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Hepatitis C Virus Core Protein Expression for Enzyme Linked Immunosorbant Assay

Khalid Pervez
Department of Biochemistry, University of Agriculture, Faisalabad-3040, Pakistan

Abstract: HCV core region, being most conserved, in the whole genome can be used as a diagnostic marker in enzyme linked immuno-sorbant assay development. However, quasi species nature of HCV during its replication in host is the major hindrance in developing an effective therapeutic agent. Recombinant fusion protein for HCV immuno-assay from core region was prepared by PCR amplification and subsequent cloning in expression vector P^{ET32a} which was expressed by iso-propyl thio-B-galactosides (IPTG) in *E. coli* strain D^{E3} followed by purification by affinity chromatography. Fusion protein as an antigen reacted with human antisera containing antibodies. This HCV specific immuno-assay development based on recombinant HCV core protein will be the foundation in developing antiviral immuno-assays in Pakistan.

Key words: HCV, ELISA, fusion protein

Introduction

Transfusion associated viral hepatitis is mostly caused by HCV (Choo *et al*., 1989) which leads to chronic hepatitis in 50-80% infected patients followed by hepato cellular carcinoma (HCC) in 19% and liver cirrhosis in 46% of patients (Saito *et al.,* 1990) Patients involved in transfusion of blood, Haemophiliacs Exposed to untreated clotting factor concentrates, Intravenous Drug Users (IVDU), needle stick exposure in health care workers, and heterosexuals are more prone-to HCV infection. '5 untranslated region ('5 UTR) of HCV is the most conserved region of the whole genome and can be used as diagnostic marker for HCV by polymerase chain reaction (PCR). '5 end encodes core© and envelope (E_1 and E_2) structural glycoproteins. E_1 and E_2 viral glycoprotein complex is a functional subunit of HCV virion envelope (Dubuisson and Rice, 1996). Hyper variable region 1 and 2 (HVR 1 and HVR 2) of E2 during replication under goes mutation and virus escapes from immune response by the host. HCV core protein possess carboxyl terminal hydrophobic regions in association with endoplasmic reticulum (EP) containing enzymes for peptide processing and glycosylation. HCV core suppresses host genes transcription and interferes expression of co-infecting genomes of hepatitis 13 virus (HBV) and human immuno deficiency virus (HIV) (Srinivas *et al.,* 1996) and act as gene regulatory protein in virus-replication and pathogenesis. Cellular immune response mediated by cytotoxic T lymphocytes (CTL) is Important in virus clearance from infected host (Riddell *et al.,* 1992)

Materials and Methods

RNA extraction was done according to the recommendations of Petrelli *et al*. (1994). DNA was synthesized as per the protocol of Tisminetzky *et al*. (1994). HCV core sequence was amplified from recombinant plasmid contains '5 UTR core, E2, and NS_2 sequences of HCV^{-1} genome. P^{RSET-a} is an expression vector. Both vector and insert (Core) were digested with BamH1 and Pst1. HCV core was ligated into P^{RSET-a} with T4 DNA Haase. Ligation mixture was transformed into E-Coli competent cells as per Sambrook *et al.* (1989). Recombinants were analysed by restriction digestion analysis. This expression vector was restricted with Ball 1 and then by Hind III. Core was lifted from vector pHCV CORE (HIS) A with Bgl-II and Hind III and ligated in pRSET-B. Half of the ligation mixture was transformed into *E. coli.* Recombinants were analysed with Bam H1 and Hind III for new construct pHCV Core HIS-B.

P^{ET32a} is an other expression vector containing T7 promotor. Sub-cloned by HCV core. Both were digested with BamHI and Hind III. Recombinants were analysed by restriction digestion with BamHI/Hind III in *E. coil* for the presence of pEHCV core. P^{ET32a} vector system has the advantage of carrying HIS tag and Provides efficient system for purification of recombinant proteins through affinity chromatography. Safford test is widely used for quantitative analysis of protein concentration which was measured spectroscopically at 595 nm wave length. Purified HCV core protein was used as antigen to react with anti-serum of HCV-infected-patients. Agglutination reaction was observed under microscope indicating successful production of fusion protein.

Results

412 by product was amplified from recombinant vector by using primers which were specific for HCV^{-1} core sequence. $P^{HCV\text{-}CORE(HIS)A}$ was constructed by directional cloning into $P^{RES\text{-}A}$ at the restriction sites Bam H1 and PST1. HCV-Core was then sub-cloned into $P^{RSET\text{-}B}$ by using same enzymes at restriction sites Bgl II and Hind III. Sub-cloning was confirmed by recombinant plasmid restriction with Bam H1 and Hind III which released the insert of desired size. Again HCV core subcloned in to P^{ET32a}. This $P^{HCV\text{-}CORE}$ was confirmed by restriction digestion with Barn H1/ Hind III for the presence of desired fragment. HCV core fusion protein was expressed in *E. coli* strain. Fusion protein expression in SOS gel after 1PTG induction indicated the appearance of 334 KDa protein fragment from HCV core recombinants. Highest expression was observed after 2.5 HRS of induction. HCV core protein was purified, through affinity chromatography, by resins prepared for histidine binding. Protein conc. of HCV core was determined through barford assay. HCV core protein as antigen and human-antisera were used giving agglutination. This reaction was decreased with increasing antiserum dilution.

Discussion

Remarkable degree of heterogeneity of HCV frequent mutations leads to quasi-species and escape mutants has made difficult for a vaccine to develop (Weiner *et al.,* 1992). Blood screening for HCV infection can leads to reduction in transfusion associated hepatitis which is the main cause of its spread. Presently, ELISA and PCR tests are used for HCV detection but serological assays based on molecular cloning and expression of viral genes for the production of recombinant proteins are

preferentially, used. We used most conserved HCV core region for recombinant protein production for HCV antibody assay to be used for diagnosis of HCV infection. HCV core region was amplified and cloned in P^{RSET-A} and then subcloned in P^{RSET-B} and finally subcloned in P^{ET32a}. Resulting clone $P^{EHCV\ CORE}$ was capable of expressing fusion protein with histidine tag. This construct was 1st transferred in non expressing host having no T7 RNA polymerase gene which is required for inducing T7 promotor. So they were virtually "off" and can't cause plasmid instability due to the production of proteins toxic to the host cell. Then plasmid was transferred in *E. coli* expression host where the cloned HCV core was expressed. SOS-PAGE analysis showed that expressed protein was 33.4 kDa while actual protein was 15.1 kDa. This increase in size was due to the addition of His tag for the production of fusion protein. Agglutination reaction between recombinant fusion protein and antisera confirmed the presence of antigenic determinants in recombinant HCV core protein. In future series of immunological assays can be constructed by using the same strategy and will help in reducing HCV prevalence through blood transfusion by HCV screening of blood donors.

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