이학석사학위논문

Interaction between nucleophosmin and HBV core protein increases HBV capsid assembly

뉴클레오포스민 단백질과 HBV 코어 단백질의 상호작용에 의한 HBV 캡시드 형성 촉진

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이 논문을 이학 석사 학위 논문으로 제출함

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Heewon Jeong 의 석사학위논문을 인준함

2013 년 12 월

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ABSTRACT

Host factors are involved in Hepatitis B virus (HBV) genome replication and capsid formation during the viral life cycle. A host factor, Nucleophosmin (B23), was found to be bound to HBV core protein dimers, but its functional role of interaction has remained to be elucidated. In this study, we used *in sillico* modeling to predict the sites of physical interaction between B23 and core protein dimers and used mutagenesis to confirm the prediction. The binding of core protein and B23 promoted HBV capsid assembly and protected the degree of capsid dissociation when subjected to detergent treatments *in vitro*. In addition, chemical inhibition of B23 reduced intracellular capsid formation resulting of reduced HBV production in HepG2.2.15 cells. These results provided important evidence that the oligomeric form of B23 acts on core capsid assembly.

Keywords: Hepatitis B virus (HBV), core protein truncated at residue 149 (Cp149), core assembly, encapsidation, host factor, nucleophosmin (B23)

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CONTENT

1. Introduction

Hepatitis B virus (HBV), a member of *Hepadnaviridae* family, is a human pathogen that causes acute and chronic hepatitis (Seeger and Mason, 2000). Among 2 billion people who have been infected by HBV, around 240 million people remain chronically infected (Ott et al., 2012). Chronic infection with HBV is closely related to the development of liver cirrhosis and hepatocellular carcinoma (Block et al., 2003).

HBV contains a 3.2kb partially double-stranded DNA genome which has four open reading frames (ORFs) encoding four viral proteins: core protein (Cp), surface protein (S), polymerase (P), and X protein (X) (Nassal, 2008). Replication of HBV genome involves reverse transcription of an RNA intermediate called pregenomic RNA (pgRNA) (Nassal, 2008). The pgRNA and HBV polymerase along with associated host factors are encapsidated by core protein (Dhason et al., 2012). The pgRNA-containing T=4 icosahedral nucleocapsid (Dhason et al., 2012) plays an essential role in the virus life cycle, given that reverse transcription regenerates the virus genome after encapsidation (Nassal, 2008). The HBV core protein, which is 183 amino acids long, consists of two domains, an N-terminal (amino acids 1-149) core assembly domain and a C-terminal (amino acids 150-183) nucleic acid-binding domain (Porterfield et al., 2010). The C-terminal truncated form of core protein, Core protein 149 (Cp149; truncated at amino acid 149), includes the assembly domain that spontaneously forms a capsid *in vitro* and *in vivo* (Ceres et al., 2004).

Given that core assembly can occur without C-terminal nucleic acid-binding domain, Cp149 has been generally used for the research of monitoring capsid assembly since 1990. (Ceres et al., 2004; Kang et al., 2008; Packianathan et al., 2010). Cp149 has advantages because of not only the efficiency with which it can be expressed and purified but also the structural similarity with full length core protein (Choi et al., 2005). During capsid formation, several host factors have been identified to interact with core protein (Lee et al., 2009; Shim et al., 2011).

Nucleophosmin (B23), which comprises an N-terminal oligomerization domain, a central acidic domain, and a C-terminal RNA binding domain, regulates ribosome biogenesis and transport, tumor suppression, and nucleic acid binding (Okuwaki, 2008). B23 also acts as a chaperone insofar as it suppresses the misfolding of target proteins and protects them against denaturation (Okuwaki, 2008). Within cells, B23 exists mostly as an oligomer and its oligomeric status might be critical for chaperone activity (Okuwaki, 2008) Especially, B23 interacts with viral proteins from several different viruses, and promotes the replication cycles of viruses. Interaction between B23 and adenoviral protein V promotes virus assembly during virion maturation (Ugai et al., 2012). B23 also form a complex with Hepatitis Delta virus (HDV) antigens to enhance replication of HDV RNA (Huang et al., 2001). Moreover, B23 binds to Human Immunodeficiency virus (HIV) protein Tat to ensure the proper nucleolar localization of Tat (Li, 1997). These interactions link B23 with the viral life cycle as a chaperone protein is advantageous for its virus replication cycle.

In our previous study, we have reported the interaction between B23 and HBV core protein *in vitro* and *in vivo* (Lee et al., 2009), but the functional role of its interaction in the HBV life cycle remains to be elucidated. On the basis of these facts, we hypothesized that B23 may affect HBV life cycle by acting as a molecular chaperone. In the present study, we aimed to determine the functional role of B23 in HBV replication. Here we report both *in vitro* and *in vivo* evidence that B23 increases capsid formation by facilitating the assembly reaction through its interaction with core protein dimers.

II. Materials and Methods

Simulation of interaction between HBV core protein and B23.

Protein structure was acquired from the RCSB PDB (PDB ID: 1QGT (cp149), 3KXS (cp149), 2VXD (B23)). C-terminal amino acid losses were recovered and invalid amino acid conformations were corrected. Docking prediction was done by ZDOCK & RDOCK algorithm and protein-protein interaction was analyzed under CHARMm forcefield with Generalized Born with Molecular Volume (GBMV) implicit solvation method. Protein structures and protein-protein interactions were visualized with Discovery Studio Client 2.5 (Accelrys).

Expression and purification of Cp149 and B23

Cp149 and Cp149 mutants were cloned directly from pET28b vector (Novagen), and B23 and B23 mutants were cloned directly from pET21a vector (Novagen). Constructs were transformed into BL21(DE3)pLysS *E. coli* (Novagen). Proteins were induced by 1mM isopropyl-β-D-thiogalactopyranoside (IPTG) and were incubated for an additional 4h at 37℃. Cp149 dimers and its mutants were purified as previously described (Choi, 2005) and stock solution of proteins [1mg/ml in 100mM glycine buffer (pH9.5) containing 10% glycerol] were stored at -70℃. The yields of proteins were varied between 10 and 20mg per g weight of starting cells. B23 and its mutants were purified as previously described (Lee et al., 2009) and stock solution of proteins [1mg/ml in 20mM Tris-HCl buffer (pH7.5) containing 10%

glycerol] were stored at -70℃. The yields of proteins were varied between 7 and 13mg per g weight of starting cells.

Site-directed mutagenesis of Cp149 and B23

Site-directed mutagenesis was performed each using pET-28b-Cp149 and pET-21a-B23 as a template. The procedure followed standard site-directed mutagenesis protocols (Qiagen). Mutations were confirmed by nucleotide sequencing. All the primers used in the experiments are listed in Supplementary Tables 1–2.

Co-immunoprecipitation (co-IP), Cp149 assembly analysis and sucrose density gradient analysis.

Cp149-WT and Cp149 point mutants W125L, W125K, and W125E (each 20μM) were mixed with B23-WT (20μM), and Cp149 dimers (20μM) were mixed with B23-WT and B23 point mutants D286A, D286E and D286K (each 20μM). The mixtures were incubated at 30℃ for 1 h in assembly buffer [shim, 2011] and were co-immunoprecipitated using anti-HBV core (Santa Cruz Biotechnology) and anti-B23 antibodies (Abcam). The immunoprecipitants were analyzed by 15% SDS-PAGE, followed by immunoblot analysis with antibodies described above. Sucrose density gradient analysis was performed by ultracentrifugation as described previously [shim, 2011], and fractions 1-10 were resolved by 15% SDS-PAGE and immunoblot analysis was performed using antibodies above.

Cell culture and treatment with B23, Hsp90, and polymerase inhibitors in HepG2.2.15 cells and detection of the HBV intracellular capsid

The human hepatoma HBV genomes expressing cell line, HepG2.2.15, was cultured in Dulbecco's modified Eagle's medium (DMEM) (Welgene) supplemented with 10% fetal bovine serum (FBS) (Invitrogen) at 37℃ in 5% CO_2. HepG2.2.15 cells were treated with 1μM NSC348884, B23 inhibitor, (Santa Cruz Biotechnology) or 4μM geldanamycin(GA), Hsp90 inhibitor, (A.G. Scientific Inc.) or 85μM lamivudine (Sigma) for 48 hours as described previously [Shim, Qi]. HepG2.2.15 cells were transfected with siRNA-B23 (Santa Cruz) at 150nM by using Fugene 6.0 transfection reagent (Roche) according to manufacturer's protocol. After 48 hours of chemical treatment, cells were lysed in lysis buffer (50mM Tris-HCl, 150mM NaCl, 5mM EDTA, and 0.5% NP-40) as described previously [Shim, 2011], and intracellular capsids from HepG2.2.15 cells were detected as described previously (Park et al., 2003).

Quantification of HBV DNA and RNA by real-time quantitative PCR

After 48h of treatment, intracellular and extracellular HBV DNA was extracted by phenol-chloroform extraction method as described previously [shim, park]. Total RNA was isolated from HepG2.2.15 cells by using RiboZol RNA extraction reagent (Amresco) according to the manufacturer's instructions and was treated with DNase I for 1h at 37℃ to eliminate residual DNA. Reverse transcription of 1μg total RNA was performed by using AMV reverse transcriptase (Promega). The resulting cDNA

and HBV DNA were measured by real-time PCR using a SYBR-Green reaction mixture (Qiagen) as previously described [shim] with following primers in Supplementary Tables 3.

Statistical analysis

Data, represented as bar graphs, are expressed as the mean and standard deviation of 3 independent experiments. Statistical analyses were performed using Student's *t* test.

III. Results

Cp149 W125 residue interacts with B23 D286 residue

To determine the binding site of HBV core protein and B23, interaction simulation was done by docking prediction from the obtained protein structures (Fig. 1A). We expected that 125 tryptophan residue (W125) of core protein and 286 aspartic acid residue (D286) of B23 played critical roles on its interaction. In order to confirm the *in sillico* predictions, point mutants of Cp149 W125 residues and B23 D286 residues were generated by site-directed mutagenesis. Cp149 and B23 mutants were investigated on their ability to from capsid and oligomers, respectively (Fig. S1). The two independent co-immunoprecipitation analyses showed that the binding affinities of Cp149 W125 mutants with B23-WT (Fig. 1B) and of Cp149-WT with B23 D286 mutants were lower than the binding affinity of Cp149-WT and B23-WT (Fig. 1C). Also, sucrose density gradient analysis showed Cp149-WT and B23-WT were sedimented together by the interaction, whereas Cp149 W125K mutant and B23 D286A mutant were not able to bind, thereby B23 mutant located in different fraction from the capsid (Fig. 1D). These results clearly demonstrated that W125 residue of Cp149 and D286 residue of B23 were strongly involved in binding of the Cp149 with B23.

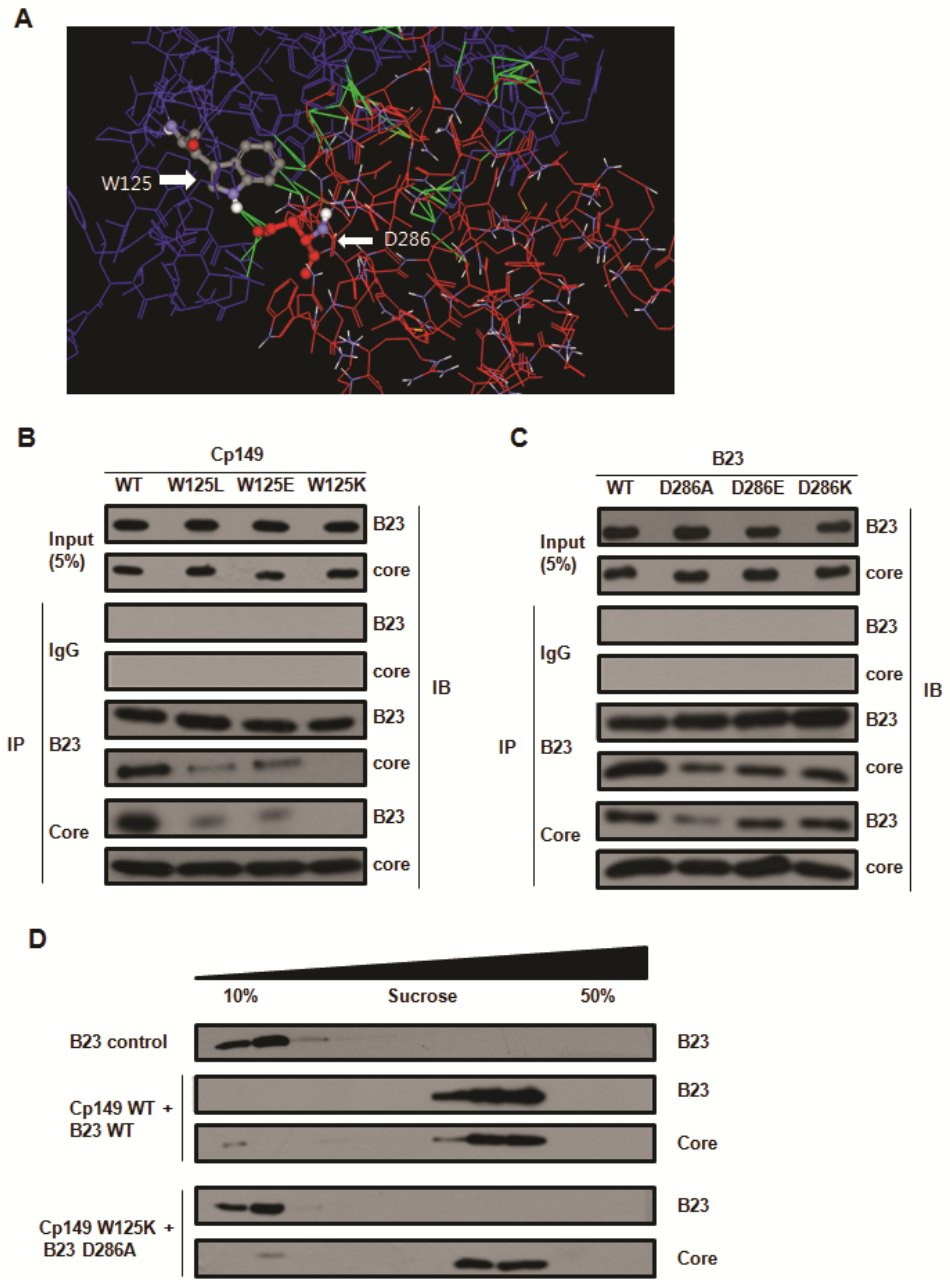


Fig. 1. Cp149 W125 residue interacts with B23 D286 residue

(A) Simulation of the interaction between Cp149 and B23. Blue corresponds to Cp149 and Red corresponds to B23. Cp149 W125 residue and B23 D286 residue are indicated by white arrow, and green lines indicate possible weak interaction sites. (B) Cp149-WT and Cp149 mutants W125L, W125K, and W125E were mixed with B23-WT, and (C) Cp149-WT were mixed with B23-WT and B23 mutants D286A, D286E and D286K. The mixture was co-immunoprecipitated using anti-HBV core and anti-B23 antibodies. 5% of total input was used as positive control and non-specific normal mouse IgG was used as negative control. (D) Sucrose density gradient analysis was performed after capsid assembly reaction either with Cp149-WT and B23-WT or with Cp149 W125K and B23 D286A.

B23 facilitates HBV core protein assembly and increases capsid stability

In vitro assembly of core protein with B23 was performed to examine how B23 affects on the functions of the Cp149 via its direct interaction with the core protein. The capsid formation increased in manner dependent on the concentration of B23 (Fig. 2A). Centrifugation through a sucrose density gradient was also used to divide samples into dimer and capsid form, and the level of capsid formation was increased in the presence of B23 (Fig. 2B). In order to compare the rate of capsid formation in the presence of B23 with that in the absence of B23, the changes in intensity of the capsid band were measured after different time intervals. Capsid formation was faster in the presence of B23 than in the presence of either bovine serum albumin (BSA) or the B23 point mutant B23 D286A (Fig. 2B). In the presence of B23, the abundance of capsid reached to the saturation level at 15 min after assembly reaction while capsid level was saturated after 120 min in the presence of either BSA or the B23 D286A (Fig. 2B). The maximum level of capsid formation at 120 min was arbitrarily assigned a value of 1, and the relative intensities of capsid were analyzed to compare the rates of assembly under the different conditions tested (Fig. 2C, right). These results illustrated that capsid assembly was facilitated when B23 was present.

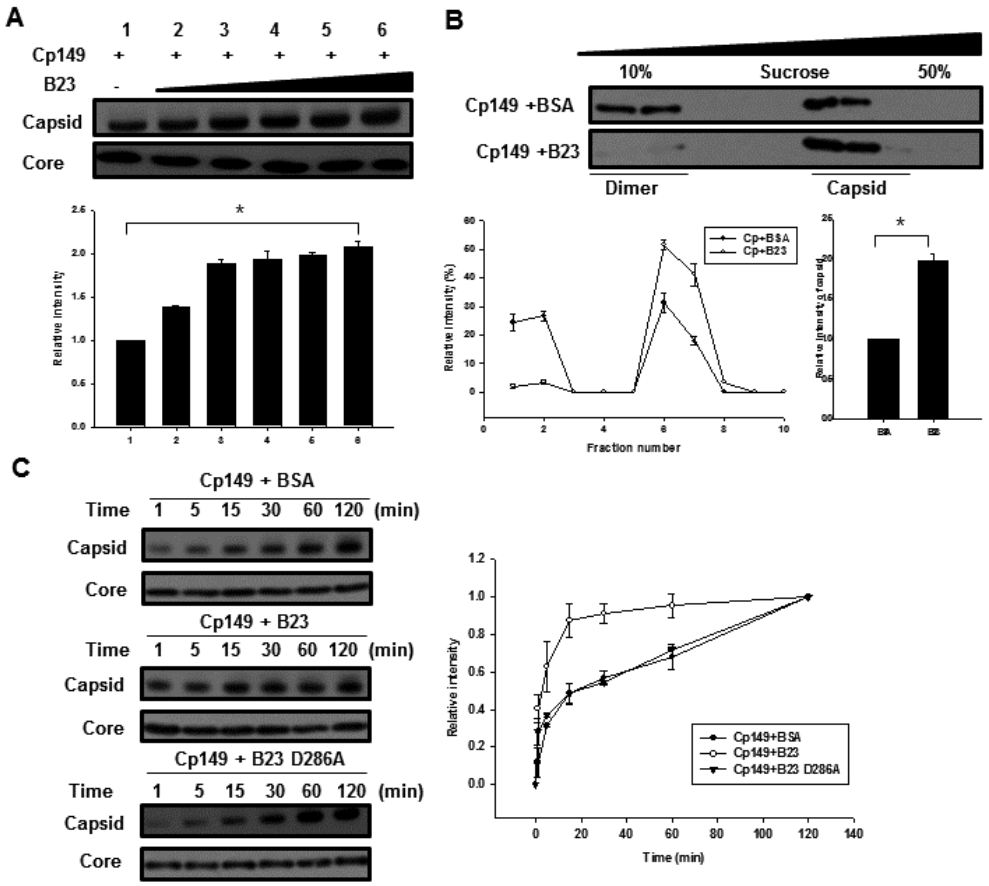


Fig. 2. B23 facilitates HBV core assembly

(A) Cp149 dimer (20μM) were incubated with increasing concentration of B23 (0-20μM) in assembly reaction buffer at 37℃ for 30 min. samples were separated by 0.9% native agarose gel electrophoresis followed by immunoblot analysis with anti-HBV core antibody. (B) Sucrose density gradient analysis was performed after capsid assembly reaction with or without B23. Cp149 incubated with BSA was used as a control (top). Fraction 1 and 2 contained dimers and fraction 6-8 contained assembled capsid. The amount of core dimer and capsid with or without B23 were

showed in the graph (bottom left). The relative capsid intensity was shown in the graph (bottom right). (C) Capsid formations in the presence of BSA, B23 and B23 D286A were monitored for 120 min using immunoblot analysis. The relative capsid intensity was determined by normalization relative to the final abundance of capsid after assembly was completed by 120 min, as shown in the graph (right). *$P<0.05$.

B23 can facilitate core capsid assembly in an environment that does not favor assembly

The HBV capsid formation is temperature-dependent (Hilmer et al., 2008), and B23 has a molecular chaperone activity which is able to suppress the misfolding and aggregation of target proteins at high temperature (Okuwaki, 2008). To examine whether B23 also had the chaperone activity toward the core protein, the effect of B23 on capsid assembly was studied in various range of temperatures. From the data, capsid formation was maximal at 37℃ in the BSA group, whereas B23 promoted capsid formation over a wide range of temperatures (Fig. 3A).

Calcium ion is necessary for the assembly and stability of capsid in many kinds of viruses including SV40 (Chromy et al., 2003). Moreover, the assembly of HBV core protein is influenced by the concentration of calcium ions both *in vitro* and *in vivo* (Choi et al., 2005). As the concentration of calcium ions increased, the levels of core assembly was increased in the presence of BSA (Fig. 3B). However, the rate of capsid formation in the B23 group was already maximal in the absence of calcium (Fig. 3B). This demonstrated that B23 mediated capsid assembly independent of the concentration of calcium ions. Taken together, B23 performed the role as a chaperone protein during capsid assembly and promoted capsid formation under assembly unfavored conditions such as at high temperature or at low concentration of calcium ions.

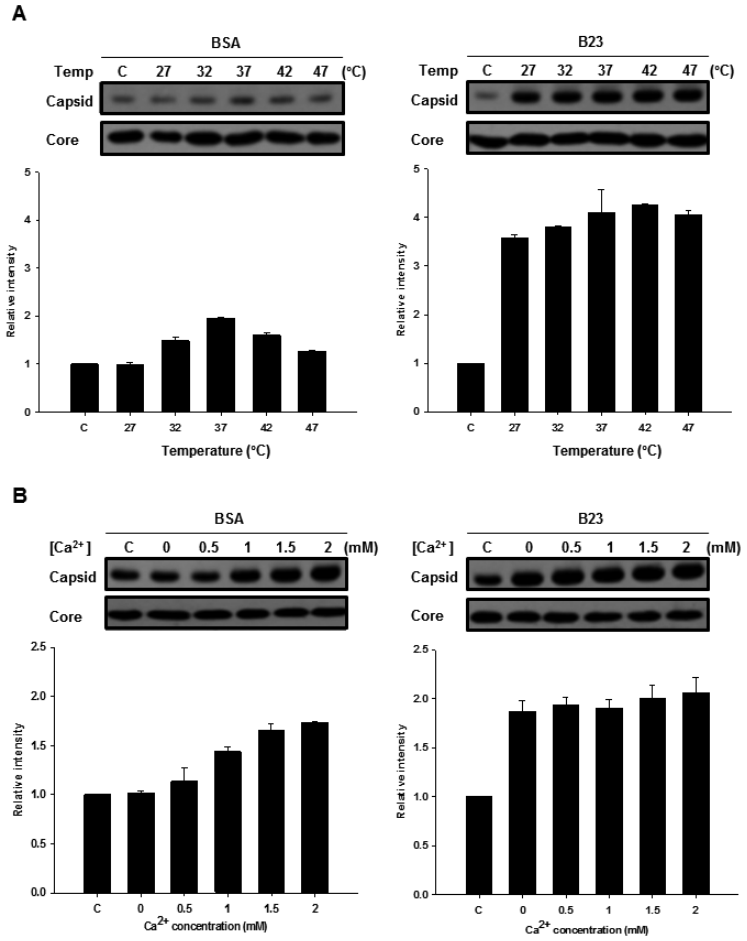


Fig. 3. B23 can facilitate core capsid assembly in environment that does not favor assembly

(A) Cp149 capsid assembly reaction with or without B23 at a wide range of temperatures (27℃-47℃) were performed. The relative capsid intensity at 27℃ without B23 was used as a standard. (B) Cp149 capsid assembly reaction in each reaction buffer with different concentration of Ca^{2+} (0-2mM) with or without B23 was performed. The relative capsid intensity in 0mM Ca^{2+} without B23 was used as a standard.

B23 can protect the degree of capsid dissociation when subjected to detergent treatments

The HBV stability was investigated under the dissociation conditions in the presence of denaturing agents, including urea and guanidine-HCl (Gu-HCl). When capsids were treated with increasing concentration of urea, capsid was dissociated and capsid levels were decreased in manner dependent on the concentration of urea (Fig. 4A). However, B23 had the protective effect on capsid dissociation, and capsid levels after 3M urea treatment were maintained 3.5-fold higher than in the presence of BSA (Fig. 4A). Also, after Gu-HCl treatment on capsid, capsid levels were decreased in the presence of BSA, whereas B23 protected the degree of capsid dissociation (Fig. 4B). These result showed that capsid stability was increased by B23 by preventing the capsid dissociation by denaturing agents.

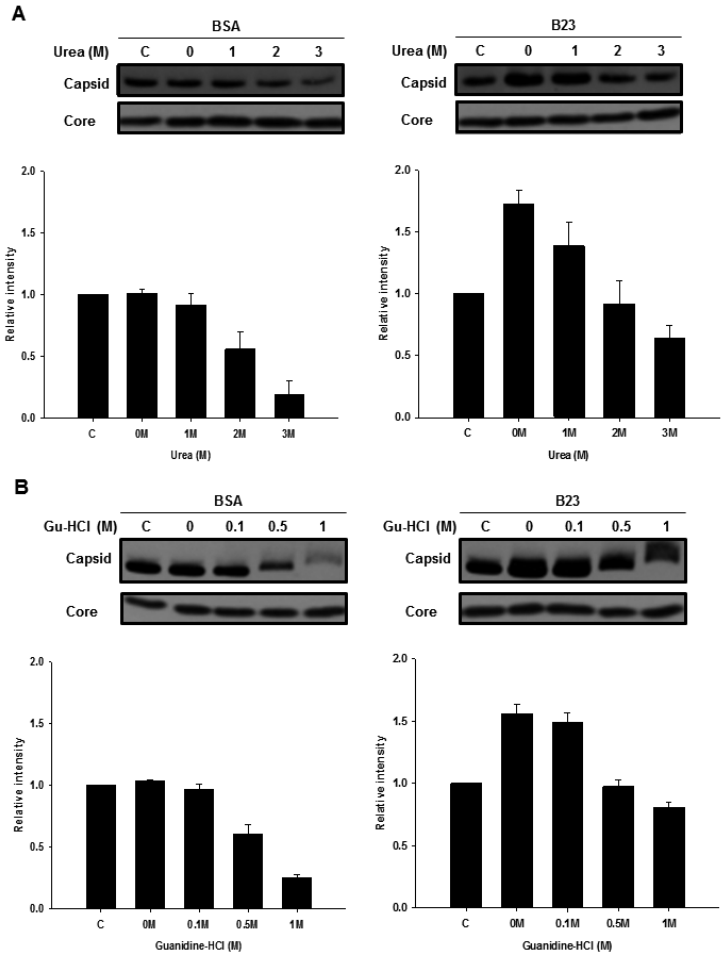
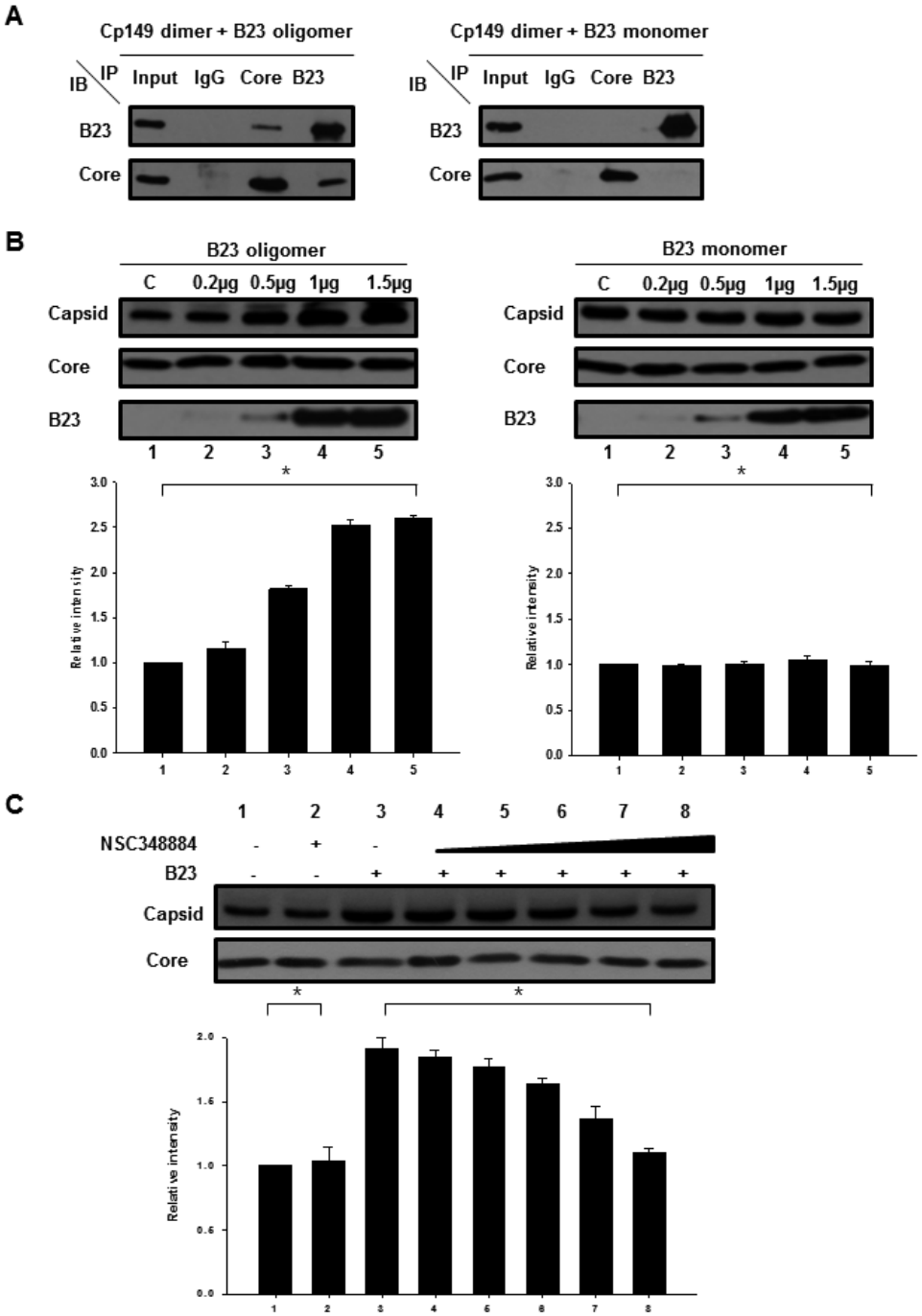


Fig. 4. B23 increases the stability of HBV capsid

(A) Capsid assembly reaction with or without B23 was performed and samples were then mixed with different concentration of urea (1M-3M). (B) Capsid assembly reaction with or without B23 was performed and samples were then mixed with different concentration of guanidine-HCl (0.1M-1M). Capsid assembly without B23 was denoted as C. The relative intensity of capsid formation was analyzed in the graph below.

Oligomerization of B23 is required for the increase in capsid assembly

When B23 functions as a chaperone protein, oligomerization of B23 is known to be an essential factor (Okuwaki, 2008). Consequently, we assumed that oligomerization of B23 might be mandatory for its chaperone activity toward HBV core protein. First, we used co-immunoprecipitation analysis to examine whether oligomerization of B23 was necessary for its interaction with core protein. Given that the B23 mutant C21F is defective in oligomerization (Prinos et al., 2011), C21F was used as a monomer in this analysis. The HBV core dimer could interact only with B23 oligomer but not with B23 monomer (Fig. 5A). These showed that the oligomerization of B23 was mandatory for its interaction with core protein. Consistent with the data, C21F (a monomeric form of B23) could not promote capsid assembly (Fig. 5B). When NSC348884, a B23 inhibitor that disrupts oligomer formation (Qi et al., 2008), was incubated with B23 during capsid assembly, the level of capsid assembly decreased to the level observed in the absence of B23 (Fig. 5C). Based on these results, B23 was able to interact with the HBV core protein and to promote capsid assembly only when B23 existed as an oligomer.


A
Cp149 dimer + B23 oligomer
IB
IP
Input
IgG
Core
B23
B23
Core
Cp149 dimer + B23 monomer
IB
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Core
B23
B23
Core
B
B23 oligomer
C
0.2μg
0.5μg
1μg
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Capsid
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Relative intensity
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Fig. 5. Oligomerization of B23 is required for B23 mediated promotion of capsid assembly

(A) After Cp149 capsid assembly reaction each with B23-WT oligomers (left panel) or B23 mutant C21F monomers (right panel), the mixture was co-immunoprecipitated using anti-HBV core and anti-B23 antibodies. (B) Capsid assembly reaction with increasing concentrations of B23-WT (0-20μM) (left panel) or B23 mutant C21F (0-20μM) (right panel) was performed. (C) After the assembly reaction with B23 along with increasing concentration of B23 inhibitor NSC348884, capsids were detected by immunoblot analysis. *$P<0.05$.

Inhibition of B23 in the HepG2.2.15 cell line reduces HBV replication

To analyze the role of B23 in HBV replication cycle, the extracellular and intracellular HBV DNA levels were quantified after knock-down or inhibition of B23 in the HepG2.2.15 cells. Inhibitors used in this study showed no effect on cell viability by MTT assay. The knock-down of B23 and treatment with NSC348884 decreased the levels of extracellular and intracellular HBV DNA by 60% (Fig. 6A), and HBV polymerase inhibitor, lamivudine (3TC), was used as a control. After treatment with NSC348884 and 3TC together, both extracellular and intracellular HBV DNA levels were reduced by 90% (Fig. 6A). The change of extracellular HBV DNA levels after B23 inhibition demonstrated that B23 affected the proliferation of HBV. Also, B23 was not able to affect the mechanisms of HBV virion secretion since the extracellular and the intracellular HBV DNA titers had no differences. To understand whether B23 affect HBV transcription, we measured the expression level of HBV RNAs. However, expression level was not affected neither by inhibition of B23 nor treatment with 3TC (Fig. 6B). The result supported clearly that B23 were not involved in the transcription of cccDNA into HBV RNAs.

After inhibition, the cells were lysed, and the intracellular capsid levels were measured by immunoblot analysis. Since Hsp90 has been proven to facilitate HBV capsid formation [12], HepG2.2.15 cells were treated with Hsp90 inhibitor, GA, in the presence or absence of NSC348884. The inhibition of B23 or Hsp90 reduced intracellular capsid formation by 50% or 40%, respectively (Fig. 6C).

Moreover, the amount of capsid formation was synergically reduced by 90% after inhibition of B23 and Hsp90 (Fig. 6C), since both Hsp90 and B23 were involved in capsid formation. However, the level of HBV core protein synthesis was not affected by the treatment of inhibitors when the expression level of the core protein was detected by immunoblot analysis (Fig. 6C). These results clearly demonstrated that B23 acted on capsid formation, but not on the translation of core protein. In summary, the inhibition of B23 interrupted to produce viral DNA by impeding capsid assembly, so that HBV polymerase could not properly function in the HepG2.2.15 cells.

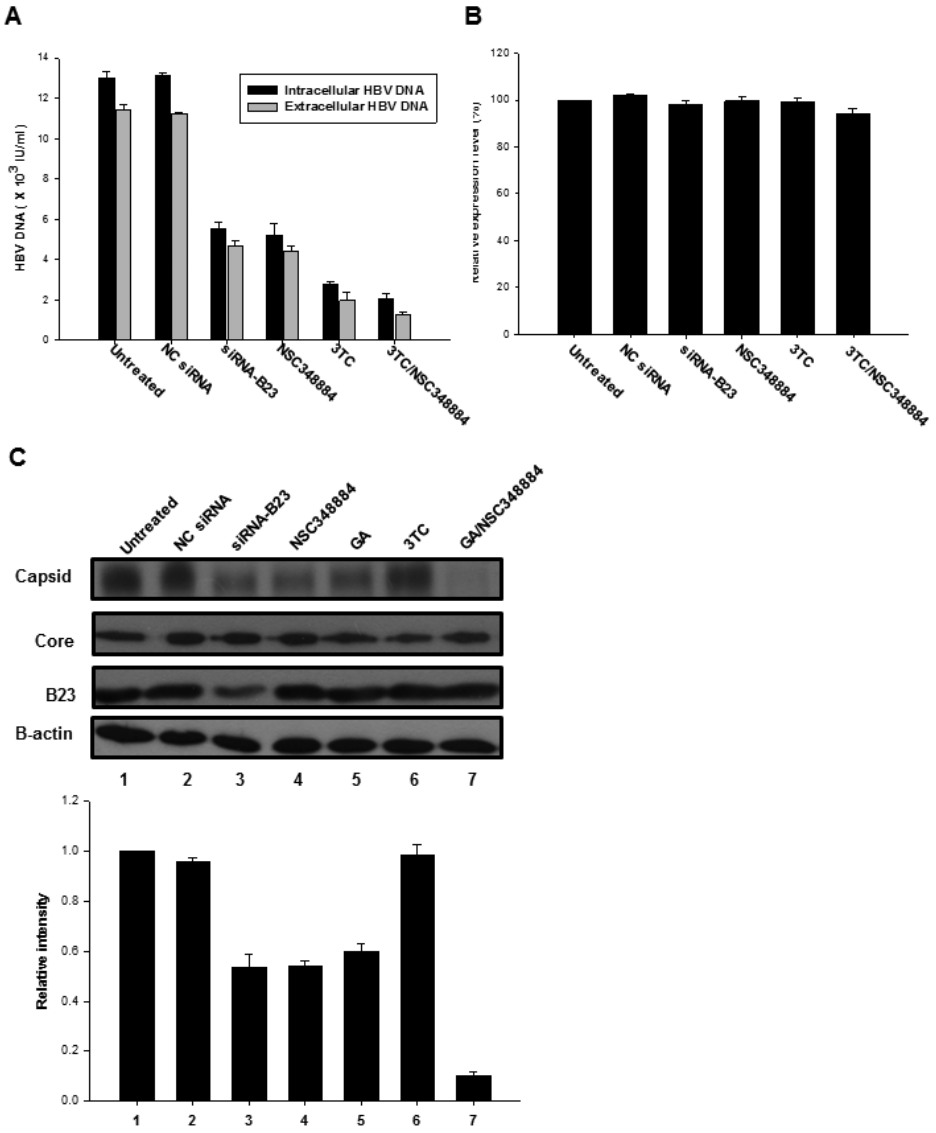

Fig. 6. Inhibition of B23 reduces HBV replication

(A) After treatment with NSC348884 and 3TC, extracellular HBV DNA was extracted from media and intracellular HBV DNA was extracted from HepG2.2.15 cells. The DNA titers were measured by qRT-PCR. (B) Intracellular total RNAs was extracted from HepG2.2.15. After reverse transcription, the relative level of RNA

expression was quantified by qRT-PCR. (C) HepG2.2.15 cells were lysed in lysis buffer and capsids were analyzed by 0.9% native agarose gel followed by immunoblot analysis using anti-HBV core antibody. Core protein, and B23 were analyzed by immunoblot analysis using anti-HBV core and anti-B23 antibodies. B-actin was used as a control.

IV. Discussion

During the HBV life cycle, HBV core proteins assemble to encapsidate pregenomic RNA (pgRNA) and the polymerase required for HBV replication (Nassal, 2008). Given the small size of the HBV genome, which encodes four viral proteins, HBV depends critically on host factors for the infection and replication (Nassal, 2008). Accordingly, host factors have been shown to interact with viral components to assist several steps in HBV replication, including priming of the HBV polymerase, promoting the interaction between polymerase and pgRNA, and encapsidating the polymerase and pgRNA (Hu et al., 2004; Kang et al., 2008; Park et al., 2003). Several host factors are involved in capsid formation. Protein kinase A (PKA) and protein kinase C (PKC) phosphorylate HBV core protein, which increases capsid assembly (Kang et al., 2008). In addition, Hsp90 binds to the HBV core protein to facilitate not only HBV capsid assembly but also the production of HBV particles (Shim et al., 2011). However, unlike what is observed for most chaperone proteins, Hsp40 decreases capsid formation and inhibits HBV replication (Sohn et al., 2006).

In our result, HBV core protein interacted with B23 oligomers (Fig 4). In living cells, nuclear-cytoplasmic shuttling of HBV core protein is regulated by phosphorylation of serine in C-terminal domain (Weigand et al., 2010). In addition, B23 is moving constantly between nuclear and cytoplasm as it contains nuclear

localization signal (NLS) and nuclear export signal (NES) sequences (Okuwaki, 2008). Therefore, we assumed a strong possibility that interaction between core protein and B23 occur in living cells. Consistent with this hypothesis, the *in vivo* interaction between B23 and core protein have been reported (Lee et al., 2009). This interaction facilitates HBV capsid formation *in vitro* and *in vivo* (Fig. 2 and 5C). Based on these results, we hypothesized that the binding of B23 to the HBV core protein may change the conformations of core proteins, which increases the affinity of core-core interaction (Packianathan et al., 2010). As a result, the increase in core dimer affinity may assist to maintain core-core interactions within the capsid and decrease the rate of capsid dissociation.

To understand the mechanism how B23 is involved to affect HBV life cycle, each step of HBV life cycle was investigated. Our results confirmed that the inhibition of B23 in the HepG2.2.15 cells decreases extracellular HBV titers by preventing the formation of HBV capsids (Fig. 5). These suggest that B23 assists in the process of capsid assembly to enhance HBV replication. Moreover, combined inhibition of B23 and Hsp90 decreased the HBV capsid formation in cooperative manner (Fig. 5C). Further study is needed to understand the mechanism of how B23 and Hsp90 cooperatively impact capsid assembly during HBV replication cycle.

Among available HBV therapies, HBV polymerase has been a target for inhibiting HBV replication, and HBV polymerase inhibitors including lamivudine, adefovir, and entecavir have been used to cure HBV patients (Stein and Loomba, 2009). However, there is a limitation of the inhibitors due to the emergence of drug

resistance (Stein and Loomba, 2009). Therefore, specific inhibitor development for B23 and HBV core protein binding is the another strategy to overcome the challenge of the drug resistance.

In conclusion, B23 is involved in HBV replication cycle as a cellular chaperone via direct interaction with core protein. The ability of B23 to facilitate HBV capsid formation *in vitro* and *in vivo* points to its role in controlling HBV proliferation *in vivo*. Given that capsid assembly is critical to complete the HBV life cycle, the mechanism of B23 mediated viral assembly may be an important step for HBV proliferation.

V. Supplementary Tables

Table 1 Sequences of the primers used for Cp149 site-directed mutagenesis

	Primer Sequence
W125L	(Sense) 5’-GTGTCTTTTGGAGTGTTGATTCGCACTCCTCCTGCT-3’ (Antisense) 5’-AGCAGGAGGAGTGCGAATCAACACTCCAAAAGACAC-3’
W125K	(Sense) 5’-GTGTCTTTTGGAGTGAAGATTCGCACTCCTCCTGCT-3’ (Antisense) 5’-AGCAGGAGGAGTGCGAATCTTCACTCCAAAAGACAC-3’
W125E	(Sense) 5’-GTGTCTTTTGGAGTGGAGATTCGCACTCCTCCTGCT-3’ (Antisense) 5’-AGCAGGAGGAGTGCGAATCTCCACTCCAAAAGACAC-3’

Mutations were indicated (underlined).

Table 2 Sequences of the primers used for B23 site-directed mutagenesis

	Primer Sequence
D286A	(Sense) 5’-GACCAAGAGGCTATTCAAGCTCTCTGGCAGTGGAGG-3’ (Antisense) 5’-CTTCCCTGCCAGAGAGCTTGAATAGCCTCTTGGTC-3’
D286K	(Sense) 5’-GACCAAGAGGCTATTCAAAAGCTCTGGCAGTGGAGG-3’ (Antisense) 5’-CTTCCACTGCCAGAGCTTTTGAATAGCCTCTTGGTC-3’.
D286E	(Sense) 5’-GACCAAGAGGCTATTCAAGAACTCTGGCAGTGGAGG-3’, (Antisense) 5’-CTTCCACTGCCAGAGTTCTTGAATAGCCTCTTGGTC-3’

Mutations were indicated (underlined).

Table 3 Sequences of the primers used for real-time RT-PCR.

	Primer Sequence
HBV DNA	(Sense) 5’-GTGTCTGCGGCGTTTTATCA-3’
	(Antisense) 5’-GACAAACGGGCAACATACCTT-3’

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VII. 국문초록

숙주요인들은 바이러스의 생활사 중 B형 간염 바이러스 유전체 복제와 캡시드 형성에 관여 합니다. 뉴클레오포스민(B23)이라고 하는 수구요인은 HBV 코어 단백질과 상호작용 하는 것으로 알려졌지만, 그 기능에 대해서는 불확실하게 남아 있습니다. 이 연구에서, *in silico* 모델링을 이용하여 B23과 코어 단백질 사이의 상호작용 위치를 예상하였고, 돌연변이 유발을 이용하여 그 결과를 확인하였습니다. 코어 단백질과 B23의 결합은 HBV 캡시드 조립을 촉진시키고, 약물처리에 따른 캡시드 분리의 정도를 감소시키는 것을 알 수 있었습니다. 또한, HepG2.2.15 세포에서 B23의 억제는 세포내의 캡시드 형성을 막아 B형 간염바이러스의 감소를 유발하였습니다. 이러한 결과들을 통해 B23이 코어 캡시드 조립에 중요한 역할을 한다는 것을 확인할 수 있었습니다.

주요어: B형 간염바이러스, 끝을 자른 코어 149 단백질, 코어 조립, 바이러스 입자를 단백질막으로 둘러싸기, 숙주요인, 뉴클레오포스민

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