Comparison of three different recombinant hepatitis B virus core particles expressed in *Escherichia coli*

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Summary. The properties of three different recombinant hepatitis B virus core proteins expressed in *Escherichia coli* were compared: an N-terminal fusion protein, a C-terminally truncated protein and a sequence-authentic protein. All three proteins assembled into capsid-like particles with typical HBe-antigenicity, sedimentation behavior and distinctive electron microscopical images. Apart from this, however, variant HBe proteins displayed properties different from sequence-authentic HBe protein p21.4. Unlike p21.4, the particles of the N-terminal fusion protein p22.2 were sensitive to proteolytic attack by trypsin at variable sites within its arginine-rich C-terminus but not in its extended N-terminus. We therefore conclude that the C-terminal region is located on the surface of the p22.2 particle. These particles also showed increased HBe-antigenicity, as did the C-terminally truncated core particles p17.6, and to an even greater extent p18* particles which were derived from p22.2 by tryptic digestion. This might be interpreted as evidence for an – albeit minor – structural change. All variant core particles were less stable and contained less RNA. Electron microscopic indication for DNA binding of C-terminal deleted p17.6 particles was obtained using an aqueous spreading technique.

Introduction

The core gene (open reading frame preC/C) of the hepatitis B virus (HBV) is capable of coding for two serologically distinguishable antigens depending on whether the first or the second ATG start codon is used: The hepatitis B core antigen (HBeAg) is synonymous with the 28-nm viral nucleocapsid or core

particle, whose single structural protein is the 21 kD HBc polypeptide (using the second ATG), consisting of 183 or 185 amino acids, depending on the HBV subtype. 180 identical subunits of HBc protein assemble with the HBV polymerase and the pregenome to form the 28-nm-diameter icosahedral nucleocapsid. The hepatitis B e antigen (HBeAg) is a nonparticulate, secreted product of the precore locus of about 17 kD [31], originally detected in the sera of HBV-infected patients [20]. It is generated from a 25 kD precore precursor protein (using the first ATG) by a series of proteolytic cleavage of the N- and C-terminus [26, 30]. However, as core particles can also display HBe-antigenicity depending on their physical state, the serological terms HBcAg and HBeAg are misleading.

It would be more correct to identify the protein by size and by the locus from which it originated (for reviews see [13, 29]). A remarkable feature of HBc is its extremely basic, arginine-rich C-terminus which resembles protamine sequences and represents the nucleic acid binding domain [24, 25]. This region is dispensable for assembly [3, 11].

In this study, we try to clarify some open questions concerning the significance of sequence elements of the core polypeptide for capsid assembly, structural requirements for HBc- and HBe-antigenicity, the localization of the C-terminus within the particle, and nucleic acid binding. For this purpose, authentic core protein as well as an N-terminal fusion variant and a C-terminal deletion variant were expressed in E. coli. These core particles were analyzed by electron microscopy, HBc- and HBe-enzyme immunoassay (EIA), electrophoresis and blot techniques, trypsin and DNAse/RNAse cleavage.

**Materials and methods**

_E. coli_ LE 392 [30] was used for expression experiments. Bacteria were grown in Luria Bertani Broth [21] in the presence of ampicillin. The expression plasmids used were based on pUC18 and pUC19 [34]. All DNA manipulations were carried out as described by Maniatis et al. [21].

The strain _E. coli_: pLIN3C grown in overnight cultures up to retardation phase, produced HBc protein constitutively. The strains of _E. coli_, harboring the plasmids ptacVR4 and ptacCR2, respectively, were induced by addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) in the late logarithmic growth phase. Cells were harvested and disrupted by sonication.

For purification, HBc protein was precipitated with ammonium sulfate and centrifuged through a continuous sucrose gradient (20 to 60% wt/vol). The fraction of the sucrose gradient with the highest HBc-reactivity was diluted to 10% sucrose in TE buffer and pelleted in order to remove free nucleic acids and protein impurities and nearly homogenous core particles were resulted.

Following SDS-polyacrylamide gel electrophoreses [18] (10–20% polyacrylamide, sample buffer included dithiothreitol), HBc proteins were detected by Western blotting [6] using polyclonal human anti-HBc antibodies.

HBc-specific immunoreactivity of recombinant core particles was detected by a self-made sandwich EIA using polyclonal human anti-HBc-IgG and anti-HBc-IgG horseradish peroxidase [9].

HBe-antigenicity was tested by a commercial EIA (ETI-EBK, SORIN Biomedica), which utilizes monoclonal antibodies 904 (anti-HBe/a) and 905 (anti-HBe/b) [15] in a sandwich design.