against time during molecular dynamics simulations.

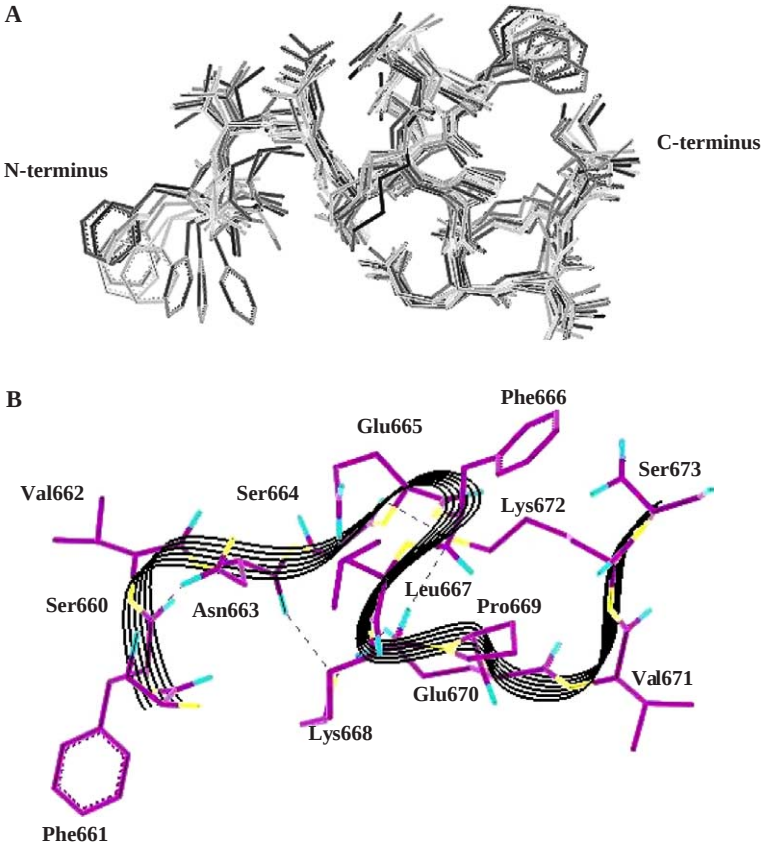

Fig. 9. (A) Superimposed heavy atoms of the 10 structures of βII V5-1 peptide and (B) its ribbon representation.

In addition to distances of the inter-residue protons, dihedral angles were calculated from the $^{3}J_{NH\alpha}$ coupling constants. Twelve dihedral angle values are listed in the right column of Table 1. In the case of Ser^{660} and Pro^{669}, the constants were not observed.

In the simulated annealing, ten structures of low total energy were obtained using the distances calculated from nOe data and the dihedral angles from $^{3}J_{NH\alpha}$. At the final simulated annealing step, the peptide was subjected to energy minimization and molecular dynamics at 300 K, 1 atm for 250 ps with 1 fs each step. A plot of the total energy against the time is shown in Fig. 8. The total energy was ranged within 100 kcal/mol. The value of the root mean square deviation (RMSD) of 10 superimposed backbone structures was 0.47 Å (Fig. 9A). The N-terminal showed the bigger fluctuation than the C-terminal because Pro^{669} is close to the C-terminal. The structure with the lowest total energy was chosen and evaluated using PROCHECK. The statistical analysis of Ramachandran plot showed that 45.5% are in the most favored region, 54.5% in the additional allowed region, and 0% in the generously allowed region and the disallowed region (Fig. 10).

As mentioned before, authors expected the peptide may have a short helix based on its chemical shift index analysis. Fig. 9B shows that there is a turn in the peptide structure between Ser^{664} and Pro^{669}. This result agrees to the consideration obtained from chemical shift index.

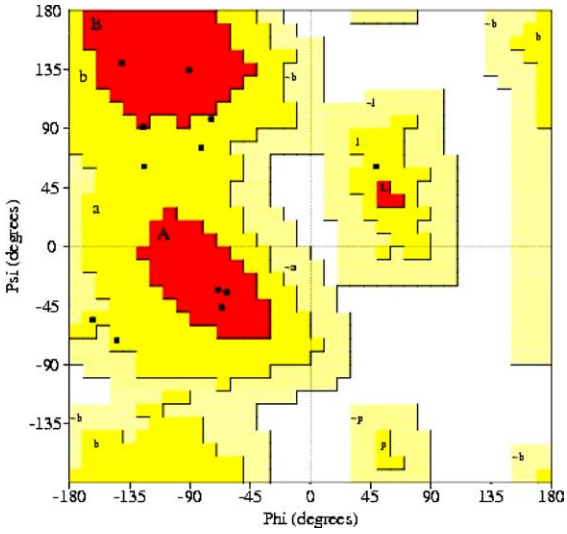

Fig. 10. Ramachandran plot of βII V5 peptide obtained from PROCHECK.

The Connolly surface of the peptide was made by InsightII, which gave information about the hydrophobicity of the peptide. This peptide consists of eight hydrophilic residues and 6 hydrophobic residues, but its surface is more hydrophobic as shown in Fig. 11. While the side chains of Phe^{661}, Val^{662}, Phe^{666}, Leu^{667}, and Val^{671} were exposed to the surface, the others were hidden inside. Especially, as

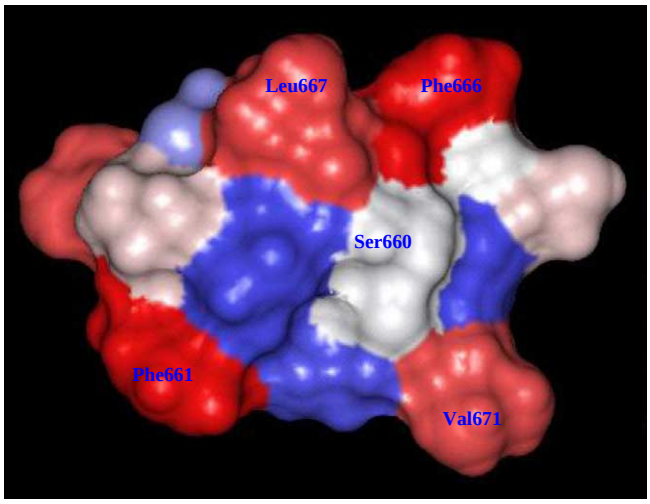

Fig. 11. Connolly surface for βII V5-1 peptide. (Red, the most hydrophobic; blue, the most hydrophilic; white, medium.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

shown in Fig. 9A the side chains of Phe^{661} and Phe^{666} are more flexible than other residues so that they contribute to the hydrophobicity of the surface. In addition, because the hydroxyl group of Ser^{660} forms H-bond with the gamma carbonyl group of Asn^{663}, Ser^{660} OH faces the core and Ser^{660} $C\beta H_2$ does to the surface. The side chain of Ser^{660} contributes to the hydrophobicity, instead of hydrophilicity. As a result, even though the binding site of RACK1 with PKC βII is not known yet, it may prefer the hydrophobic surface such as PKC βII V5-1 region.

Acknowledgments

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