DNA-containing and empty hepatitis B virus core particles bind similarly to envelope protein domains

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DNA synthesis within the hepatitis B virus (HBV) nucleocapsid appears to be coupled to nucleocapsid envelopment. The nature of the envelopment signal is unknown, but is thought to involve a conformational change at the surface of the capsid that facilitates interaction with HBV envelope proteins. In binding assays in vitro, it was found that empty HBV core particles bound synthetic peptides corresponding to HBV envelope protein domains with the same affinity as did HBV DNA-containing core particles. This suggests that the selection of replication-competent nucleocapsids for envelopment is not related to the capacity of DNA-containing core particles to bind specifically to HBV envelope proteins, and that there must be an alternative mechanism.

Human hepatitis B virus (HBV) is a member of the hepadnaviruses, a family of small, enveloped viruses with partially double-stranded, circular DNA genomes that replicate by reverse transcription (Ganem & Varmus, 1987). During initiation of infection, virus particles deliver their DNA to the nucleus, where it is converted to a covalently closed, circular DNA molecule that acts as a transcriptional template for the production of an RNA genome (the pregenome) (Tuttleman et al., 1986). The RNA pregenome and the viral polymerase are encapsidated in the cytoplasm by viral capsid proteins to form immature nucleocapsid particles within which viral DNA synthesis occurs. Initially, a minus-strand DNA is synthesized by reverse transcription of the pregenome. This minus-strand DNA is then used as a template for plus-strand synthesis (reviewed by Nassal, 1996). Thereafter, nucleocapsids interact with surface proteins embedded in a lipid bilayer of a post-endoplasmic reticulum, pre-Golgi compartment and bud into the lumen of this compartment to form mature virions (Roingeard et al., 1990; Bruss & Ganem, 1991; Huovila et al., 1992).

Previous studies have shown that immature nucleocapsids containing pregenomic RNA are incompetent for envelopment and that minus-strand DNA synthesis must be completed for core particle envelopment to occur (Wei et al., 1996; Gerelsaikhan et al., 1996). This suggests that DNA synthesis within the capsid is coupled to the appearance of a signal on the exterior of the nucleocapsid that is essential for nucleocapsid envelopment. The molecular nature of this envelopment signal is unknown, but it is thought to involve the specific binding of DNA-containing core particles, but not of RNA-containing core particles, to HBV envelope proteins (Hui et al., 1999; Nassal, 1999). We have recently investigated the core–envelope interactions involved in the maturation of HBV, using in vitro assays to determine the capacity of HBV core particles to bind a panel of synthetic peptides that map to HBV envelope proteins (Poisson et al., 1997). A short domain of the large (L) envelope protein (aa 96–116 in the ayw subtype) and a short domain in the cytosolic loop of the small (S) envelope protein (aa 56–80) were found to bind HBV core particles efficiently. The L envelope domain found to interact with HBV core particles was subsequently shown to be essential for virion morphogenesis in two independent studies with envelope mutants, in which this domain was substituted by random sequence (Bruss, 1997) or deleted (Le Seyec et al., 1998). A recent study with the S domain confirmed that deletions and/or amino acid substitutions within this domain hinder subviral particle morphogenesis and therefore provide no information about the role of this domain in virion packaging (Jenna & Sureau, 1998).

Our previous assays of the binding of HBV core particles to HBV envelope peptides were performed with purified, liver-derived core particles containing HBV DNA (Poisson et al., 1997). In this study, we used similar assays to compare the binding of these DNA-containing core particles with that of empty HBV core particles, obtained by using the baculovirus expression system. These particles, called HBVΔ core particles, were obtained by expression of the sequence encoding the first 144 amino acids of the HBV core gene (excluding the arginine-
rich C-terminal stretch of 39 amino acids responsible for pregenome binding) and have been described elsewhere (Touzé et al., 1999). HBV envelope peptides (Fig. 1) and binding assays were described previously (Poisson et al., 1997; Hourioux et al., 1998). We used two 25-residue peptides, S1 and S2, that together covered the cytosolic loop of the HBV S protein. To cover the cytosolic domain of the HBV L protein, we used five peptides of 28, 28, 29, 23 and 55 residues named L1, L2, L3, L4 and L5, respectively (L1—L4 corresponded to the pre-S1 domain and L5 corresponded to the pre-S2 domain). Two additional peptides overlapping peptide L4 were used: peptide L4a (the last 10 residues of peptide L3 plus the first eight residues of peptide L4) and peptide L4b (the last 13 residues of peptide L4 plus the first eight residues of peptide L5).

Dynatech ELISA plates were coated with the various peptides coupled to BSA (or with BSA alone) at a concentration of 20 µg/ml in 0.05 M bicarbonate buffer (pH 9.6) for 24 h at 4 °C (200 µl per well). Plates were washed three times with 0.01 M PBS containing 0.15 M NaCl, pH 7.4, and 0.5% Tween 20 and were blocked by adding 300 µl PBS containing 2% newborn calf serum (NBCS) and incubating for 45 min at 37 °C. The plates were washed once and the HBV core preparations were adjusted to the same concentration (estimated by immunocapture ELISA and electron microscopy to be around 10⁶ core particles/ml) in 1 mM PBS plus 15 mM NaCl, pH 7.4, and were incubated for 24 h at 4 °C with the peptides. The plates were washed three times and 100 µl of anti-HBc monoclonal antibody cC6A1, diluted 1:10000 in 0.05 M PBS, 0.75% NaCl, pH 7.4, containing 5% NBCS, 5% BSA and 0.5% Tween 20, was added. The plates were incubated for 1 h at room temperature and then washed three times and 100 µl horseradish peroxidase-conjugated goat anti-mouse F(ab’2)² (Tago), diluted 1:10000 in the same buffer, was added. Plates were incubated for 30 min at 37 °C and washed three times. A mixture of hydrogen peroxide and α-phenylenediamine was added and the plates were left for 30 min in the dark at room temperature. Colour development was stopped with 1 M H₂SO₄ and absorbance was read at 492 nm. The cut-off value chosen was twice the mean value obtained from eight control wells containing BSA alone (2 × 0.035 in a representative experiment). Binding activity for a given peptide was expressed as the absorbance/cut-off value.

We found that HBVΔ core particles bound the L and S HBV envelope peptide domains with the same affinity as liver-derived, HBV DNA-containing capsids (Table 1). We cannot rule out the hypothesis that, in the absence of their C-terminal part, our truncated capsids could adopt a structure that resembles that of DNA-containing capsids, thus explaining their binding to envelope peptides. This is unlikely, however, because others have demonstrated the ability of full-length, E. coli-derived HBV capsids to bind to HBV L envelope protein expressed in rabbit reticuloocyte lysates (Tan et al., 1999).

Table 1. Binding of BSA-coupled HBV envelope peptides to HBV DNA-containing, liver-derived HBV core particles and to empty, baculovirus-derived HBV core particles

<table>
<thead>
<tr>
<th>Peptide</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
<th>L4a</th>
<th>L4b</th>
<th>L5</th>
<th>S1</th>
<th>S2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV-L</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.9 ± 0.6</td>
<td>–</td>
<td>6.8 ± 0.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HBVΔ</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.7 ± 0.1</td>
<td>–</td>
<td>9.7 ± 1.0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Results are given as absorbance/cut-off value. The cut-off value is twice the mean absorbance obtained with BSA alone. Values presented here are the means and SD from four independent experiments performed with all peptides. HBV-L indicates liver-derived core particles; HBVΔ indicates baculovirus-derived core particles. –, Absorbance was below the cut-off value.
particular capacity of DNA-containing nucleocapsids to bind HBV envelope proteins.

Alternatively, HBV DNA-containing capsids may interact with a cellular factor, such as a molecular chaperone or an element of the cytoskeleton involved in cell trafficking. We recently analysed HBV morphogenesis in HepG2 cells transfected with HBV DNA and showed that nucleocapsids seem to regroup and bud into specific areas of dilated host cell compartments (Roingeard & Sureau, 1998). This suggests that there may be a specific transport of DNA-containing core particles towards these cellular compartments. Such a mechanism may depend on host cell factors binding specifically to DNA-containing core particles, resulting in the selection of these core particles for envelopment. In vitro binding assays will be of value for identifying these host factors that potentially bind HBV core particles. Such studies may reveal novel targets for specific antiviral strategies.

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References


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