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# Heat shock protein 90 facilitates formation of the HBV capsid via interacting with the HBV core protein dimers

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ABSTRACT

The mechanism by which host factors contribute to hepatitis B virus (HBV) capsid formation during the viral life cycle remains unclear. This study analyzed the interaction between heat shock protein 90 (Hsp90), a host factor, and the HBV core protein. Hsp90 was found to bind to HBV core protein dimers, which was then encapsidated into the HBV capsid. Furthermore, activated Hsp90 may facilitate the formation of the human HBV capsid by catalyzing core assembly and reducing the degree of capsid dissociation at various temperatures, both *in vitro* and *in vivo*, and when subjected to detergent treatments *in vitro*. In addition, inhibition or downregulation of Hsp90 reduced HBV production in HepG2.2.15 cells. These results showed that Hsp90 plays an important role in HBV capsid stabilization and HBV formation.



## Introduction

Human hepatitis B virus (HBV) is a member of the *Hepadnaviridae* family and has infected over 2 billion people worldwide (Vanlandschoot et al., 2003). Chronic HBV infection is associated with liver disease, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC), particularly in Asian and African cases (Zuckerman, 1999).

The HBV genome consists of a partially double-stranded 3.2-kb circular DNA that encodes four proteins: a surface protein (HBs), a core protein (HBc), a polymerase (HBV pol), and an X protein (HBx) (Seeger and Mason, 2000). The core protein plays an essential role in the HBV life cycle by packaging HBV pol, pre-genomic RNA (pgRNA), and other components, such as heat shock proteins and protein kinases (Seeger and Mason, 2000). Since the virus is generated by the assembly of core proteins with the viral genome and other proteins, core assembly is a critical step in HBV replication.

The core protein consists of 183–185 amino acids and is organized into two domains: an N-terminal domain (amino acids 1–149; Cp149), which is involved in core assembly, and a C-terminal domain (amino acids 150–183 or 185), which regulates viral replication (Seeger and Mason, 2000). Cp149, a 34-residue, C-terminal truncated form, spontaneously forms a capsid under suitable conditions *in vitro* and *in vivo* (Kim et al., 2001). This protein has been used to study capsid assembly because it can be overexpressed in *Escherichia coli* more efficiently than the full-length core protein and is structurally similar to the HBV core protein (Biermer et al., 2003). Capsid formation is a key event in the viral life cycle because the capsid protects packaged viral and host factors. The identity of the host factors, such as heat shock proteins, that interact with the core protein and affect HBV capsid formation is unknown.

Heat shock protein 90 (Hsp90) is composed of three domains: an N-terminal ATP-binding domain, a middle domain, and a C-terminal dimerization domain (Prodromou et al., 1997; Stebbins et al., 1997; Terasawa et al., 2005). Hsp90, which functions in an ATP-dependent manner and often together with a co-chaperone, interacts with various proteins, including kinases and transcription factors, and controls biological processes by stabilizing protein folding (Cho et al., 2000a,b; Clark et al., 2009; Ganem and Varmus, 1987; Hu et al., 2004, 2002). Geldanamycin (GA) binds directly to the ATP-binding domain of Hsp90, thereby preventing ATP binding and reducing its affinity for client proteins (Buchner, 1999; Ujino et al., 2009).

Hsp90 is known to be involved in duck HBV replication. During duck HBV replication, Hsp90 and the co-chaperone p23 bind together and affect HBV pol activity. Moreover, Hsp90 becomes encapsidated with the duck HBV pol/pgRNA complex (Clark et al., 2009; Hu et al., 1997). Human and duck HBV replication are similar in that Hsp90 binds to HBV pol and affects HBV pol activity in both strains of the

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virus (Cho et al., 2000a,b; Hu et al., 2004). However, human HBV differs from duck HBV in that Hsp90 makes human HBV pol competent for *in vitro* priming rather than simply maintaining the human HBV pol/pgRNA complex (Gyoo Park et al., 2002).

HBV pol is known to bind to human HBV core proteins before being packaged into capsids (Lott et al., 2000), and also to Hsp90 and p23 (Hu et al., 1997). However, the relationship between Hsp90 and human HBV core proteins is unknown.

In this study, we established that Hsp90 binds to core protein dimers, but not to the capsid surface, and that Hsp90 is internalized into the human HBV capsid when Hsp90-bound core protein dimers form the capsid. This work demonstrates that Hsp90 can be packaged into the capsid by interacting not only with the HBV pol/pgRNA complex but also with core proteins. Furthermore, the presence of activated Hsp90 and HBV core protein dimers not only facilitated capsid formation but also reduced capsid dissociation when subjected to various temperatures and detergent treatments.

## Results

### *Hsp90 binds to HBV core protein dimers*

To determine if Hsp90 binds to HBV core proteins, a co-immunoprecipitation (co-IP) analysis was performed. The results show that Hsp90 binds to the HBV core protein (Fig. 1A). The location of the interaction between the Hsp90 and the HBV core protein was investigated by co-IP and binding assays using purified proteins. Hsp90 was found to bind to Cp149 dimers but not to the capsid surface (Fig. 1B and C). In addition, each binding site was identified (Fig. S1).

### *Hsp90 is incorporated into the HBV capsid through binding with Cp149 dimers*

In ducks, Hsp90 is packaged into the capsid by interacting with the duck HBV pol/pgRNA complex (Hu et al., 1997; Nassal, 1999). We sought to determine whether an alternative pathway of Hsp90 packaging into human HBV capsid exists other than the pathway described for duck HBV. Since Hsp90 binds to Cp149 dimers, we assumed that this may be the mechanism by which Hsp90 is packaged into the capsid. Sucrose density gradient analysis showed that when Hsp90 was mixed with Cp149 dimers, Hsp90 was present in fractions 8–10. The capsid was also detected in these fractions after the assembly reaction (Fig. 2A). When Hsp90 was mixed with BSA (control), it was detected in fractions 2–4 (data not shown). Two samples each from fractions 3 and 8 (Fig. 2A) were examined under non-denaturing and denaturing conditions to confirm Hsp90 packaging into the capsid. SDS-PAGE revealed the presence of Hsp90 in fractions 3 and 8 (Fig. 2B, lanes 3 and 4, bottom panel). However, dot blot and native agarose gel analyses, which do not interfere with capsid formation, showed no detectable Hsp90 in the fraction 3 and 8 samples (Fig. 2B, top and middle panels). Hsp90 may be undetectable in the fraction 8 sample due to its localization inside the capsid. Since native agarose gel electrophoresis can only detect the capsid, Cp149 was only detectable in the fraction 8 sample (Fig. 2B, lane 4 of middle panel). In addition, dot blot analysis detected Hsp90 and Cp149 dimers (Fig. S2). From these results, we conclude an alternative packaging pathway where Hsp90 is packaged into the capsid by binding to Cp149 dimers.

### *Activated Hsp90 facilitates HBV core protein assembly*

HBV core assembly begins with Cp149 homodimer formation through a cysteine disulfide bond between the 61$^{st}$ amino acid of each subunit (Nassal et al., 1992; Zheng et al., 1992). Hsp90 activity requires protein p23 binding and ATP, and its activity facilitates maturation of the client protein (Sullivan et al., 1997; Woo et al., 2009). To examine whether activated Hsp90 affects HBV core assembly, an assembly reaction with Cp149 dimers or a core protein point mutant (C61A) in the presence of Hsp90 was performed. The presence of activated Hsp90 facilitated capsid formation (Fig. 3A) not

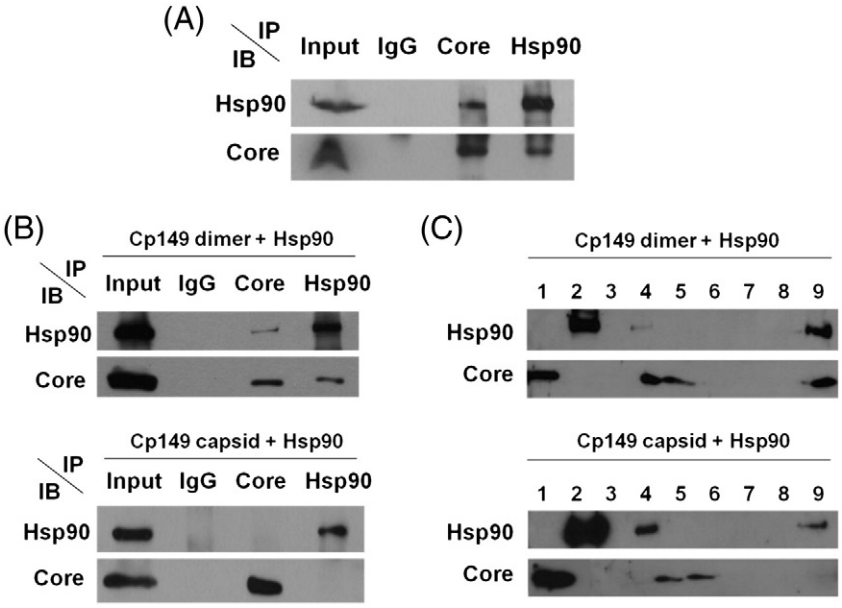


**Fig. 1.** Hsp90 binds to the HBV core protein. (A) The human hepatoma cell line, Huh7, was transfected with pCMV/Flag-core to express the HBV core protein. Lysates from transfected cells were co-immunoprecipitated using anti-HBV core Ab and anti-Hsp90 Ab. Immunoblot analysis was performed with anti-Flag M2 Ab and anti-Hsp90 Ab. An input of 4% of the total protein mixture was used as the positive control. Non-specific IgG was used as the negative control. (B) Cp149 dimers were mixed with Hsp90 and incubated at 30 °C for 1 h in reaction buffer. The mixture was co-immunoprecipitated using anti-HBV core Ab and anti-Hsp90 Ab and analyzed by 15% SDS-PAGE. Immunoblot analysis was performed using anti-HBV core Ab and anti-Hsp90 Ab (top panel). Capsid was formed in an assembly reaction with 20 μM Cp149 dimers and Hsp90. Co-immunoprecipitation was performed as above (bottom panel). An input of 5% of the total protein mixture was used as the positive control. Non-specific IgG was used as the negative control. (C) His-tagged Hsp90 was immobilized on Ni-NTA agarose, and Cp149 dimers (top panel) and capsid (bottom panel) were independently loaded on the agarose. Lane 1, purified Cp149; lane 2, purified Hsp90; lane 3, Ni-NTA agarose alone; lane 4, Hsp90 immobilized on agarose (3%); lane 5, flow-through; lanes 6–8, 50 mM imidazole washes to show protein that did not bind to agarose; and lane 9, 500 mM imidazole elution.