Chemical shift assignments of a fusion protein comprising the C-terminal-deleted hepatitis B virus X protein BH3-like motif peptide and Bcl-xL

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Abstract
Chronic hepatitis B virus (HBV) infection is a major risk factor for the development of liver diseases including fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). HBV has the multifunctional protein, HBV X protein (HBx, 154 residues), which plays key roles in HBV replication and liver disease development. Interaction of HBx through its BH3-like motif with the anti-apoptotic protein Bcl-xL leads to HBV replication and induction of apoptosis, resulting in HCC development. Our previous nuclear magnetic resonance (NMR) study revealed that the HBx BH3-like motif peptide (residues 101–136) binds to the common BH3-binding groove of Bcl-xL. Importantly, a C-terminal-truncated HBx, e.g., residues 1–120 of HBx, is strongly associated with the increased risk of HBV-related HCC development. However, the interaction mode between the C-terminal-truncated HBx and Bcl-xL remains unclear. To elucidate this interaction mode, the C-terminal-deleted HBx BH3-like motif peptide (residues 101–120) was used as a model peptide in this study. To facilitate the NMR analysis, we prepared a fusion protein of HBx (101–120) and Bcl-xL connected with five repeats of the glycine-serine dipeptide as a linker. Here, we report the 1H, 13C, and 15N resonance assignments of the fusion protein. This is the first step for the elucidation of the pathogenesis of liver diseases caused by the interaction between the C-terminal-truncated HBx and Bcl-xL.

Keywords Bcl-xL · BH3-like motif · Fusion protein · HBx

Biological context
Chronic hepatitis B virus (HBV) infection causes liver diseases such as hepatocellular carcinoma (HCC), which is a worldwide health problem (Tsuge 2021). One of the HBV proteins, HBV X protein (HBx), plays important roles in HBV replication and liver diseases including the development of HCC (Feitelson and Duan 1997). Additionally, a C-terminal-truncated HBx, e.g., residues 1–120 poses a higher risk factor for HBV-related HCC development than intact HBx (Ma et al. 2008). Therefore, to suppress viral replication and prevent progression of liver diseases, it is important to understand the mode of interaction between the C-terminal-truncated HBx and host proteins.

HBx can interact with anti-apoptotic proteins Bcl-2 and Bcl-xL, leading to HBV replication and induction of apoptosis, which are associated with HCC development and progression (Geng et al. 2012a, b). We have previously reported that the HBx BH3-like motif, residues 101–136 of HBx [HBx(101–136)], can bind directly to the common BH3-binding groove of Bcl-xL (Kusunoki et al. 2019). This result is consistent with the complex structure of the HBx BH3-like motif peptide (residues 113–135) and Bcl-xL, which was determined by X-ray crystallography (Zhang et al. 2019).

The fusion protein of HBx(101–136) and Bcl-xL connected with five repeats of the glycine-serine dipeptide, [HBx(101–136)-(GS)5-Bcl-xL], demonstrated stable
intramolecular docking and retained the native conformation of the complex between them (Kusunoki et al. 2017). Furthermore, such a fusion protein was used to successfully determine the complex structure of the Beclin-1 BH3 peptide and Bcl-xL by nuclear magnetic resonance (NMR) (Feng et al. 2007).

In this study, to examine the detailed interaction between the C-terminal-truncated HBx and Bcl-xL, we designed and constructed a fusion protein consisting of HBx(101–120) and Bcl-xL connected by five repeats of a glycine-serine dipeptide [HBx(101–120)-(GS)_5-Bcl-xL]. Herein, we report the backbone and side-chain resonance assignments of the fusion protein.

Methods and experiments

Plasmid construction and protein preparation

The Bcl-xL (residues 1–44 and 85–196) used in this study lacked residues 45–84 of the long flexible loop and residues 197–233 at the C-terminus (Kusunoki et al. 2017). We refer to Bcl-xL as Bcl-xL. The construct of the fusion protein, pET15b-HBx(101–120)-(GS)_5-Bcl-xL, was generated by a KOD-Plus Mutagenesis Kit (TOYOBO, Osaka, Japan) using the pET15b-HBx(101–136)-(GS)_5-Bcl-xL plasmid as the template (Kusunoki et al. 2017). The obtained construct was confirmed to be the target DNA sequence by DNA sequencing.

The fusion protein, HBx(101–120)-(GS)_5-Bcl-xL, was expressed in Escherichia coli BL21(DE3)pLysS cells as a His6-tagged protein at the N-terminus. Cells were grown in M9 minimal medium containing 2.5 g/L 13C6-D-glucose/1 g/L 15NH4Cl as the sole carbon and nitrogen sources for the preparation of uniformly 13C/15N-labeled fusion protein. The fusion protein was purified as described previously (Kusunoki et al. 2017). First, the fusion protein was purified by a Ni-NTA agarose column (FUJIFILM-Wako pure chemical corporation, Osaka, Japan) or TALON metal affinity column (Takara Bio USA, Inc., San Jose, CA, USA), and then the histidine-tag was cleaved by thrombin overnight at 4 °C. The resulting sample

![Fig. 1 The 2D 1H-15N HSQC spectrum of 0.5 mM 13C/15N-labeled HBx(101–120)-(GS)_5-Bcl-xL at 35 °C. The side-chains of asparagine and glutamine are connected by solid lines. The assignment is labeled with each amino acid name and residue number. The HBx(101–120)-derived amide resonances are surrounded by red rectangles. The amide resonance of residue Ala119 of Bcl-xL is shown in a gray asterisk because of a weaker amide signal. The four vector-derived and the glycine-serine (GS) linker-derived residues are not labeled for clarity. The figure was created by the NMRFAM-Sparky software (Lee et al. 2015).](image-url)
contained four extra vector-derived residues (Gly-Ser-His-Met) at the N-terminus. The sample was further purified with a Resource Q anion exchange column (GE Healthcare, Pittsburgh, PA, USA). Finally, the buffer was exchanged to an NMR buffer (50 mM potassium phosphate, pH 6.8, 50 mM NaCl, and 1 mM DTT) by repeated concentration and dilution with the Amicon Ultra-4 centrifugal filter unit (NMWL 3 kDa; Merck Millipore Ltd., Tullagreen, Ireland). Sample concentrations were determined by a Nanodrop 200 UV–Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) using UV absorbance at 280 nm. All the purified samples were stored at −85 °C until use.

**NMR experiments**

Both D2O (final concentration of 5%) and 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS; final concentration of 0.02 mM) were added into approximately 0.5 mM 13C/15N-labeled sample in the NMR buffer. All NMR experiments were performed at 35 °C using a Bruker AVANCE III HD 600 MHz spectrometer (Bruker, Billerica, MA, USA) equipped with a cryogenic probe. Assignment of backbone and side-chain resonances was obtained by analyzing the following spectra: 2D 1H–15N HSQC, 3D HNCACB, CBCACONH, HNCO, 2D 1H-13C CT-HSQC, and 3D CCH- and HCCH-TOCSY, where 3D spectra were obtained using a non-uniform sampling scheme (Ferentz and Wagner 2000). Proton chemical shifts were referenced directly to DSS at 0 ppm, and 13C and 15N chemical shifts were referenced indirectly to the absolute frequency ratio 13C/1H = 0.251449530 and 15N/1H = 0.101329118, respectively (Wishart et al. 1995). The NMR data were processed using NMR Pipe/NMRDraw and qMDD (Delaglio et al. 1995; Orekhov and Jaravine 2011). Spectral analysis was performed by MagRO-NMRView (Johnson and Blevins 1994; Kobayashi et al. 2007, 2012). The secondary structure prediction was performed by the TALOS + software (Shen and Bax 2013).

**Extent of assignments and data deposition**

The 13C/15N-labeled HBx(101–120)-(GS)5-Bcl-xL fusion protein was obtained at approximately 2 mg per M9 liter culture. Figure 1 shows the 2D 1H–15N HSQC spectrum of the fusion protein at 35 °C. The amide signals in the spectrum were well resolved, and the backbone and side-chain resonances of the fusion protein were nearly completely assigned. The amide signals of the HBx(101–120) portion were assigned, except for Ser101, Ala102, and Thr105, and those of the Bcl-xL portion were assigned except for Ser2, Gln3, Arg103, His113, Gly117, Thr118, Phe123, and three proline residues (Pro38, Pro116, and Pro180). In the HBx(101–120) portion and the Bcl-xL portion, the observable backbone resonances (94% of 15N and HN, 97% of 13Cα, 97% of 13Cβ, 90% of carbonyl 13C', and 94% of Hα) were assigned. Additionally, more than 90% of the observable side-chain resonances were assigned.
The assignments have been deposited in the BioMagResBank with ID 26333.

Secondary structure prediction was performed by the backbone chemical shift resonances processed using the TALOS+ software (Shen and Bax 2013). The result showed that Bcl-xL may form eight helices (Fig. 2), which is very similar to the three-dimensional structure of Bcl-xL (PDB code 1MAZ) previously reported by X-ray crystallography (Muchmore et al. 1996). By contrast, HBx(101–120) forms a short α-helix (residues Asp107–Cys115) in the fusion protein. The complex structure of the HBx BH3-like motif peptide (residues 113–135) and Bcl-xL revealed that the HBx peptide forms a short α-helix consisting of residues Trp120–Ile127 when bound to Bcl-xL (Zhang et al. 2019). The TALOS+ result strongly suggests that the Bcl-xL-binding manner of the C-terminal-deleted HBx BH3-like motif has a similarity to some extent to that of the HBx BH3-like motif, although the Bcl-xL-binding region is different between them. These data will help characterize the interaction manner between the C-terminal-truncated HBx and Bcl-xL.

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Author contribution HK designed the research; HK and TN performed experiments; TN analyzed the NMR data; NK developed the NMR analytical tools; HK and TN wrote the manuscript; All authors read and approved the final manuscript.

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Data availability The 1H, 13C, and 15N chemical shift assignments of the HBx(101–120)-(GS)5-Bcl-xL fusion protein have been deposited in the BioMagResBank under accession number 26333.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval The experiments comply with the current laws of Japan.

Informed Consent Not applicable.

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