Cloning, Expression and Biological Characterization of a Recombinant Multiepitope for Hepatitis C Diagnosis


Introduction: Hepatitis C virus (HCV) has emerged as the major pathogen of liver diseases in recent years leading worldwide blood–transmitted chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Accurate diagnosis for differentiation of hepatitis C from others virus is thus of pivotal importance for proper treatment. Material and Methods: In this work, a recombinant multiepitope protein (rMEHCV) was developed in order to for hepatitis C diagnostic. It was possible based on conserved and immunodominant epitopes from core, NS3, NS4A, NS4B, and NS5 regions of the virus polyprotein of genotypes 1a, 1b and 3a - the most prevalent genotypes in South America (especially in Brazil). Results and Discussion A synthetic gene was designed to encode eight epitopes in tandem separated by a flexible linker and bearing a his-tag at the C-terminal. The recombinant protein was produced in Escherichia coli and purified in a single affinity chromatographic step with > 95% purity. Purified rMEHCV was used to perform an ELISA assay which showed that the recombinant protein was recognized by IgG and IgM from human serum samples. Conclusions: In this work the structural stability of rMEHCV was investigated in neutral and alkaline conditions by circular dichroism (CD) spectroscopy. The careful choice of epitopes (genotypes and subtypes), the high epitope density coupled with simple purification offer a promising alternative option for hepatitis C diagnosis, with potential for development of an inexpensive diagnostic test with high degree of sensitivity and specificity.

Keywords: Cloning, Multiepitope, Hepatitis C, Diagnosis.
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