UNDERSTANDING THE MECHANISMS INVOLVED ON HEPATITIS C VIRUS ASSEMBLY

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Hepatitis C is a worldwide public health problem since more than 3% of the population is infected by Hepatitis C virus (HCV) and current therapies are inefficient. The HCV core protein (HCVCP) has been described as an important target because it is essential for viral assembly, besides to be involved in viral and cellular processes. This work aims a better understanding of the mechanisms of HCV assembly, providing additional information to the development of more efficient antiviral drugs. Here we express the C-terminal truncated HCVCP and its GFP (green fluorescent protein) fused form in order to investigate the *in vitro* assembly in the presence of a nonspecific poly(GC) DNA and a consensus p53 DNA using electron microscopy, fluorescence polarization, spectrophotometry, calorimetry and gel shift assay. The formation of nucleocapsid-like particles (NLPs) was observed by electron microscopy for both forms in the absence and in the presence of both DNA sequences. Our data also show that the formation of NLPs was dependent on protein and DNA concentrations, as measured by spectrophotometry and fluorescence polarization. Isothermal titration calorimetry (ITC) data have shown that the poly(GC) DNA-protein and protein-protein interactions are enthalpically driven. Gel shift assays show that no assembly intermediate form was observed for HCVCP, indicating a cooperative process. Chromatography analysis show that NLPs are unstable since it disassembles after dilution. In conclusion, our results show that a nonspecific DNA sequence is able to trigger the formation of NLPs *in vitro*, the assembly process is highly cooperative, enthalpically favored, dependent on the protein and DNA concentration, and that it is not altered by the presence of GFP.
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