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Report:

Hepatitis B is human pathogen of major importance. There are estimated to be 300 million carriers worldwide. Chronic infection usually leads to cirrhosis and cancer of the liver. Hepatitis B virus is a small enveloped DNA virus. The protein capsid is made up of a single particle (HBcAg). When expressed in E. coli, the protein assembles into shells like those in native virions. Cryoelectron microscopy has been used to study these viral capsids, and has shown that there are two sizes of particle, one with 180 copies of HBcAg arranged with T=3 icosahedral symmetry and the other with 240 copies and T=4 symmetry (Crowther et al.). We wish to determine the atomic structure of one or both of these viral shells, as part of a more general program aimed at understanding the molecular biology of the whole virus. This in turn may provide a basis for the design of therapeutic agents that block viral assembly.

We have grown crystals of the T=4 protein capsids using material expressed in *E.coli*. Depending on the exact crystallisation conditions, some crystals are triclinic (cell 285x337x396Å, α=91, β=90, γ=93) while others are monoclinic (P21, cell 293x341x387Å, β=95), or when grown in the presence of cryoprotectants the spacegroup is C2, cell 538x353x370Å, β=132.3º. Diffraction to 7.5Å resolution is observed using a laboratory source.

Initial diffraction experiments at the Daresbury synchrotron indicated that the unfrozen crystals diffract to at least 3.5Å resolution. However, these crystals decayed rapidly in the X-ray beam.

Diffraction from the unfrozen crystals at BL4 was significantly worse than the diffraction observed at the SRS, Daresbury. Most crystals only diffracted to 8Å resolution, while the best diffracted to 5Å. In addition, the crystal lifetime was much shorter, allowing only a single (10 second) exposure per crystal while several exposures per crystal had been possible at the SRS. It is possible that temperature or pressure changes during transport to Grenoble (by air in hanging drop trays) may have produced the deterioration in crystal quality.

However crystals grown in the presence of the cryoprotectant butane diol and flash frozen at 100K in the N2 gas stream diffracted to between 3.6Å and 3.9Å resolution. It was possible to collect a complete dataset to a nominal resolution of 3.6Å from four crystals, using up to 3 positions per crystal. The overall Rmerge was 16.2% (60% at 3.6Å) and the mean $F/\sigma(F)$ was 11.6 (4 at 3.6Å). There was significant radiation damage, and translating the crystals did not restore the original quality of the diffraction, even if the two positions were separated by several times the size of the incident beam. However this level of radiation damage was tolerated in order to be able to collect a complete dataset within the time available. A low resolution (8Å) dataset was subsequently collected in house in order to improve the completeness of the synchrotron dataset at low resolution. The final dataset was 96% complete to 3.6Å.

The 7.5Å resolution structure determined by cryo electron-microscopy (Bottcher et al) was used as an initial phasing model, after determining the particle orientation from a self-rotation function. This model gave an excellent fit (R-factor 22%) to the 7.581 resolution X-ray data. There is one half of the virus capsid in the crystallographic asymmetric subunit, and the 30-fold non-crystallographic symmetry was used to extend the phasing to 3.6Å. An atomic model for the four icosahedrally independent subunits has been built into the resulting electron density map. This has largely confirmed the protein fold proposed on the basis of the 7.5Å cryo electron-microscopy. The path of the polypeptide backbone is unambigous, although there are a few regions where the exact main chain conformation is not clear.

References

B. Bottcher, S.A.Wynne and R.A. Crowther (1997) Determination of the fold of the core protein of hepatitis B virus by electron cryomicroscopy. Nature 386, 88-91.

R.A. Crowther, N.A. Kiselev, B. Böttcher, J.A. Berriman, G.P. Borisova, V. Ose and P. Pumpens. (1994) Three-dimensional structure of hepatitis B virus core particles determined by electron microscopy. Cell, 77, 943-950.