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To Probe the Conformational Adaptability of Conserved G-P-G-R Segment in the V3 Loop of HIV-1

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Abstract

To design an effective HIV vaccine it is important to study the relationship between sequence diversity and conformations of principal neutralizing determinant (PND) peptides, the hypervariable region of gp120. Two fragments 318-327 and 315-329 of gp120 are mapped as PND and can induce HIV-1 specific cytotoxic lymphocyte activity that could kill virus infected cells. Consequently, the design of more ordered and biologically relevant conformation of immunogenic region from gp120-V3 loop may aid in the design of more effective immunogens for HIV-1 vaccine development. In order to enlighten optimal structural preferences we have explored the conformational adaptability of two fragments from V3 loop of gp120 that contains G-P-G-R sequence encompassing mainly the crown region. Nuclear Magnetic Resonance (NMR) spectroscopy and molecular dynamics (MD) simulations have been used to map out the conformation in diverse solvent systems such as water and hexafluroacetone (HFA). In HFA, the larger fragment, 315-329 shows some degree of inclination towards the formation of secondary structure. This indicates that length of the fragment is crucial in attaining secondary structure, which may be responsible for V3 loop's hypervariable status. However, in water, both the fragments do not show any specific conformational preferences.

Keywords: HIV-1; Gp120; V3 loop; NMR; Molecular dynamics

Introduction

The acquired immunodeficiency syndrome (AIDS) is one of the most lethal disease and is caused by human immunodeficiency virus (HIV) [1]. The mature virion contains cone shaped capsid which encapsulates two strands of genomic RNA, cellular proteins and enzymes. The capsid is enclosed within an envelope glycoprotein which contains two subunits. The external gp120 binds to the $CD4^+$ receptor as well as to co-receptor CCR5 or CXCR4 [2,3] on the surface of target cells. The inner gp41 plays major role in viral fusion. The structural information regarding the various conserved and variable fragments of HIV envelope protein chiefly gp120 is vital in the development of HIV vaccine, as it is known target for neutralizing antibodies. The mechanism of virus neutralizing activity is not yet clear. Gp120 is further subdivided into five variable (V1-V5) and five constant regions (C1-C5). Among all, third variable loop (V3) mapped as PND [4] of the virus, is of importance due to its hyper-variability and crucial relevance to the co-receptor usage. It is 34-36 residues long, occupying 296 - 331 positions relative to HXB2 [5]. Residues 306-320 designated as V3 crown and residues 296-305 and 321-330 as N and C terminal strands of V3 stem. The crown and stem of V3 are two functionally different domains. V3 stem alone mediates soluble gp120 binding to CCR5 and V3 crown alone determines co-receptor usage. Upon binding $CD4^+$, gp 120 undergoes conformational changes involving V3 loop which facilitates subsequent interactions with the co-receptors CCR5/CXCR4. The amino acid sequence of the V3 loop is highly variable among different isolates especially in the regions flanking the highly conserved G-P-G (97%) [6]. G-P-G-(R/K/Q) sequence is situated in the crown (or crest) region at the center of the neutralizing domain. It is reported that G-P-G crest forms a β turn, with the flanking regions as two strands of an antiparallel β sheet [7-9]. Sequence changes close to the G-P-G motif can alter the stability of the β sheet and/or alter the surface accessibility thereby, influencing co-receptor usage. V3 loop derived peptides have been found to be structurally similar to distinct chemokines, the natural ligands of CCR5 and CXCR4 [10]. This suggests that alternative V3 conformations are responsible for selective interactions with the co-receptors. Thus understanding the conformational need of the most variable loop in HIV-1 virus, having conserved sequence will add the important information for AIDS vaccine development. So far it has been shown that the optimal length of the amino acid sequence may play a crucial role in its function [11]. Thus it will be significant to study the optimal requirement of the peptide fragment by selecting a combination of peptides with conserved region of PND that has a specific structural framework. It may produce candidate vaccines that induce antibodies capable of neutralizing the majority of HIV-1 strains.

In order to enlighten optimal structural preference we have explored the peptide sequence of V3 loop of gp120 that contains G-P-G-R sequence which encompass mainly the crown region using diverse solvent systems such as water and hexafluroacetone (HFA) with the help of Nuclear Magnetic Resonance (NMR) spectroscopy and restrained molecular dynamics (MD) simulations. In addition to understand the effect of solvated system on conformational adaptability of V3 loop fragments, we have carried out MD simulations without any restrains using water solvated system on four different fragments of varying length. The fragments studied were molecule 1: Arg-Gly-Pro-Gly-Arg-Ala (6 residues), molecule 2: Gln-Arg-Gly-Pro-Gly-Arg-Ala-Phe (8 residues), molecule 3: Arg-Gly-Pro-Gly-Arg-Ala-Phe-Val-Thr-Ile (10 residues, same as V3 loop fragment 318-327) and molecule 4 (15 residues, same as V3 loop fragment 318- 329). Fragment 318-327

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of V3 is reported as PND region. This might induce HIV-1 specific cytotoxic lymphocyte activity [11] and thus can act as immunogen to induce a cytotoxic response that could effectively kill virus infected cells. Another fragment 315-329 of gp120 is capable of inducing HIV-1 specific cytotoxic T lymphocyte response that could kill virus infected cells [12]. Consequently, the design of more ordered and biologically relevant conformation of immunogenic region from gp120-V3 loop may aid in the design of more effective immunogens for HIV-1 vaccine development. To design an effective HIV vaccine it is important to study the relationship between sequence diversity and conformations of the PND.

Materials and Methods

Materials

Peptide fragments 318-327 and 315-329 were purchased from Bachem (Switzerland) while solvent purchased from Sigma chemical Co. (USA).

Sample preparation for NMR

2 mg each of the fragment containing the G-P-G-R sequence (fragment 318-327: and fragment 315-329) was dissolved in 90% water with pH adjusted to 4.68 (10% D_2O) separately to give 600 µl sample volume. At these concentrations, no aggregation was observed by NMR for either peptide. Various 1D and 2D NMR experiments were done on Varian Inova 600 and Bruker 500 machine. For resonance assignments two dimensional DQF-COSY [13] and TOCSY [14] were recorded. The mixing pulse (80 ms) in TOCSY was achieved by the MLEV-17 scheme. For deducing the conformation 2D-NOESY [15] and ROESY [16] at different mixing times was recorded. The number of transients was 32 for TOCSY, NOESY/ROESY and 64 for DQF-COSY. All 2D NMR experiments were recorded with the conventional pulse sequences and a 1.5 s relaxation delay. The 2D data were processed using Felix software (v97.0 MSI, USA) running on Silicon Graphics O2 workstation. Then the same sample was lyophilized, dissolved in 40% HFA and carried out all above mentioned NMR experiments on it. Relevant data such as temperature coefficients of NH chemical shifts, $^3J_{NH\alpha}$ coupling constants, CSI values, dihedral and distance restrains were fetched from NMR data.

Molecular Dynamics (MD) simulations

All NMR data incorporated in a restrained MD simulation on 2.8 GHZ D Intel P4 computer using Accelrys DS 2.1 [17] with CHARMm force field [18]. MD simulations without any restrains on solvated system (water as solvent) on four different fragments of varying length were also performed in the similar manner. The fragments studied were molecule 1: Arg-Gly-Pro-Gly-Arg-Ala (6 residues), molecule 2: Gln-Arg-Gly-Pro-Gly-Arg-Ala-Phe (8 residues), molecule 3: Arg-Gly-Pro-Gly-Arg-Ala-Phe-Val-Thr-Ile (10 residues, same as V3 loop fragment318-327) and molecule 4 (15 residues, same as V3 loop fragment 318- 329). Before performing MD simulations the extended structure of all fragments was thoroughly minimized using "Smart Minimzer" method in energy minimization protocol in DS 2.1 that performs steepest descents and conjugate gradients with the CHARMm force field to a gradient of 0.0001 kcal/mol/Å. The protocol minimizes the energy of a structure through geometry optimization. Minimization using steepest descent and conjugate gradient were performed at 5000 cycles and 10000 cycles respectively. For unrestrained MD simulation of above four fragments, solvation of each fragment was done in solvent water with "explicit periodic boundary" conditions on. The complexes were created with a 10 Å solvation shell of water around the protein. MD simulation on all solvated fragments was done using standard dynamics cascade protocol of DS 2.1 which performs the following steps. Step1 dynamics with heating where each peptide was gradually heated from 0K to 300K over 5 ps. Step 2 was 10 ps equilibration dynamics followed by step 3 production dynamics was performed for 100 ps using NVT canonical ensemble and trajectory frames were saved every ps. SHAKE algorithm was applied to immobilize all bonds involving hydrogen atoms throughout the MD simulation. Time step was set to 1 fs for all MD stages. The dynamics (heating or cooling) protocol allows controlling the temperature of a system when performing a molecular dynamics simulation. The lowest energy structure from the 300 K trajectory was then subjected to a final round of minimization, using the "Smart Minimzer" method (10000 iterations) in energy minimization protocol in DS 2.1 to a gradient 0.0001 kcal/mol/Å.

Distance restraints: The cross peaks obtained from ROESY were categorized as strong, intermediate and weak with corresponding distance ranges (1.8-2.7 Å), (1.8-3.5 Å) and (1.8-5.0 Å) set for respective protons. These distances were modified for methyl and methylene groups and for aromatic rings that rotate fast on the NMR time scale. For such groups of protons, it is necessary to define a pseudoatom which is used as a reference point for the distance restraint. Corrections for the distances to such pseudoatoms were made according to the rules initially formulated by Wüthrich [19]. Force constants for distance restraints ranged from 25 to 30 kcal/mol/Å^2.

Dihedral restraints: The $^3J_{NH\alpha}$ coupling constant is related to the dihedral angle φ as expressed by the relationship [20]: $^3J_{NH\alpha}=6.7\cos^2\varphi-1.3\cos\varphi+1.5$.

The coupling constants were transformed to φ, ϕ values which were introduced as dihedral restraints allowing a range of ±10° on the calculated values, with a force constant of 25-50 kcal/mol/rad^2.

Result and Discussion

MD simulation

The unrestrained MD simulations for all four fragments of varying lengths in water solvated system indicate random coil conformation (Figure 1). Out of these four fragments, molecule 3 and 4 studied broadly using multidimensional NMR in water and HFA. The NMR data is used as restrains in MD simulations of these two fragments 318-327 and 315-329 in both solvent systems. A representation of structures obtained from restrained MD simulation for fragment 318-327 and 315-329 in water and HFA is shown in figures 2 and 3. The backbone torsion angles (φ, ϕ values) averaged over the ensembles of the structures obtained from restrained and unrestrained MD simulations for fragment 318-327 indicates that the peptide adopt a random coil structure (Figure 4). The dihedral angle values obtained from MD simulations done in water and HFA for fragment 318-327 using NMR restrains as well as unrestrained MD by solvating the fragment with water is tabulated in table 1.

NMR

The amino acid residues of both fragments 318-327 and 315-329 were easily identified in the TOCSY spectra of respective peptides and sequential assignments in both solvents were achieved by the standard procedures using the ROESY spectra as elaborated (Figures 5-7). The unique spin systems such as Gly, Ala, Val, Thr, and Ile in case of both fragments could be distinguished easily. Phe in both fragments was identified from the ROESY cross peaks arising from its aromatic protons to α and β protons. Arg was confirmed from peaks from its εNH resonance to its α, β, γ and δ protons. The presence of Pro residue

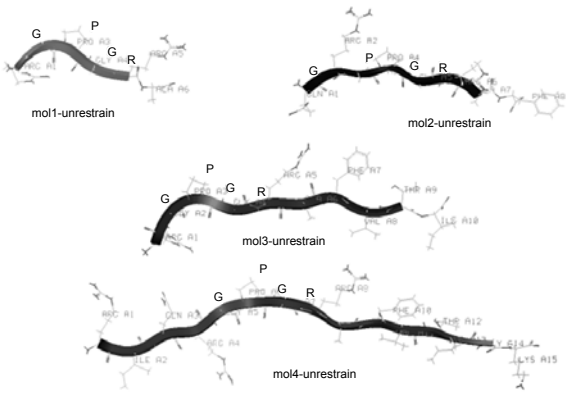

Figure 1: Conformation obtained from unrestrained MD simulation for molecule 1 to molecule 4 with variable fragment length.

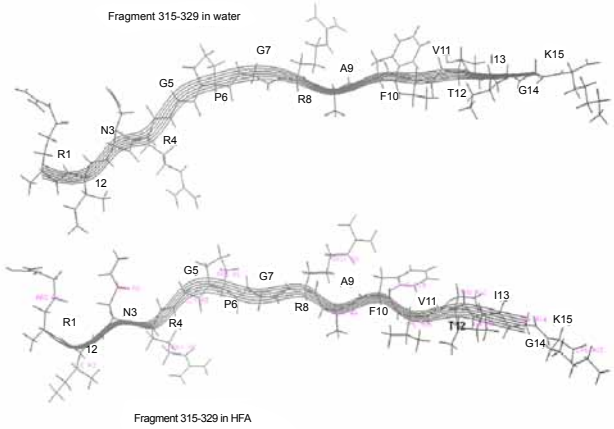

Figure 2: Global conformation of fragment 318-327 in water and HFA. The ribbon traces the backbone atoms.

at position 3 in fragment 318-327 and 5 in fragment 315-329 caused a break in the assignment. The observation of cross peak between Gly[3] CαH and Pro[3] CδH mended this gap and also indicated 'trans' state of Gly-Pro amide bond. Pro residue of fragment 315-329 also indicated similar cross peak between Gly[5] CαH and Pro[6] CδH observation showing 'trans' state of amide bond. Once the proton assignment was complete the ^{13}C assignments were done from the HSQC spectrum by correlating the assigned proton NMR signals to the ^{13}C NMR signals. The ^{13}C assignments helped to further confirm all residues assigned.

1D temperature coefficients of NH for fragment 318-327 in water as well as in HFA indicated solvent exposed amides for all residues dΔ/dT > 6 ppm/°K. Most of the residues indicate J-values for NH's ($^3J_{NH}$) above 7.0 Hz indicating presence of random coil. The ^1H chemical shifts, observed temperature coefficient of the NH and $^3J_{NH}$ for both fragments in water and HFA are listed in tables 2 and 3. Both fragments in water as well as HFA exhibit a second set of weak peaks that could be attributed to minor conformations. Secondary structural characteristics through NOEs, $^3J_{NH}$, δ values and CSI values indicate that the studied peptide fragments mainly exist in random coil conformation in water and HFA at 25°C. In case of fragment 315-329, in HFA, medium range peak between R4 NH-G7 NH was observed that indicates presence of minor conformations probably inducing the turn like structure for this fragment. Overall observations suggest that Arg at the N-terminal and the C-terminal pentapeptide sequence Ala-Phe-Val-Thr-Ile are insufficient to maintain the G-P-G-R segment in β-turn conformation even after increasing the length of the sequence from ten to fifteen amino acid residues as in case of fragment 315-329**.** These observations

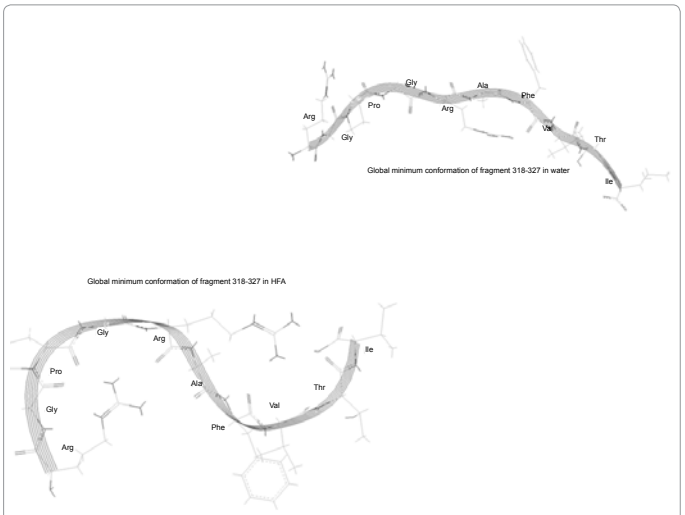

Figure 3: Global conformation of fragment 315-329 in water and HFA. The ribbon traces the backbone atoms.

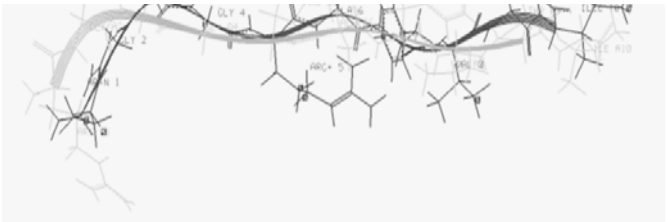

Figure 4: Conformation obtained from restrained MD simulation using NMR data (black) and unrestrained MD simulation (grey) for fragment 318-327 in water.

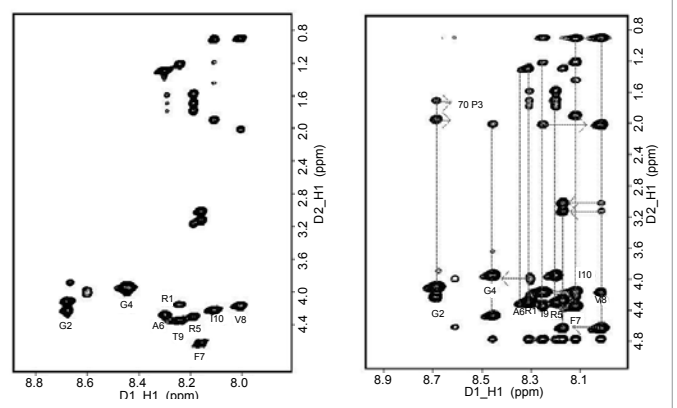

Figure 5: 600 MHz TOCSY and ROESY spectrum of fragment 318-327 in water at 298 K.

were supported by MD simulations done for both fragments using NMR restrains as well as unrestrained MD by solvating the fragment with water.

It is reported that the fragments with conserved secondary structure (Type II β-turn) are responsible for specific actions [21]. In contrast, our results indicate that both the fragments adapt much random conformation than a specific secondary structure in water. Even the use of helix inducer solvent such as HFA does not maintain a β turn conformation for both peptide fragments.

Residue	MD from NMR data				From unrestrained MD	
	HFA		Water		Water	
	ϕ	φ	ϕ	φ	ϕ	φ
Arg		-134		-137		162
Gly	169	-137	-179	-159	-163	-169
Pro	-50	163	-76	-85	-72	-167
Gly	109	-156	-150	-135	-178	172
Arg	-78	-173	-147	-172	-177	-160
Ala	-83	-131	-148	-129	-174	-119
Phe	-80	-164	-143	-132	-118	114
Val	-85	179	-151	178	-132	146
Thr	62	-140	-151	-140	-153	136
Ile	-84		-146		-115	

Table 1: Observed dihedral angles of fragment 318-327 residue in water and HFA obtained from restrained and unrestrained MD.

Aminoacid	Solvent	NH	^{1}H Chemical Shifts			Temp Coff	$^3J_{NH}$
			α	β	others		
Arg	HFA	-	4.11	1.97	γCH_2 1.72, δCH_2 3.19 NH 7.14	-	-
	Water	8.28	4.26	1.78	γCH_2 1.57, δCH_2 3.17 NH 7.15	7	-
Gly	HFA	8.49	4.11, 4.18	-	-	7	-
	Water	8.67	4.21, 4.10	-	-	6.8	10.9
Pro	HFA	-	4.46	2.26	γCH_2 2.02, $\delta_1 CH_2$ 3.64 $\delta_2 CH_2$ 3.54	-	-
	Water	-	4.44	1.9	γCH_2 1.68, $\delta_1 CH_2$ 3.63	-	-
Gly Gly'	HFA	8.09 8.23	3.96 4.01	-	-	8	-
	Water	8.4	3.9	-	-	6.9	10.4
Arg	HFA	7.97	4.33	1.81	γCH_2 1. 72, 1.61, δCH_2 3.17, NH 7.05	8	4.48
	Water	8.18	4.28	1.87	γCH_2 1. 77, 1.57, δCH_2 3.15, NH 7.15	6.8	7.9
Ala	HFA	8.03	4.27	1.32	-	8	4.37
	Water	8.29	4.26	1.27	-	7	7.4
Phe	HFA	7.80	4.63	3.17, 3.07	-	6	9.14
	Water	8.15	4.6	3.10, 3	7.39, 7.34	7	7.5
Val	HFA	6.27	4.20	2.08	γCH_3 0.91	9	6.70
	Water	7.99	4.15	2.0	γCH_3 0.88	6.1	8.4
Thr	HFA	7.90	4.43	4.25	γCH_3 1.22	7	6.64
	Water	8.23	4.33	4.14	γCH_3 1.2	7.4	8.4
Ile	HFA	7.72	4.29	1.91	$\gamma_1 CH_2$ 1.47, $\gamma_2 CH_2$ 1.20 γCH_3 0.93	8	5.85
	Water	8.1	4.19	-	$\gamma_1 CH_2$ 1.43, 1.16	7.6	9.9

Table 2: ^{1}H Chemical Shifts, Temperature coefficient of amide proton and $^3J_{NH}$ for fragment 318-327 of V3 loop of HIV-1 in water and HFA.

Conclusion

To understand the flexibility of hyper variable region of V3 loop, it is necessary to identify the conformational preference of crucial conserved fragment of V3. We have explored the conformational adaptability for two fragments of V3 loop with different size, in variable solvent environment like water and HFA. The longer fragment 315-329 in HFA showed some degree of inclination towards the formation of secondary structure. Though it is known that in water, peptide generally adopts random structure, HFA usually enhances helical propensity of the peptide by providing hydrophobic environment. But interestingly both fragments under study have not showed any specific conformational preference in water. Our study indicates that in order to adopt preferential secondary conformation, length of the fragment is very important which might express further V3 loop's hyper variable status.

Aminoacid	Solvent	NH	^{1}H Chemical Shifts			Temp Coff	$^3J_{NH}$
			α	β	others		
Arg	Water	8.31	4.28	-	-	-	-
	HFA	7.61	4.11	-	-	-	-
Ile	Water	8.67	4.21	-	γ_1CH_2 1.18, γCH_3 0.93	6	6.5
	HFA	8.44	4.36	1.83	γ_1CH_2 1.19, γCH_3 0.91	9	7.2
Gln	Water	8.63	4.37	-	-	7	8.4
	HFA	8.49	4.48	2.08	γCH_2 2.35, 1.97, δCH_2 3.17	10	5.6
Arg	Water	8.49	4.39	-	γCH_2 1.65, δCH_2 3.17	8	-
	HFA	8.24	4.42	1.87, 1.78	γCH_2 1.65, δCH_2 3.17	8	-
Gly	Water	8.35	4.16, 4.04	-	-	7	10.3
	HFA	8.28	4.01	-	-	8	-
Pro	Water	-	4.44	2.3	γCH_2 2.02, δ_1CH_2 3.62	-	-
	HFA	-	4.48	2.3	γCH_2 2.02, δ_1CH_2 3.60, δ_2CH_2 3.54	-	-
Gly Gly'	Water	8.48	3.94	-	-	7.5	-
	HFA	8.09	3.99, 3.93	-	-	7	-
Arg	Water	8.16	4.29	1.87	γCH_2 1.68, δCH_2 1.76, NH 7.09	6.9	-
	HFA	7.99	4.30	1.81	γCH_2 1.62, δCH_2 1.74, NH 7.05	7	-
Ala	Water	8.28	4.27	1.27	-	7	-
	HFA	8.07	4.29	1.35	-	7	-
Phe	Water	8.20	4.63	3.0, 3.11	7.27, 7.30	8	8.3
	HFA	7.93	4.67	3.08, 3.17	-	8	6.8
Val	Water	8.05	4.20	2.10	γCH_3 0.96	7	7.1
	HFA	7.82	4.22	2.12	γCH_3 0.96	12	-
Thr	Water	8.27	4.43	4.12	γCH_3 1.17	8	-
	HFA	7.97	4.45	4.27		8	-
Ile	Water	8.24	4.29	1.9	γ_1CH_2 1.18, γ_2CH_2 0.85, γCH_3 0.98	9	-
	HFA	7.88	4.36	1.90	γ_1CH_2 1.23, γ_2CH_2 1.53, γCH_3 0.96	8	-
Gly	Water	8.62	3.98	-	-	7	9.9
	HFA	8.19	4.03, 3.93	-	-	7.8	10.2
Lys	Water	8.06	4.29	1.90	1.45, 1.69, 3.83	11	-
	HFA	7.75	4.36	1.89	1.43, 1.78, 3.92	7	-

Table 3: ^{1}H Chemical Shifts, Temperature coefficient of amide proton for fragment 315-329 of V3 loop of HIV1 in water and HFA.

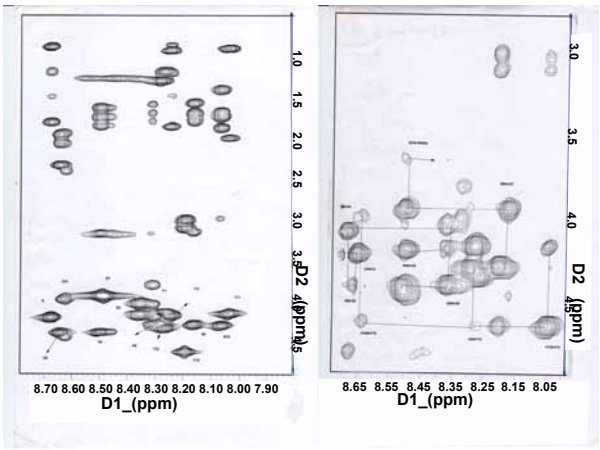

Figure 6: 600 MHz TOCSY and ROESY spectrum of fragment 315-329 in water at 298 K.

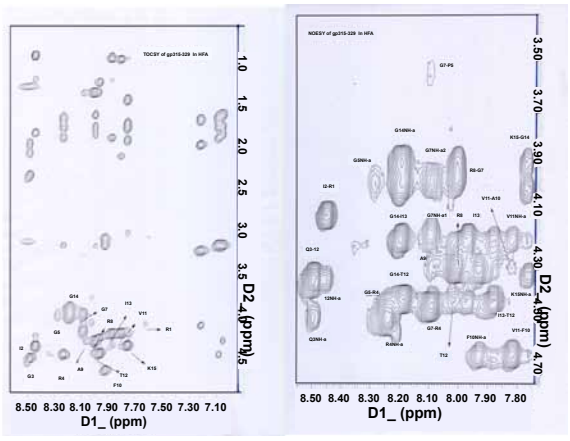

Figure 7: 600 MHz TOCSY and ROESY spectrum of fragment 315-329 in HFA at 298 K.

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