Interaction of wild-type and naturally occurring deleted variants of hepatitis B virus core polypeptides leads to formation of mosaic particles

Petra Preikschat[a,1], Andris Kazaks[b,1], Andris Dishlers[b], Paul Pumpens[b], Detlev H. Krüger[a], Helga Meisel[a,*]

[a] *Institute of Virology, Humboldt University School of Medicine (Charité), Schumannstr. 20/21, D-10117 Berlin, Germany*
[b] *Biomedical Research and Study Centre, University of Latvia, Riga, Latvia*

Received 29 March 2000; revised 27 June 2000; accepted 29 June 2000

Edited by Takashi Gojobori

Abstract The simultaneous presence of hepatitis B virus (HBV) genomes carrying wild-type (wt) and in-frame deleted variants of the HBV core gene has been identified as a typical feature of HBV-infected renal transplant patients with severe liver disease. To investigate possible interactions of wt and deleted core polypeptides a two-vector *Escherichia coli* expression system ensuring their concomitant synthesis has been developed. Co-expression of wt and a mutant core lacking 17 amino acid residues (77–93) within the immunodominant region led to the formation of mosaic particles, whereas the mutant alone was incapable of self-assembly.

Key words: Hepatitis B virus; C gene deletion; Dimer formation; Mosaic particle

1. Introduction

With regard to their employment as carriers of foreign epitopes in vaccine development, core particles of hepatitis B virus (HBV) have been well characterized by biochemical and immunological methods during the last years [1–4]. The HBV core particle is composed of homodimers that can be stabilized by disulfide links between the Cys61 residues in two monomers [5,6]. Recently, the crystal structure of the T = 4 HBV core particle has been solved at 3.3 Å resolution, revealing its composition of dimers with protruding spikes composed of a four-helix bundle, the tip of them representing the immunodominant region [7]. According to epitope mapping data, amino acid (aa) residues crucial for formation of the immunodominant region lie within the Gly73–Gly94 stretch of the core polypeptide (for references see [4]). Clinical studies have demonstrated that the emergence and accumulation of mutated HBV genomes carrying deletions in the central region of the HBV core gene (C gene) are accompanied by the progression of liver disease in long-term immunosuppressed renal transplant recipients [8,9]. During immunosuppression such mutated HBV genomes seem to have a selective advantage for replication over wild-type (wt) genomes [8,10] and can represent up to 90% of the total HBV DNA population in patient sera (unpublished data). Preferential appearance of such C gene mutants during immunosuppression makes their possible relation to immune escape mechanisms unlikely.

To understand the folding and particle assembly properties of mutated core polypeptides, the expression of a set of deleted C genes has been undertaken in *Escherichia coli* [11]. As a result, the majority of mutated core polypeptides with deletions protruding from Gly73 in the N-terminal direction and from Gly94 in the C-terminal direction, respectively, was incapable of correct folding and self-assembly. In agreement with this, deleted core polypeptides were not found within infected hepatocytes (unpublished data). Recent data from eukaryotic in vitro systems support these results; after transfection of hepatoma-derived cell lines with HBV genomes carrying in-frame C gene deletions, no corresponding deleted core derivatives could be detected [12,13].

However, it remained to be elucidated whether core deletion variants could co-assemble with wt core polypeptides to form mosaic particles consisting of different homo- and/or heterodimers as subunits [3]. The simultaneous presence of wt HBV genomes and virus variants carrying C gene deletions in the sera and liver of immunosuppressed patients over long periods [8,9] could reflect a functional interaction with possible pathophysiological consequences.

In the work presented here, the simultaneous expression of wt and mutated forms of the C gene in *E. coli* was investigated. Using a two-vector expression system, the interaction of wt with a mutant core polypeptide #77/93 carrying a deletion of 17 aa residues (positions 77–93) was demonstrated. This interaction leads to the formation of mosaic particles within the bacterial cell, whereas the mutant #77/93 polypeptide alone is incapable of self-assembly.

2. Materials and methods

2.1. Construction of expression vectors

Full-length HBV genomes were amplified from sera and liver samples of patients with severe liver disease followed by cloning and sequencing as described earlier [14]. C gene fragments carrying deletions of 8, 17, 21 and 30 codons (designated as #86/93, #77/93, #75/95 and #82/111, respectively, according to the positions of codons deleted) were expressed in *E. coli* under the control of tandem tryptophan promoters. For that purpose, ColE1-replicon-based expression plasmids pHBc3 (for expression of full-length core polypeptide as 'long form', aa 1–183) [15] and pT31 (for expression of C-terminally truncated core polypeptide as 'short form', aa 1–144) [16] (Fig. 1) were used. To ensure simultaneous synthesis of both wt and deleted

*Corresponding author. Fax: (49)-30-2802 2180.
E-mail: helga.meisel@charite.de

[1] Equal contribution.

Abbreviations: aa, amino acid; C gene, HBV core gene; HBV, hepatitis B virus; wt, wild-type

PII: S0014-5793(00)01836-6

core proteins within the same cell, a two-vector system was developed using the compatible p15A-replicon-based vector pREP4 (Qiagen, Hilden, Germany) in combination with ColE1-replicon-derived plasmids (Fig. 1).

2.2. Extraction and purification of core particles

The synthesis of wt and deleted core polypeptides was directed by the expression/co-expression of the appropriate C gene variants from ColE1- and/or p15A-replicon plasmids in the *E. coli* strain K802. For co-expression experiments, deleted C genes were expressed in long and short form from pREPc3 and pREPpT, respectively, whereas wt-C genes were expressed from pBR-based plasmids pHBc3 or pT31. For co-expression of both (short and long) wt core genes, plasmids pHBc3 and pREPpT were used. Cells were grown and lysed as described earlier [11]. Core particles were purified by ammonium sulfate precipitation and by subsequent sucrose gradient (20–50%) centrifugation.

2.3. SDS–PAGE and immunoblotting

Sample preparation and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE; 15%) were done according to Laemmli [17]. Western blotting was performed as described by Towbin et al. [18]. Core proteins were detected with the monoclonal anti-HBc/HBe antibody 239F5 (Sorin Biomedical, Saluggia, Italy) recognizing a region between aa 130–140 and anti-mouse IgG peroxidase conjugate (Dako Diagnostika, Hamburg, Germany).

2.4. Electron microscopy

Sucrose gradient fractions were investigated by electron microscopy of uranyl acetate negative contrasted samples as described by Gelderblom et al. [19] using a Zeiss EM 10A electron microscope.

2.5. Immunoprecipitation

Immunoprecipitation was carried out by overnight incubation of purified core particles with the monoclonal antibody C1-5 (recognizing aa 78–83) [20] coupled to protein A-Sepharose CL-4B (Amersham Pharmacia Biotech, Freiburg, Germany) at 4°C on a rotary shaker. After washing three times with TNE buffer (0.02 M Tris, 0.15 M NaCl, 0.01 M EDTA, pH 8.0), the Sepharose-bound material was eluted with Laemmli SDS-sample buffer containing 5% β-mercaptoethanol. Sepharose-unbound material was pooled and concentrated from washing buffer by ammonium sulfate precipitation, before solubilization in SDS-sample buffer.

2.6. Cross-linking of dimers

To cross-link non-covalently connected core monomers by the intermolecular disulfide bridge of Cys61 residues located at the dimer interface, $CuSO_4$ was used as an oxidizing agent in a procedure according to Nassal et al. [5]. Portions of purified particles were mixed with various amounts of a 1 mM $CuSO_4$ stock solution to determine the optimal Cu^{2+} concentrations necessary for visualization of dimers of each core variant by SDS–PAGE and Western blot. After 20 min incubation at room temperature, the reactions were stopped by adding 4 μl of 50 mM EDTA and 24 μl of the SDS-sample buffer without β-mercaptoethanol. The samples were analyzed by SDS–PAGE and by Western blotting.

2.7. Native agarose gel electrophoresis

Electrophoresis of purified particles was performed on 1.5% agarose gels in TBE buffer, pH 7.6. Protein bands were stained with Coomassie brilliant blue.

3. Results

3.1. Co-expression of wt and deleted C genes in E. coli

Four HBV core variants with different deletions and distinct characteristics when expressed alone [11] were chosen to study the interaction with wt core in co-expression experiments using the *E. coli* two-vector expression system (Table 1). Due to the different protein expression levels of the full-length compared with the C-terminally truncated form of the C gene [11], both forms were included in the analysis. The synthesis of the four mutant core polypeptides expressed alone

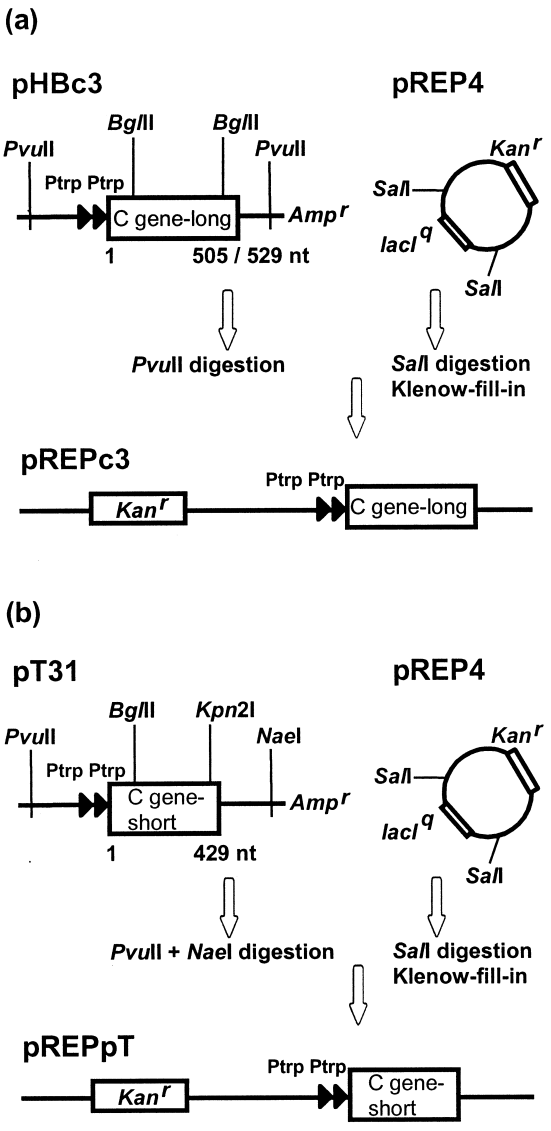

Fig. 1. Constructs for *E. coli* expression/co-expression of wt and deleted C genes in long (a) and short (b) forms. *Bgl*II and *Bgl*II/*Kpn*2I fragments from cloned full-length genomes of HBV mutants were used to substitute the corresponding fragments in pHBc3 and pT31, as described previously [11]. Using *Pvu*II and *Nae*I restriction sites, deleted C genes in both short and long forms were cloned (along with Ptrp) into pREP4.

or co-expressed with wt cores were analyzed by SDS–PAGE and Western blotting of *E. coli* lysates (Table 1). The combinations of short and long forms of core proteins enabled us to easily differentiate between two polypeptide forms present in the same cell lysate.

In agreement with our previous study [11], no protein products could be detected for deletion variants #75/95-long, #75/95-short and #82/111-long when expressed alone. Also, no detection of these deleted proteins was obtained by co-expression with wt cores in the short and long form, respectively (not shown). In contrast, for deletion mutants #86/93-long, #86/93-short and #77/93-short efficient synthesis was found by single expression, as well as by co-expression with wt cores in both forms. Interestingly, the synthesis and solubility of #77/93-long were markedly enhanced by co-expression of long or short wt-core proteins. In most cases of successful

Table 1
Synthesis and assembly-competence of deleted core polypeptides as detected by single expression or by co-expression with wt core

Deletion variant	Expressed alone		Co-expressed with wt cores	
	synthesis	assembly	synthesis	assembly
#86/93-long	+++	+++	+++	+++
#86/93-short	+++	+++	+++	+++
#77/93-long	+	−	+++	+++
#77/93-short	+++	+	+++	+++
#75/95-long	−	−	−	−
#75/95-short	−	−	−	−
#82/111-long	−	−	−	−

Co-expression experiments were performed with four distinct core deletion variants exhibiting different levels of synthesis and self-assembly, when expressed alone. Deletion variants are designated according to the positions of aa deleted. Levels of synthesis are shown as: +++, strong, high solubility; +, low, poor solubility as compared to wt core protein; −, no protein detectable. Assembly as determined by electron microscopy was scored as: +++, level similar to wt; +, traces of capsids; −, no detectable capsids.

co-expression both wt and deleted proteins were detected in comparable amounts in crude *E. coli* lysates (not shown). Only in the case of the co-expression variant #77/93-long with wt-long or -short, the deleted core polypeptide was detected in lower amounts in comparison to the wt. An additional approach was chosen to examine whether these proteins interact on the level of homo- or heterodimers to form mosaic particles.

Although both #86/93 and #77/93 variants could be used for further investigations concerning the possible formation of mosaic core particles, variant #77/93 was chosen for the following reasons: (i) during a period of 2 years #77/93 remained the major C gene deletion variant in sera and liver of an immunosuppressed renal transplant patient with severe liver disease, whereas #86/93 represented only a minor variant from the same patient; (ii) the synthesis of #77/93-long was improved in the presence of wt cores; (iii) the monoclonal antibody C1-5, recognizing aa residues 78–83 absent in #77/93 (but not in #86/93), should allow the immunoprecipitation of putative mosaic particles by the recognition of their wt constituents.

3.2. *Purification and immunoprecipitation of mosaic core particles*

To identify putative mosaic particles by an immunoprecipitation approach using the monoclonal antibody C1-5, four combinations of sucrose gradient purified co-expression products were studied: #77/93-long+wt-long, #77/93-short+wt-long, #77/93-long+wt-short, #77/93-short+wt-short. In addition, expression products of #77/93-short alone as well as of the combination of wt core-short+wt-long were included as controls. Due to the differences in molecular weight of the short and long forms of the core protein, the used combinations should facilitate the differentiation between core polypeptides by Western blotting.

After sucrose gradient purification, particle formation of #77/93+wt co-expression products in comparison to wt particles was confirmed by electron microscopy. Because no obvious quantitative or qualitative differences have been observed, Fig. 2 shows only two examples of particles derived from #77/93-short+wt-long or #77/93-long+wt-long co-expression variants.

All sucrose gradient fractions were analyzed for the content of wt and deleted core polypeptides, and only particle-containing fractions showing the highest antibody-reactivity in Western blot analysis (Fig. 3a) were loaded onto protein A-Sepharose CL-4B coupled with C1-5 antibody. Antibody-bound and -unbound material were analyzed by SDS–PAGE and by immunoblotting (Fig. 3b). Only wt and mosaic particles consisting of #77/93+wt polypeptides could be retained by the antibody. As expected, the #77/93-short variant alone was found in the concentrated unbound material (Fig.

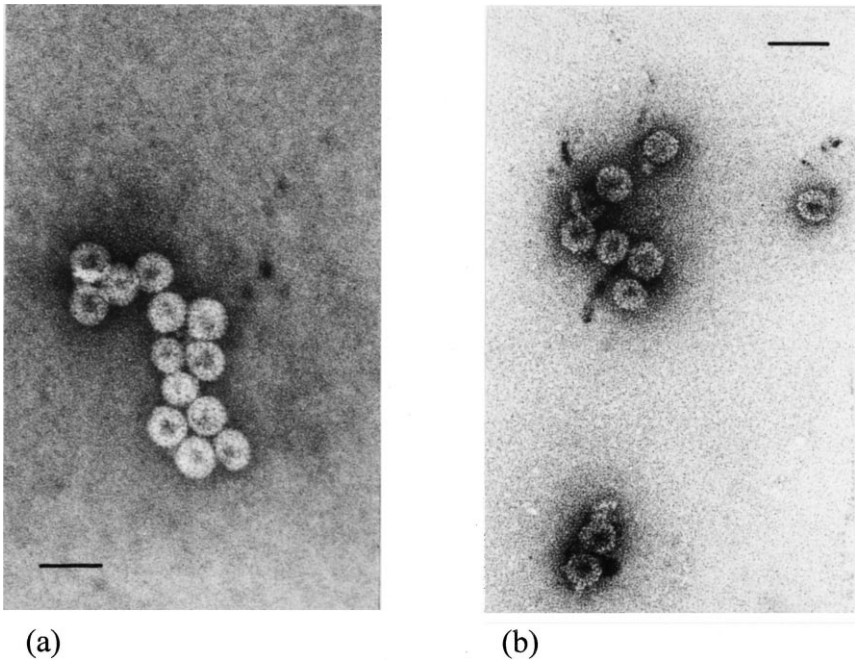

Fig. 2. Electron microscopy of purified core particles derived from (a) #77/93-short+wt-long and (b) #77/93-long+wt-long co-expression. Bar: 50 nm.

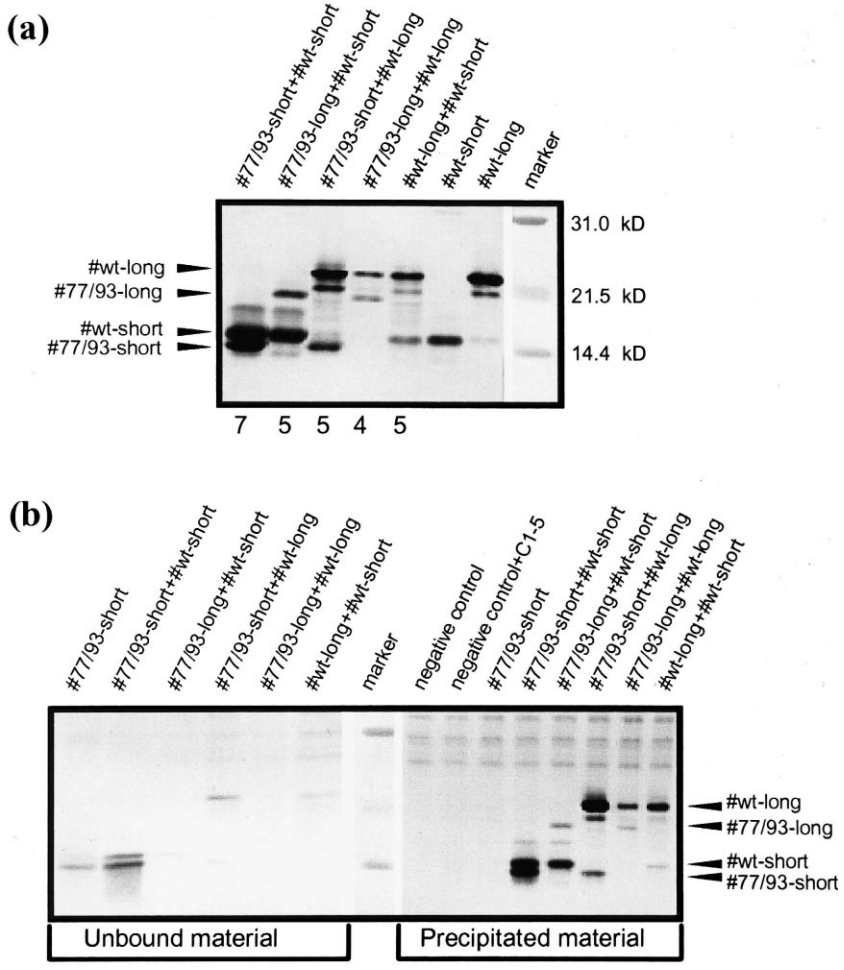

Fig. 3. Mosaic structure of core particles consisting of wt and #77/93 polypeptides, as shown by immunoprecipitation with the monoclonal antibody C1-5. (a) Western blot of sucrose gradient fractions subjected to precipitation. Numbers below the lanes correspond to the numbers of appropriate fractions (fraction 1 has the highest density). The specific protein product of #wt-long was represented by two bands, the lower of them probably representing a degradation product. (b) SDS–PAGE and Western blotting of cores after binding to C1-5-coupled Sepharose CL-4B: antibody-bound (right) and -unbound (left) material.

3b, left). Antibody-bound co-expression products of all four combinations of wt and #77/93 contained both wt and deleted polypeptides (Fig. 3b, right). This indicates that in all cases of co-expression at least a part of the purified particles represents mosaic ones. No obvious differences in the ratios of wt to deleted proteins were detected when precipitates were compared with original sucrose gradient fractions (Fig. 3a).

To analyze co-expression products for the presence of different types of particles as wt, mutant and mosaic particles, respectively, the same sucrose gradient samples were applied to native agarose gel electrophoresis (Fig. 4). To test whether this method is suitable to distinguish between different types of particles contained in a mixture, purified wt-short particles were mixed with purified particles derived from co-expression (#77–93-short+wt-short) and included as control. The appearance of two distinct bands (Fig. 4, lane 8) proved the feasibility of this method to differentiate between two kinds of particles. In contrast to the artificial mixture, particles obtained as a result of co-expression were characterized by slightly diffuse, but definite bands with different electrophoretic mobility in comparison to each other (Fig. 4, lanes 3–7) and to sharp distinct bands of wt core particles (Fig. 4, lanes 1, 2). The higher mobility of the wt-long particles in comparison to their short counterparts could be attributed to different particle surface charges. Because no wt-corresponding bands have been observed in lanes of co-expression particles (Fig. 4, lanes 3–7) it is supposed that particles derived from

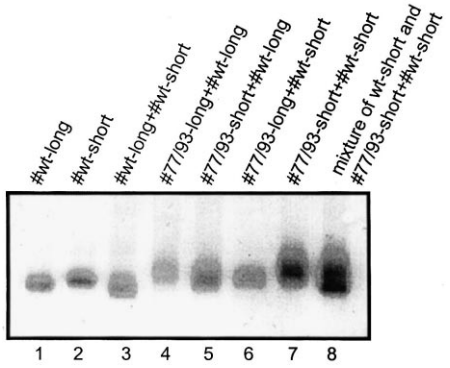

Fig. 4. Native agarose gel electrophoresis of purified mosaic core particles obtained after expression/co-expression of wt and deleted C genes.

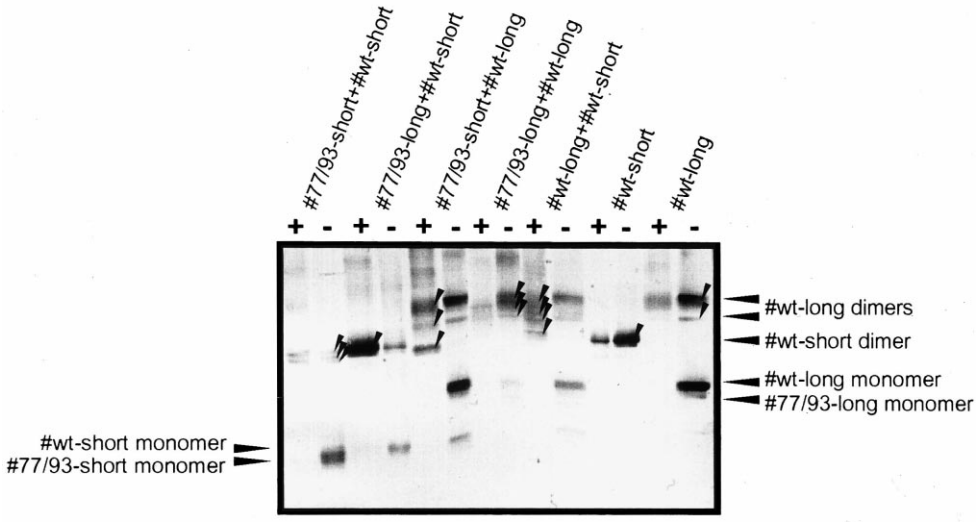

Fig. 5. Western blot of core-protein products obtained after disruption of purified wt and mosaic core particles under non-reducing conditions with ('+') or without ('−') prior oxidation by Cu^{2+}. Products with dimer-corresponding molecular weight are marked with arrowheads.

co-expression are rather a mixture of mosaic ones. Whether such mixtures contain mosaic particles consisting exclusively of wt-homodimers as well as of homodimers of deleted proteins or/and of heterodimers remained unclear.

3.3. Formation of homo- and heterodimers during co-expression of wt and #77/93

To characterize the composition of dimers forming mosaic particles, the latter were disrupted under non-reducing conditions and subjected to SDS–PAGE and immunoblotting (Fig. 5). To achieve a stable dimer formation, one portion of each kind of purified particle was oxidized to fix intermolecular disulfide bridges between monomers. The best dimer separation was achieved for #77/93-short when it was co-expressed with wt-long or wt-short. For these two cases, dimer-corresponding bands were cut from PAGE and the proteins were eluted and analyzed by immunoblotting. Preliminary experiments showed the presence of heterodimers in addition to both homodimeric forms in the particles (data not shown here).

4. Discussion

HBV core particles, like other icosahedral shells, are built from homodimers as basic structural subunits [7]. The dimers are stabilized by a hydrophobic protein core, which is highly conserved among human HBV variants. There are a few reports on the interaction of diverse core monomers derived from different hepadnaviruses or structurally changed by insertions of foreign epitopes. Using the *Xenopus* oocyte expression system it was shown that co-injection of HBV core transcripts with woodchuck hepatitis virus or ground squirrel hepatitis virus core transcripts leads to the formation of hybrid particles consisting preferentially of homodimers of core polypeptides from different hepadnaviruses [21]. The formation of mosaic particles, where one of the constituents is represented by a C-terminally modified variant of the initial parent monomer ('helper'), was recently shown for HBV cores [22] and for RNA phage Qβ capsids [23]. Such recombinant mosaic particles are proposed to be novel carriers for vaccine and gene therapy purposes [3,4]. However, it remained unclear whether such mosaic particles are composed of homodimers or/and heterodimers.

A broad spectrum of natural C gene deletion variants coexisting with the wt, was isolated from HBV-infected patients [8,10,11,13]. Under immunosuppression in renal transplant patients virus variants carrying in-frame deletions in the central region of the C gene seem to have a selective advantage and their accumulation correlates with the severity of liver disease ([8,9,11] and unpublished results). However, such C gene deletion variants have also been detected for non-immunosuppressed chronic carriers without an evident association with the outcome of the disease [10]. It has been documented that mutated core polypeptide variants can interfere with the wt virus in hepatoma-derived cell lines. Thus, C-terminally modified cores of HBV [24] and duck HBV [25–28] are able to inhibit viral replication and are suggested as potential antiviral agents.

Here, the possible interference of wt and deleted core polypeptides within the same cell was studied. The *E. coli* system was chosen for the expression of deleted C genes, since it ensures highly efficient synthesis [15,16] and correct folding and self-assembly [29,30] of recombinant cores. Moreover, some core mutants unable to assemble in human hepatoma cells revealed assembly competence in *E. coli* [31]. Short forms (aa 1–144) of both wt and deleted core proteins were included in the investigation, as C-terminal aa 145–183 are dispensable for self-assembly [32,33]. As we have shown previously for the #77/93 variant, a much better protein synthesis was obtained for expression of the short form [11] (Table 1).

An efficient two-vector system was established allowing the co-expression of wt and deleted core proteins in *E. coli* resulting in the formation of mosaic core particles at least in the case of the #77/93 deletion variant. The formation of mosaic particles was confirmed by immunoprecipitation using a monoclonal antibody recognizing the aa sequence 78–83 present in the wt polypeptide. Native agarose gel electrophoresis of purified particles was carried out to show whether one or different types of particles are formed during co-expression. The more diffuse bands in the case of mosaic particles in

comparison to wt particles indicates the formation of mosaic particles with varying ratios of wt and deleted polypeptides, rather than of heterogeneous mixtures of different types of exclusively wt, mutant or mosaic particles.

Moreover, to investigate the dimer content, particles were disrupted under non-reducing conditions. Besides homodimers formed between wt (long or short) and the #77/93-short polypeptides, there is also preliminary evidence for the presence of heterodimers in mosaic particles. It can be speculated, that the #77/93 polypeptide is rescued by wt 'helper' core from degradation or from segregation into insoluble fractions. The wt homodimeric form was detected exclusively only in the case of #77/93-long+wt-short. This may be due to the small content of the deletion variant within these particles. In addition, the oxidation of dimers containing long monomers leads to oligomerization due to the interactions of C-terminal Cys183 residues [5]. To avoid this, it will be necessary to replace the Cys183 by another amino acid residue.

One can speculate that the implication of the #77/93 deleted core polypeptide in the assembly process via interaction with the wt partner could contribute to liver destruction through intracellular accumulation of aberrant, cytopathic core particles, or by interference with substantial cellular proteins. However, for core mutants with longer deletions (#75/95, #82/111) such rescue phenomenon in the presence of wt polypeptides was not observed at least in *E. coli*. Obviously, this reflects the rather complex situation in liver pathogenesis of immunosuppressed renal transplant patients. Nevertheless, the generation of the two-vector expression system opens a way for more extensive investigations of the mechanisms involved in virus assembly.

Acknowledgements: We thank H. Gelderblom for performing the electron microscopy, Astrid Schories for excellent technical assistance and R. Ulrich, K. Leitmeyer and M. Shaw for critical reading of the manuscript. This work was supported by grants from the BMBF (project No. LET-00496 and No. 01KI9861/0), the European Community (IC15-CT98-0319) and by the Humboldt University Berlin.

References

[1] Bichko, V., Schödel, F., Nassal, M., Gren, E., Berzinsh, I., Borisova, G., Miska, S., Peterson, D.L. and Pushko, P. (1993) Mol. Immunol. 30, 221–231.
[2] Schödel, F., Peterson, D. and Milich, D. (1996) Intervirology 39, 104–110.
[3] Ulrich, R., Nassal, M., Meisel, H. and Krüger, D.H. (1998) Adv. Virus Res. 50, 141–182.
[4] Pumpens, P. and Grens, E. (1999) FEBS Lett. 442, 1–6.
[5] Nassal, M., Rieger, A. and Steinau, O. (1992) J. Mol. Biol. 225, 1013–1025.
[6] Zheng, J., Schödel, F. and Peterson, D.L. (1992) J. Biol. Chem. 267, 9422–9429.
[7] Wynne, S.A., Crowther, R.A. and Leslie, A.G. (1999) Mol. Cell 3, 771–780.
[8] Günther, S., Baginski, S., Kissel, H., Reinke, P., Krüger, D.H., Will, H. and Meisel, H. (1996) Hepatology 24, 751–758.
[9] Preikschat, P., Meisel, H., Will, H. and Günther, S. (1999) J. Gen. Virol. 80, 2685–2691.
[10] Marinos, G., Torre, F., Günther, S., Thomas, M.G., Will, H., Williams, R. and Naoumov, N.V. (1996) Gastroenterology 111, 183–192.
[11] Preikschat, P., Borisova, G., Borschukova, O., Dislers, A., Mezule, G., Grens, E., Krüger, D.H., Pumpens, P. and Meisel, H. (1999) J. Gen. Virol. 80, 1777–1788.
[12] Yuan, T.T., Lin, M.H., Chen, D.S. and Shih, C. (1998) J. Virol. 72, 578–584.
[13] Yuan, T.T., Lin, M.H., Qiu, S.M. and Shih, C. (1998) J. Virol. 72, 2168–2176.
[14] Günther, S., Li, B.C., Miska, S., Krüger, D.H., Meisel, H. and Will, H. (1995) J. Virol. 69, 5437–5444.
[15] Borisova, G.P., Pumpen, P.P., Bychko, V.V., Pushko, P.M., Kalis, J.V., Dishler, A.V., Gren, E.J., Tsibinogin, V.V. and Kukaine, R.A. (1984) Dokl. Akad. Nauk SSSR 279, 1245–1249.
[16] Borisova, G.P., Kalis, I.V., Pushko, P.M., Tsibinogin, V.V., Loseva, V.J., Ose, V.P., Stankevica, E.I., Dreimane, A.J., Snikere, D.J., Grinstein, E.E., Pumpen, P.P. and Gren, E.J. (1988) Dokl. Akad. Nauk SSSR 298, 1474–1478.
[17] Lämmli, U.K. (1970) Nature 227, 680–685.
[18] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[19] Gelderblom, H., Bauer, H., Frank, H. and Wigand, R. (1967) J. Gen. Virol. 1, 553–560.
[20] Skrivelis, V., Steinberg, Yu., Bichko, V., Gren, E. and Tsimanis, A. (1993) Scand. J. Immunol. 37, 637–643.
[21] Chang, C., Zhou, S., Ganem, D. and Standring, D.N. (1994) J. Virol. 68, 5225–5231.
[22] Koletzki, D., Zankl, A., Gelderblom, H.R., Meisel, H., Dislers, A., Borisova, G., Pumpens, P., Krüger, D.H. and Ulrich, R. (1997) J. Gen. Virol. 78, 2049–2053.
[23] Vasiljeva, I., Kozlovska, T., Cielens, I., Strelnikova, A., Kazaks, A., Ose, V. and Pumpens, P. (1998) FEBS Lett. 431, 7–11.
[24] Scaglioni, P.P., Melegari, M. and Wands, J.R. (1994) Virology 205, 112–120.
[25] von Weizsäcker, F., Wieland, S. and Blum, H.E. (1996) Hepatology 24, 294–299.
[26] Scaglioni, P., Melegari, M., Takahashi, M., Chowdhury, J.R. and Wands, J. (1996) Hepatology 24, 1010–1017.
[27] Köck, J., Wieland, S., Blum, H.E. and von Weizsäcker, F. (1998) J. Virol. 72, 9116–9120.
[28] von Weizsäcker, F., Köck, J., Wieland, S., Offensperger, W.B. and Blum, H.E. (1999) Hepatology 30, 308–315.
[29] Cohen, B.J. and Richmond, J.E. (1982) Nature 296, 677–679.
[30] Crowther, R.A., Kiselev, N.A., Böttcher, B., Berriman, J.A., Borisova, G.P., Ose, V. and Pumpens, P. (1994) Cell 77, 943–950.
[31] Koschel, M., Oed, D., Gerelsaikhan, T., Thomssen, R. and Bruss, V. (2000) J. Virol. 74, 1–7.
[32] Zlotnick, A., Cheng, N., Conway, J.F., Booy, F.P., Steven, A.C., Stahl, S.J. and Wingfield, P.T. (1996) Biochemistry 35, 7412–7421.
[33] Gallina, A., Bonelli, F., Zentilin, L., Rindi, G., Muttini, M. and Milanesi, G. (1989) J. Virol. 63, 4645–4652.