Proline-138 is essential for the assembly of hepatitis B virus core protein

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In small RNA viruses, arm-like segments located at the N or C termini have been suggested as mediators in the assembly of the capsid proteins. In many cases the arms of several subunits converge at a common point (the symmetry axis). Recent advances in studies of the hepatitis B virus (HBV) core protein attest the convergence of the segments preceding the protamine region, around the symmetry axis, where five or six HBc protein subunits converge. We report a mutation study of the region that we have suggested forms an arm-like structure, which reveals that a single mutation, Pro-138 → Gly, prevents the full-length HBV core protein self-assembling into particles.

In many viruses, extended conformations frequently found at the N terminus of the capsid protein are supposed to play an important role in the assembly of the viral particles (Harrison, 1992). In simian virus 40, for example, the capsid contains 72 pentamers of the VP1 protein. The pentamers are linked by the interaction of the C-terminal arms of the VP1 protein with five or six neighbouring pentamers (Liddington et al., 1991). These interactions supplant the absence of complementary interfaces between pentamers.

The human hepatitis B virus (HBV) has been shown to form particles of two different sizes, one major population of capsids containing 240 copies of HBc protein (T = 4 capsids, where T is the triangulation number) and a minor population of capsids containing 180 copies (T = 3 capsids) (Crowthers et al., 1994; Kenney et al., 1995; Zlotnick et al., 1996). In both cases, the capsids reveal tightly clustered dimers composed of a shell domain and a protruding spike. Two recent papers (Böttcher et al., 1997; Conway et al., 1997) have made an important contribution to the knowledge of the structure of the HBV capsid. It has been revealed that the previously reported protruding spike of the HBV core capsid consists of a four-helix bundle, as proposed in a model developed by one of us (Bringas, 1997). This model suggests that the region preceding the protamine domain of the HBc protein converges at the point where five or six HBc dimers come together (fivefold and sixfold symmetry axes). This prediction was demonstrated in the cryomicroscopy reports of Böttcher et al. (1997) and Conway et al. (1997). In both cases, an arm-like conformation is observed around the fivefold and sixfold symmetry axes.

Mutation studies of HBc variants by Birnbaum & Nassal (1990) have shown that the assembly capability of the HBV core protein resides in its first 140 to 144 amino acids and that the entire basic C-terminal domain is not necessary for assembly. HBc variants containing the first 139 or less residues are not able to assemble into particles, while variants containing 144 residues or more are still capable of assembling. Additionally, Zlotnick et al. (1996) found that the region between residues 140 and 147 is important in determining whether HBc protein will produce predominantly T = 4 or T = 3 capsids. These results imply that these residues are implicated in inter-subunit interaction.

The model of the HBc protein developed by Bringas (1997) proposes the existence of an arm-like structure in the region preceding the protamine domain. This region is abundant in proline residues. As it happens in some plant viruses, this arm would separate the protamine domain from the shell domain (Harrison et al., 1978; Abad-Zapatero et al., 1980; Hogle et al., 1986). In the HBV core protein, it has been suggested that this segment plays a key role in the virus assembly process, acting as hinge facilitating conformational changes that accompany nucleic acid and dimer packaging during particle assembly (Seifer & Standring, 1994). In order to test the possible role of prolines in such segments we present a mutational study of prolines preceding the protamine domain.

Vaccinia virus recombinants encoding the wild-type HBc and HBe proteins have been described by Schlütter & Schaller (1989). The other HBc mutants are based upon a construct that contains only the HBc gene sequences. Stop codons and Pro → Gly mutations were introduced by PCR mutagenesis.

Cultivation and infection of HepG2 cells were carried out as follows: HepG2 cells (Aden et al., 1979) (ca. 6 × 10⁶ cells
**Fig. 1.** The region preceding the protamine domain. The Pro → Gly exchanges were introduced at positions 134/135, 138 and 144 as indicated. Stop codons (indicated by stars) were introduced at positions 138, 140, 142, 144 and 146.

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129 PPAYRPPNAPILSTLPETTV150 HBC
↓↓ ↓  ↓  ↓
GG G  G
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**Fig. 2.** Analysis of core particle assembly. HepG2 cells were infected with the indicated recombinant vaccinia viruses encoding either wild-type core gene products, variants with stop codons at the indicated positions (a) or variants with Pro → Gly mutations (b). Particulate core proteins were isolated from cell lysates by a combination of sedimentation and CsCl equilibrium centrifugation. Fractions 3–14 were tested for particulate core gene products by using an HBc/e-specific diagnostic radioimmunoassay. Each chart shows results from one of the mutants (●), compared with wild-type HBc (■) and HBe proteins (▲). HBc and HBe proteins are used as positive and negative controls for the assembly, respectively.
Mutational analysis of Hbc assembly

The wild-type Hbc protein show a similar behaviour in SDS–PAGE under non-reducing conditions (data not shown). This means that all three mutants still form dimers and indicates that the Pro-138 → Gly mutation does not affect the dimer interface. In the partial sequence alignment shown in Fig. 3, Pro-138 is conserved in all the sequences, including isolates from the less-related duck hepatitis virus (DHBV), while prolines at positions 134, 135 and 144 are not. These results suggest that residues following position 139 are implicated in interactions with neighbouring dimers around the symmetry axes. Replacing Pro-138 by Gly results in cessation of these interactions. This implies that Pro-138 is essential for keeping an assembly competent conformation.

Despite the recent achievements in determination of the structure of the HBV capsid, there are still many unanswered questions related to its structure. The involvement of Pro-138 in the proposed arm-like structure will be corroborated only with the solution of the three dimensional structure at high resolution, but our proposal and the interpretation of results presented here are consistent with the current knowledge of virus capsid structures. The proposed arms may provide the switching mechanism for the assembly of the two sizes of particles (Bringas, 1997), and Pro-138 may play a crucial role in this mechanism. Additional mutations, e.g. replacing Pro-138 by residues less flexible than glycine, may provide assembly competent mutants that would yield preferentially T = 3 capsids as in the case of C-terminal truncated mutants (Zlotnick et al., 1996). The mutation of the proline residue in the core protein from avian isolates that is equivalent to Pro-138 may also provide additional information on the role of this conserved residue in hepadnaviruses.

Following submission of this manuscript, the X-ray structure of Hbc protein was presented by Dr Crowthers during the ‘Molecular Biology of Hepatitis B Virus’ Meeting held in the Institut Pasteur, Paris, France. The existence of the arm-like structure and its convergence around the fivefold and sixfold symmetry axes was definitively confirmed. Proline-138 is located immediately before the arms from adjacent subunits get into contact around the symmetry axes.
References


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