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Tertiary Structure of PreS1(21-47) of Hepatitis B Virus Studied by NMR Spectroscopy

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Abstract: To design more efficient peptide antagonist against the HBV, preS1(21-47) which carries the HBV receptor binding site for hepatocytes was synthesized and the solution structure of preS1(21-47) was investigated using CD spectroscopy and NMR spectroscopy in membrane-mimicking environments. According to CD spectra, preS1(21-47) has a random structure in aqueous solution, while conformational change was induced by addition of TFE and SDS micelle. Tertiary structure as determined by NMR spectroscopy shows that preS1(21-47) has a very flexible structure even in SDS micelle.

INTRODUCTION

Hepatitis B virus (HBV) has not yet been propagated in vitro, and knowledge concerning its reaction with receptors on target cells remained scant[1-5]. But a sequence mediating the attachment of HBV to human hepatoma HepG2 cells was located within the HBV envelope proteins. A synthetic peptide analog preS1(21-47) (PLGFFPDHQLDPAFGA NSNNPDWDFNP) is recognized by both cell receptors and anti-HBV antibodies and elicits antibodies reacting with native HBV[2]. The synthetic peptide is a promising immunogen expected to elicit protective antibodies based on the concept of the attachment blockade pathway of virus neutralization. Therefore, developement of anti-viral strategy based on anti-peptide antisera is popular and has been tried by many research groups[3,4]. On the other aspect, peptide analogs which bind to cell receptors is another candidate for the developement of the antiviral agent for the delineation of cell receptor binding sites on viruses[5]. To design more efficient peptide antagonist against the HBV receptor it is necessary to identify the structural

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features of the peptides which bind to the receptor and characterize the important binding sites of the peptide ligand and the receptor. Therefore, we investigated the tertiary structure of preS1(21-47) in the present study. Membrane may induce a specific conformation onto the polypeptide backbone of the preS1(21-47) prior to its interacting with its receptor. Binding with phopholipids induces a change in the three-dimensional structure of many peptides such as mastoparan B, neuropeptides, gramicidin and mellitin.[6-10] It has been further postulated that these conformational alterations should be an essential step for the recognition by the receptors. In this study sodiumdodecylsulfate (SDS) micelle was selected for the model membrane system because it can be used to mimic the amphiphilic local chemical environment. We determined the tertiary structure of preS1(21-47) in 120 mM SDS micelles by NMR spectroscopy.

EXPERIMENTALS

Sample preparation.

Peptides were synthesized on Rink Amide MBHA resin as C-terminal amides by the solid phase method using Fmoc-chemistry, and were purified by a preparative reverse-phase C_{18} column. Trifluoroethanol (TFE) was purchased from ALDRICH chemical co. and perdeuterated sodium dodecyl sulfate (SDS-d_{25}) was obtained from Cambridge Isotope Inc. For NMR experiments in SDS micelle, peptide was dissolved in 0.45mL of 120mM SDS micelle to make a final concentration of 2.0 mM.

CD Experiments

CD measurements of 100 μM peptide solutions were performed on a J720 spectropolarimeter (Japan, Jasco) between 190 and 250 nm at 25°C. In order to investigate the conformations in membrane-like environment, peptides were dissolved in 20%-60% (v/v) TFE-containing aqueous solution and 15mM-120mM SDS micelle.

NMR spectroscopy

All of the NMR experiments for the sample in SDS micelle were performed at 298K. All the phase sensitive two-dimensional experiments such as DQF-COSY, TOCSY, PE-COSY, and NOESY experiments were performed using time-proportional phase incrementation (TPPI) method[11-16]. For these experiments, 400-512 transients with 2K complex data points were collected for each of the increments with a relaxation delay of 1.2-1.5 sec between the successive transients and the data along the t_1 dimension were zerofilled to 1K before 2D-Fourier transformation. TOCSY experiment was performed using 80-100 msec, MLEV-17 spin-lock mixing pulse. NOESY experiments were performed using mixing time of 150 and 250 msec.

All NMR spectra were recorded on Bruker DPX-400 spectrometer in Konkuk University and Bruker DMX-600 spectrometer in Korea Basic Science Institute. All NMR spectra were processed off-line using the FELIX software package (Molecular Simulations Inc., San Diego) on SGI workstation in our laboratory.

Chemical shifts of the samples were measured relative to the methyl resonance of internal 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) at 0 ppm. $^3J_{HN\alpha}$ coupling constants were either measured in 1D spectrum or calculated by the formula derived by Kim and Prestegard from the separation of absorptive peaks and dispersive peaks in DQF-COSY spectrum[17]. DQF-COSY spectrum was processed to the 4K×2K matrix and used to measure peak-to-peak separations. P.E.COSY experiment was executed to obtain $^3J_{\alpha\beta}$ coupling constants[16]. To identify slowly exchanging amide protons, a series of 1D spectra were acquired after deuterium oxide was added to the sample.

Structure calculation.

Structure calculations were carried out using X-PLOR version 3.851[18]. All the NOE intensities are divided into three classes, i.e., strong, medium, and weak with the distance ranges of 1.8-2.7, 1.8-3.5, and 1.8-5.0Å, respectively. Standard pseudoatom corrections[19] were applied to the non-stereospecifically assigned restraints, and the additional 0.5 Å was added to the upper bounds for NOEs involving methyl protons. Standard distance geometry-dynamical simulated annealing hybrid protocol[20-23] was imployed to generate structures. Center averaging was used to correct distances involving methyl groups and non-stereospecifically assigned methylene. The target function that is minimized during simulated annealing comprises only quadratic harmonic potential terms for covalent geometry, square-well quadratic potentials for the experimental distance and torsion angle restraints, and a quartic van der Waals repulsion term for the nonbonded contacts.

RESULTS and DISCUSSION

Figure 1 shows the CD spectrum of preS1 in water, 40% TFE/water solution (v/v), 90 mM SDS micelle. PreS1 has a random coil structure in water. Addition of TFE and SDS induced structural changes, but PreS1 in TFE/water solution and in SDS micelles does not show any distinct secondary structure in CD spectrum. In order to investigate the structural changes induced by the SDS micelles, we determined the tertiary structure of preS1(21-47) in SDS micelles using NMR spectroscopy.

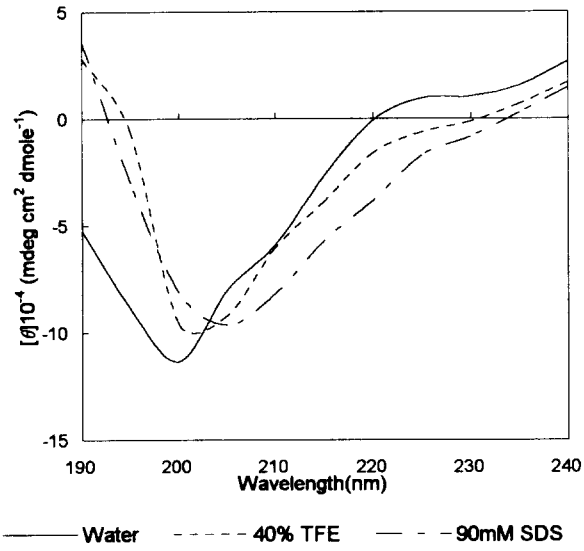

Fig. 1. CD spectra of preS1(21-47) a) in water, b) 40% TFE/water solution, and c) in 90mM SDS micelles.

Using standard sequential assignment strategy[24], all the proton resonances were assigned. TOCSY and DQF-COSY spectra were used to assign spin systems of most of the amino acid residues. By direct comparison of TOCSY and NOESY spectra, sequence-specific resonance assignments were completed. Table 1 lists the complete assignments of the proton chemical shifts of pres1 in 120 mM SDS micelle at 303K. The sequential nOe connectivities in the fingerprint region of NOESY spectra of preS1(21-47) in SDS micelle are illustrated in Fig. 1.

Fig. 2 illustrates the summary of the NOE connectivities of preS1(21-47) in TFE/H_2O(1:1, v/v) solution, which were extracted directly from NOESY spectrum recorded with a mixing time of 250msec. Medium-range, i.e., *i*-*i*+2, *i*-*i*+3 NOE connectivities are observed. $d_{\alpha N}(i,i+3)$ connectivities observed from Ala^{13} to Asn^{20}. Also, *i*-*i*+3 connectivities are found between the aliphatic protons from Gly^{3} to Leu^{10}. Even though a dense grouping of four or more -1 values of chemical shift indices not interrupted by a +1 in preS1(21-47) can indicate the presence of an α-helix, the size of all coupling constants, $^{3}J_{HN\alpha}$ is larger than 6Hz which is too large for the α-helical structure. Total of 50 structures were generated by hybrid distance geometry-dynamical simulated annealing algorithm, and 20 structures having lowest

Table 1. ^{1}H chemical shifts (ppm) for preS1(21-47) in SDS micelle solution at303K

Residue	*Chemical shift(ppm)*[a]			
	NH	αH	βH	Others
Pro[1]		4.35	2.43, 1.28	γ 1.95, 1.55; δ 3.30
Leu[2]	8.52	4.09	1.66, 1.41	γ 1.10; δ 0.83
Gly[3]	8.06	3.74, 3.68		
Phe[4]	7.33	4.29	2.71, 2.60	2H, 6H 6.85
Phe[5]	7.93	4.71	2.49, 2.41	2H, 6H 6.62; 3H, 5H 7.21
Pro[6]		4.22	2.10, 1.53	γ 1.80; δ 3.46, 3.19
Asp[7]	7.80	4.18	2.00, 1.86	
His[8]	7.60	4.40	3.01	2H, 4H 7.39
Gln[9]	8.09	4.23	1.96, 1.83	γ 2.18; δ 7.17
Leu[10]	7.82	4.12	1.75	γ 1.54; δ 1.27, 1.17
Asp[11]	8.15	4.83	2.77, 2.58	
Pro[12]		4.25	2.26, 1.67	γ 1.86, 1.77; δ 3.47, 3.27
Ala[13]	8.26	4.25	1.14	
Phe[14]	7.72	4.51	3.05, 2.94	2H, 6H 7.13
Gly[15]	8.12	3.88, 3.77		
Ala[16]	7.91	4.13	1.25	
Asn[17]	7.93	4.52	3.02, 2.90	γ 7.19, 6.61
Ser[18]	7.87	4.17	3.65	
His[19]	7.28	4.31	2.71, 2.64	
Asn[20]	7.87	4.70	3.03, 2.89	γ 7.19
Pro[21]		4.12	1.99, 1.86	γ 1.73, 1.52; δ 3.49, 3.18
Asp[22]	7.97	4.26	2.22, 1.85	
Trp[23]	7.47	4.49	2.99	2H 6.99; 4H 7.39; 5H 6.71; 6H 6.85; 7H 7.10
Asp[24]	8.05	4.21	1.56, 1.51	
phe[25]	7.55	4.39	2.85,2.75	3H, 5H 7.04
Asn[26]	8.03	4.84	2.61, 2.44	γ 7.04, 6.86
Pro[27]		4.36	2.43, 1.85	γ 1.98, 1.92; δ 3.31, 3.18

[a]Chemical shifts are relative to DSS(0ppm)

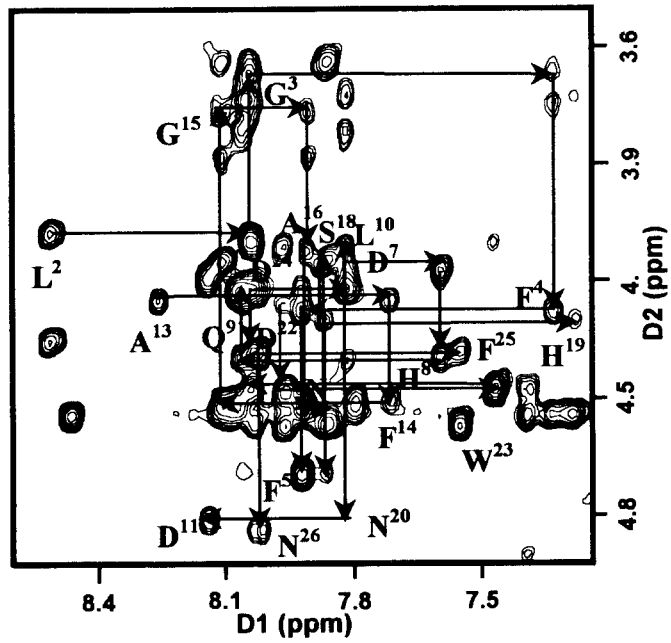

Fig. 2. NH-CαH region of a 250msec mixing time NOESY spectrum of preS1(21-47) in 120mM SDs micelle. For the sake of clarity, only the intraresidual NH-CαH cross peaks are labeled.

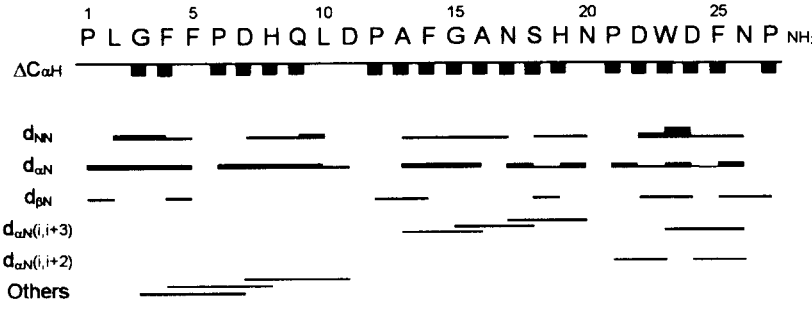

Fig. 3. Schematic representation of NOE connectivities of preS1 in 120mM SDS micelle, at 298K. The relative thickness of the lines represent the intensities of the NOEs with three classes (strong, medium, and weak NOEs).

Fig. 4. The ribbon structure of the lowest energy conformation of preS1 in SDS micelles showing all atoms.

energies were selected for further analysis. All structures satisfy the experimental NOEs well within 0.02 Å. All structures display good covalent geometry and small NMR constraint violations. The mean structure was obtained by restrained minimization of the averaged coordinates of the 20 final structures of each peptide. 20 final structures were fitted over the backbone heavy atoms of Phe^4-Leu^{10} of the mean structures and rmsd from the mean structure of preS1 were 0.6 Å for the backbone atoms (N, Cα, C′, O) and 1.7 Å for all heavy atoms. These high rmsd values might come from the lack of NOEs for preS1(21-47).

Figure 4 shows the ribbon structure of the lowest energy structure with all atoms. Structures were analyzed using PROCHECK and there is a 3_{10} helix from Phe^4 to Leu^{10} in the N-terminus. Conformational changes induced by SDS micelles may be primarily due to the hydrophobic interaction between Phe^4 and Phe^5 in PreS1 and the hydrophobic acyl chains of SDS in the micelles. According to our CD and NMR data, preS1 in the presence of 120mM SDS micelles has a very flexible structure even though it adopts a 3_{10}-helix from Phe^4 to

Leu[10]. The aqueous solution in the presence of SDS micelles may represent a good model for defining the conformation of preS1(21-47) recognized by the receptor near anionic membrane. Tertiary structure of preS1(21-47) determined here will be used to design more efficient peptide antagonist against the HBV receptor

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