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Structure of Hepatitis B Surface Antigen from Subviral Tubes Determined by Electron Cryomicroscopy

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Hepatitis B virus consists of an icosahedral core containing the double-stranded DNA genome, enveloped by a membrane with embedded surface proteins. The crystal structure of the core protein has been solved but little information about the structure of the surface proteins has so far been available. There are three sizes of surface protein, small (S), medium (M) and large (L), which form disulfide-bonded homo- and heterodimers. The three proteins, expressed from different start sites in the coding sequence, share the common C-terminal S region; the M protein contains an additional preS2 sequence N-terminal to S, and the L protein a further preS1 sequence N-terminal to M. In infected individuals, the surface proteins are produced in huge excess over the amount needed for viral envelopment and are secreted as a heterogeneous mixture of isometric and tubular subviral particles. We have used electron cryomicroscopy to study tubular particles extracted from human serum. Helical Fourier–Bessel analysis was used to calculate a low-resolution map, although it showed that the tubes were quite disordered. From the symmetry derived from this analysis, we used single-particle methods to improve the resolution. We found that the tubes had a diameter of approximately 250 Å, with spike-like features projecting from the membrane. In the plane of the membrane the proteins appear to be close packed. We propose a model for the packing arrangement of surface protein dimers in the tubes.

Keywords: hepatitis B virus; surface proteins; tubular HBsAg; cryomicroscopy; helical image processing

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Introduction

Hepatitis B virus (HBV) is a major cause of human disease with an estimated 350 million carriers worldwide. Chronic infection can lead to liver damage and hepatocellular carcinoma. The virus consists of an icosahedral nucleocapsid or core containing the genome, surrounded by an envelope containing virally encoded surface proteins.[1] During virus assembly (for a review, see Bruss),[2] the core protein polymerizes around a complex of the viral polymerase and pre-genomic mRNA to form an immature core. The polymerase reverse-transcribes the RNA into partially double-stranded DNA, producing a mature core.[3,4] The mature core is then able to interact with the cytoplasmic parts of the surface proteins, already inserted into an inner cellular membrane. The core gains its envelope by budding through this membrane and is secreted as an enveloped virion.

There are three sizes of surface protein, called large (L), medium (M) or small (S) (for a review, see Bruss).[2] The three proteins share the common C-terminal S domain and the M and L proteins have an N-terminal preS2 or preS1 + preS2 extension respectively, giving proteins of 389 or 400 (L, depending on genotype), 281 (M) and 226 (S) amino acids. The surface proteins are variably glycosylated and the L protein is N-terminally myristylated. The S and L proteins are essential for virus morphogenesis and

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Abbreviations used: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; IHRSR, iterative helical real space refinement; TBS, Tris-buffered saline.

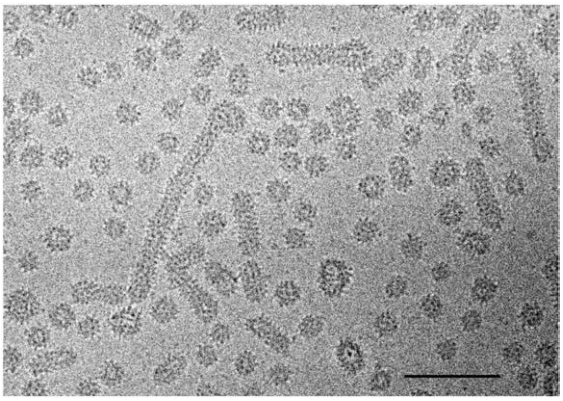

Fig. 1. Cryomicrograph of an HBsAg preparation showing a variety of isometric and tubular particles. The scale bar represents 1000 Å.

for the infectivity of the assembled virions but M is dispensable.[5] During morphogenesis, the preS region of the L protein adopts a mixed topology, with some molecules having the preS region internal (i-preS), on the cytoplasmic/core facing side of the membrane, and others with it external (e-preS), on the luminal/outer surface of the membrane.[6–8] In the e-preS configuration, the preS region is exposed on the surface of the virus to interact with the cellular receptor, whereas in the i-preS configuration the preS region can interact with the core.

In infected individuals, the surface proteins, referred to as hepatitis B surface antigen (HBsAg), are produced and secreted in huge excess over virions, such that in serum there are ~10,000-fold more HBsAg particles than virions. The morphology of HBsAg is extremely heterogeneous with roughly spherical particles of about 22 nm diameter or larger and tubular particles about 22 nm wide and of variable length. Given the heterogeneity in size, glycosylation and topology of the surface proteins, it is not surprising that the assembled particles are so variable. Biochemical studies indicate that the surface proteins in HBsAg particles form disulfide-linked homo- and heterodimers.[9] HBsAg particles extracted from the plasma of carriers contain about 25% of lipid by weight.[10] The tubular HBsAg structures are estimated to contain L, M and S proteins in the approximate ratio 1:1:4.[11,12]

A detailed structure is available for the bacterially expressed core protein[13] and a lower-resolution structure for the DNA-containing mature core[4] but rather little is known about the structure of the surface proteins. Sequence analysis and modelling have indicated that the S protein is likely to have four transmembrane spanning helices.[14] The N- and C-termini of the S protein are on the outside of the membrane, as are those of the M protein and of the e-preS form of the L protein. Unlike the core, the surface proteins are not icosahedrally organized in virions, so studies of whole virions have not been particularly informative about the structure of the surface proteins.[15,16] Analysis by cryomicroscopy of spherical 22-nm HBsAg particles made of S protein expressed in a transgenic mouse line has suggested that they have octahedral symmetry, with two sizes of particle showing different kinds of dimer packing of the S protein.[17]

Here we have used electron cryomicroscopy to analyse the structure of HBsAg tubes that were extracted from infected human blood donations. Initial analysis with conventional helical methods allowed the helical parameters to be determined but showed that the symmetry was variable and that the tubes were not well ordered. The maps were therefore improved by using iterative helical real space refinement (IHRSR).[18] The maps display a regular series of spikes on the outside of the tube with an array of close-packed protein domains in the membrane region of the tube. The volumes of the various regions and the area occupied in the membrane indicate that the packing unit is likely to be a tetramer of the surface proteins.

Results and Discussion

A micrograph of the surface antigen preparation (Fig. 1) demonstrates the heterogeneity of the sample, with isometric particles of various sizes and tubular or filamentous particles of various lengths. The filaments themselves were disordered and heterogeneous from tube to tube. Furthermore, Fourier transforms from different parts of a single tube sometimes showed different helical symmetry. The helical pitch showed a large variation, as indicated by the averaged power spectrum (Fig. 2), and stacked discs were also observed. Where it was possible to index the Fourier transforms, we deduced that different families of tube are formed as one-, two- and three-start helices with an average of about 10 subunits per turn and a pitch varying from 100 to 500 Å (Fig. 3). In general, only three layer

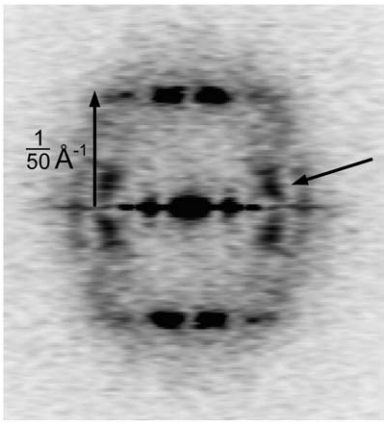

Fig. 2. Averaged power spectrum from 114 HBsAg tubes. The wide variation between tubes in the pitch of the longer-pitch helices, spanning a range of 100–500 Å, is indicated by the smear of intensities near the equator (arrow). The short-pitch helices, corresponding to the intensities near the meridian at a spacing of about 50 Å, are more sharply defined.