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High Plasticity of the Hepatitis B Virus Capsid Revealed by Conformational Stress

Bettina Böttcher[1]*, Maren Vogel[2], Martin Ploss[1] and Michael Nassal[2]

[1]*European Molecular Biology Laboratory, Meyerhofstr. 1 D-69117 Heidelberg, Germany*

[2]*University Hospital Freiburg Internal Medicine II / Molecular Biology, Hugstetter Str. 55 D-79106 Freiburg, Germany*

**Corresponding author*

Hepatitis B virus (HBV) replicates through reverse transcription inside its icosahedral nucleocapsid. The internal genome status is signaled to the capsid surface, predicting regulated conformational changes in the capsid structure. To probe their nature and extent, we imposed local conformational stress on the outer surface of HBV capsid-like particles, and monitored its consequences by electron cryomicroscopy and image reconstruction. The capsid structure had an enormous flexibility and robustness as a whole, as well as within the subunits, whose spikes were able to rotate by as much as 40° against the distal interdimer contact sites. The likely hinge for the swiveling movement was the conserved Gly111 residue at the inner surface of the capsid. The stress imposed from the outside also affected the internal capsid organization, implying a specific route for the flow of conformational information between capsid interior and exterior as required for signaling of the genome status.

Keywords: hepatitis B core protein; electron cryo-microscopy; capsid dynamics; image reconstruction; conformational flexibility

Introduction

Hepatitis B virus (HBV), the causative agent of B-type hepatitis in humans,[1] consists of an icosahedral nucleocapsid (core particle) harboring the 3 kb genome and the viral reverse transcriptase, and an outer lipid envelope into which three types of surface proteins are embedded. The functions of the HBV capsid extend way beyond that of a protective transport container for the virus genome: Reverse transcription of the initially packaged pregenomic (pg) RNA into a partially double-stranded circular DNA is confined to the interior of intact nucleocapsids.[2,3] The capsid surface, on the other hand, provides specific interaction sites for the preS domain of the large surface protein,[4] enabling the envelopment that is essential for egress from and entry into the host cell. Only DNA-containing mature, but not RNA-containing immature, HBV nucleocapsids are enveloped efficiently.[5] Hence, information on the internal genome status is transmittable to the capsid surface. This, and the active role in replication, imply that the capsid can undergo regulated conformational changes of currently unknown nature.

HBV capsids consist of a single type of capsid protein, also termed core protein, or HBc antigen. The first ~140 of its 183 amino acid residues form the capsid shell.[6,7] This assembly domain is followed by a linker of about ten amino acid residues,[8] and by a basic nucleic acid-binding C-terminal domain (CTD).[6,9] Assembly of authentic nucleocapsids, requiring the CTD,[10] is thought to initiate by binding of the core protein to a complex of the reverse transcriptase and pgRNA,[11] co-sequestering the enzyme and its template into the proper environment for reverse transcription. However, core protein can assemble on its own, providing access to recombinant capsid-like particles (CLPs) in sufficient quantity for high-resolution studies.

Such CLPs occur in a large (triangulation number $T=4$) and a small form ($T=3$), consisting of 240 and 180 subunits, respectively.[12] Their protein shells are very similar to those of liver-derived nucleocapsids for which, at present, only low-resolution data are available.[13] The fraction of large CLPs is about 50% for full-length protein CLPs, increases to more than 95% when the CTD is deleted after position 149,[14] and decreases again upon further truncation.[7] Details of the CLP structures were revealed by electron microscopy and X-ray crystallography.[14–16]

Abbreviations used: HBV, hepatitis B virus; CTD, C-terminal domain; CLP, capsid-like particle; GFP, green fluorescent protein.

E-mail address of the corresponding author: boettcher@embl-heidelberg.de

The most striking features are surface spikes, flanked at either side by holes. Each spike can be arranged in one of two non-identical environments, being part of either two hexagons, or of a hexagon and a pentagon (Figure 1(a)).

The spikes are created by core protein dimers.[14–16] Two antiparallel α-helices from each subunit, connected by a loop around position 80 that overlaps with the immunodominant c/e1 B cell epitope,[17–19] associate into a four-helix bundle (Figure 1(b)). Helix 3 (residues 50–73)[16] is proximal to the 2-fold axis of the dimer and forms an inner, disulphide bridge.[20] The kinked helix 4 (residues 79–110) is more distant. The spikes and/or residues around their base are likely contact points for the preS domain during envelopment.[21,22] The inter-dimer contacts are mediated by a hand-like subdomain in the C-terminal half of the subunit, which is spatially remote from the spike-region that stabilizes the inner dimer contacts.[23] Residues downstream of position 143 point into the particle interior.[24]

These data describe only one possible state of the capsid, which can hardly explain the dynamic changes during nucleocapsid maturation. Here, we exploited a novel approach, developed originally for the generation of CLP-based vaccines,[25,26] for investigating the structural flexibility of the HBV capsid. Selected foreign sequences can be inserted into the loop at the tip of the spike without abolishing particle formation.[27–30] Inserts, differing in sequence, size, and length of the connecting linkers (Figure 1(c)) allowed us to impose varying degrees of local conformational stress on the outer CLP surface, and then to monitor *via* electron cryomicroscopy and image analysis the impact on the capsid structure. Our data reveal an astounding plasticity in the capsid structure as a whole as well as within its subunits. They provide direct evidence for conformational cross-talk between the capsid lumen and surface.

Results

Experimental design

All fusion constructs were based on an HBV core protein truncated after position 149 (HBc1-149). Due to the uniform size distribution of the corresponding CLPs, a potential shift in the $T=4$ to $T=3$ ratio provides a sensitive indicator for insertion-induced alterations at the inter-dimer contacts. At the C terminus, a His_6 tag was added for improved particle formation of insert-carrying fusions.[29] We refer to this construct, HBc1-149_H6, as the icosahedral carrier protein.

To impose controlled stress, different heterologous sequences were inserted into the c/e1 loop (Figure 1(c)), with local splaying of the central helices as the expected primary consequence. The green fluorescent protein (GFP) as insert is compatible with CLP formation when connected to

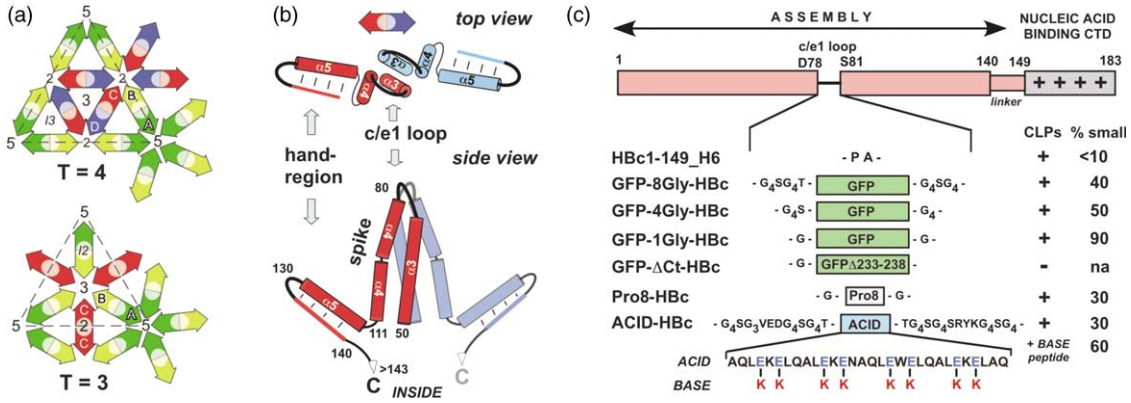

Figure 1. Structural features of HBV CLPs and the core protein, and variants used in this study. (a) Architecture of HBV CLPs. The subunit arrangement in large ($T=4$) and small ($T=3$) CLPs is shown. Each double-arrow represents one dimer, the circular white areas represent the spikes. Monomers adopt one of four ($T=4$), or three ($T=3$) non-equivalent positions, giving rise to AB (green+yellow) and CD (red+blue) dimers in the large, and AB and CC dimers in the small CLPs.[7] Symmetry axes are indicated by numbers. The broken line shows one of the 20 triangular facets of the icosahedron. The curvature induced at the 5-fold axes is reflected in an imperfect match between the hexagon and pentagon in this two-dimensional representation. (b) The core protein dimer. The X-ray-based[16] representations highlight the spike-forming four-helix bundle mediating the intra-dimer, and the hand regions mediating the inter-dimer contacts. Numbers represent amino acid residue positions; the first 49 residues are omitted for clarity. The thick black line around position 80 symbolizes the c/e1 loop used for insertions; and that around position 130 symbolizes a Pro-rich turn that connects helix 5 and downstream residues to form the hand region. Residues past position 143 were not resolved in the X-ray structure but point into the particle interior. (c) Constructs used. The domain structure of the core protein is indicated at the top. The basic CTD (+ signs representing four arginine clusters) was replaced by a His_6-tag after position 149. All insertions (not drawn to scale), with their flanking linkers shown, replaced the authentic P79 and A80 residues. The ability of the constructs to form CLPs and the fraction of small particles in the total population is shown on the right; na, not applicable. The BASE peptide differs from the inserted ACID sequence by the indicated K *versus* E exchanges.