EM analysis of expression of recombinant Hepatitis B virus core antigen in E. coli and assembly to core particles

K. Himmelsbach, S. Schille, S. Akhras, R. Eberle, E. Hildt and K. Boller
Paul-Ehrlich-Institut, Paul-Ehrlich-Str. 51 – 59, 63225 Langen, Germany

Klaus.Boller@pei.de
Hepatitis B virus, virus assembly, immunoelectron microscopy

Introduction
Hepatitis B Virus (HBV) is a major cause of liver diseases including cirrhosis and cancer. About one million people die of the consequences of an HBV infection each year. We study the HBV life cycle in order to obtain closer insight into disease development and to improve vaccination strategies.

HBV cores represent a well-characterized capsid like particle (CLP) model system. These capsids are built of dimers of HBV core antigen (HbcAg) that assemble spontaneously to viral cores. For induction of a robust T-cell response to HBV core antigen, a prerequisite for a working vaccine, cell permeable CLPs were designed, that enter the cytoplasm to enable direct degradation and subsequent presentation by MHC class I molecules. To construct such CLPs, HBV core protein was fused with a protein transduction domain, the TLM (translocation motive) and expressed in E. coli. Capsids assembled from this recombinant protein translocate easily through membranes by a receptor- and energy-independent process [1].

Here, we study the expression of recombinant HbcAg and formation of CLPs by transformed bacteria with electron microscopical methods. For immunogold labeling, two different antibodies were used: A rabbit antiserum to HbcAg that reacts with soluble HbcAg monomeric protein but not with core particles, and a monoclonal antibody 3120 that binds exclusively to assembled capsids [2]. We used these two antibodies to monitor expression and assembly of core particles inside or outside the bacterial cell, and to survey purification and enrichment of capsid particles.

Methods
For embedding in Epon, bacterial cells were fixed in 2.5 % glutaraldehyde, dehydrated and embedded in Epon according to standard methods. 70 nm sections were cut and stained with 2% uranylacetate and 1 % lead citrate. Immunogold labelling was performed on ultrathin frozen sections according to Tokuyasu [3] or on ultrathin sections of cells after embedding at low temperature (PLT) in Lowicryl K4M [4]. For negative staining, capsid preparations were adsorbed to carbon coated formvar grids and stained with 2% uranylacetate.

Results
After induction, bacterial cells develop intracellular structures resembling inclusion bodies. Capsid like particles cannot be observed. These inclusion bodies are labelled with rabbit antiserum to HbcAg. Besides labelling of these structures, the anti HbcAg antiserum binds to the membrane and to the cytoplasmic part of the cell periphery. In contrast, monoclonal antibody 3120, that has been shown to bind exclusively to assembled particles, binds to a relatively small area inside the cell. This observation can be confirmed by immunogold double labelling on frozen sections as well as on Lowicryl sections. Interestingly, also in areas were Mab 3120 binds, no capsid structures are visible. After disruption of the cells and density centrifugation, HBV capsids of the typical size of 30-34 nm can be observed by negative staining. These observations may be helpful to improve conditions for capsid formation and to obtain high yields of capsids for vaccination development.

References
Figure 1. Immunogold labelling of ultrathin frozen sections of E. coli. transfected with HBV core protein A) 4 h after induction of protein expression, labelled with antibody anti HBcAg Ab1 which recognizes the soluble protein. B) same as A, but labelled with monoclonal antibody 3120, which binds exclusively to assembled core particles. C) double labelling with both antibodies. Rabbit antiserum to HBcAg was detected with anti rabbit IgG coupled to 10 nm gold, whereas Mab 3120 was revealed with 5 nm gold coupled anti mouse IgG. Arrows point to areas where Mab 3120 localizes HBcAg, no assembled capsids are visible. D) control of specificity: Double labelling as in C, but before induction of bacteria.