EXPRESSION OF HEPATITIS B VIRUS POLYMERASE GENE IN E. coli.

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SUMMARY

The polymerase gene of the hepatitis B virus was fused with the E. coli Maltose Binding Protein (MBP) and expressed as a fusion protein. Reverse transcriptase activity was detected in purified P protein with the assay conditions of 50mM Mg concentration at 30°C yielding the best results.

Introduction

Hepatitis B virus (HBV) infection has been a big threat to human health, which causes acute and chronic hepatitis and is strongly associated with the development of hepatocellular carcinoma (Tiollais et al., 1985). HBV is characterized by containing a DNA genome of 3.2kb which is circular but not covalently closed and only partially double stranded (Summers et al., 1975). Within the genome, there are four open reading frames (ORF): presurface/surface gene, a core antigen, polymerase, and the X protein (Ganem and Varmas, 1987). The ORF of the P gene occupies 80% of the whole genome and codes for 845 amino acids.

Polymerase activity appears to be present within the virus particle (Galibert et al., 1979). Through the replication of Duck Hepatitis B virus (DHBV), which has a similar genome structure of human HBV, it was discovered that HBV carries out reverse transcription having RNA as an intermediate (Summers and Mason, 1982, Miller et al., 1984). By comparing the amino acids of the P ORF of human HBV and that of the reverse transcriptase present in Rous sarcoma virus and Moloney Murine leukemia virus, it was discovered that they are partially conserved (Toh et al., 1983).

The evidence in support of the reverse transcriptase activity of the P ORF demonstrated that by changing one of the amino acid sequences of the Pol domain and RNaseH domain and placed it in the DHBV P ORF carboxyl terminal, viral DNA synthesis decreased dramatically (Chang et al., 1990). Furthermore, based on the result obtained from the activity gel analysis of the HBV particle, which was secreted from a HBV transfected cell line, reverse transcriptase activity was present (Bavand and Laub, 1988).
Polymerase is present in very small amounts. To overcome this problem, a phosphorylation site was installed within the P ORF and expressed through a vaccinia virus expression system. By making of the phosphorylation site radioactive it could be detected (Bartenschlager et al., 1992). This method can sensitively detect any presence of the P ORF product, however, it has disadvantage as the enzymatic activity of the P ORF product is lost. Despite the many assumptions made of the reverse transcriptase activity of the HBV P ORF, direct enzymatic activity from the P ORF product has not yet been confirmed. In order to demonstrate that the P ORF has polymerase activity as a reverse transcriptase, whole P ORF and MBP are fused in E. coli. P protein which was expressed here is used to discover the presence of reverse transcriptase activity of the human HBV. This result is to contribute to the screening of HBV DNA replication inhibitor.

MATERIALS AND METHODS

Strains. E. coli NM522 (F' lacI "(lacZ)M5 pro AB/supE thiA (lac-proAB)A (hsdR MS-acl B) 5 (r~ m~ VetB~)) was used for the manipulation and amplification of the plasmid. E. coli Y1090 (ΔlacU169, proA+, Δlon, araD139, strA, supF[papC 22::Tn 10(pMW9)]) were used as the host for the expression of Hepatitis B Virus P gene.

Plasmids. Plasmid pMAL-c2 was the fusion protein expression vector which expresses fusion proteins from male to lacZ by using the tac promoter.

Culture medium. The culture medium was a glucose rich media (trypton 10g/L, yeast ext. 5g/L, NaCl 5g/L).

Sequencing. To confirm the recombination plasmid sequence, sequenase was used for sequencing (Sanger, 1977).

Separation of MBP and fused HBV P protein. E. coli Y1090, in which pMPLX has been transformed, was cultured overnight. It was diluted by a ratio of 1/100 in the glucose rich media of 500ml and inoculated. OD600 0.5, IPTG was added to the final concentration of 0.3mM and cultured for 12hrs at 23°C. All purification steps were carried out at 4°C. Cell lysate prepared from cells growing at the stationary growth phase were resuspended in column buffer (10mM Tris-cl, 200mM NaCl, 1mM EDTA, pH 8.0) and were disrupted by freezing and thawing and sonic treatment. After the centrifugation (12000G) of the cell lysate for 15 minutes at 4°C, supernatant flowed to an amylose resin column. To elute the purified protein, 10mM maltose buffer flowed to the amylose resin column. By adding the protease factor Xa to the purified protein, HBV P protein was separated from the MBP.

Electrophoresis and Immunoblot. The molecular weight of purified protein was conducted by SDS-PAGE electrophoresis according to the Laemmli method (Laemmli, 1970). Measurement of the protein was carried out by the Lowry method (Lowry, 1951). Plasmid pMAL-c2 contains the fusion protein MBP, so the antibody of this protein was used in the western blot process.

Enzyme activity assay. HBV P protein, which is separated from the cell lysate, was mixed in a reverse transcriptase reaction buffer (50mM Tris-HCl, pH 8.3, 50mM KCl, 5mM DTT, 0.1% NP40, 10μg/ml poly rA/oligo dT18), at different temperatures and Mg concentrations, in a total volume of 100μL. After terminating the reaction by immersing the samples in boiling water for 1 min, the samples were processed on a DE-81 disk filter by washing three times in 5% Na2HPO4 solution and radioactivity incorporated determined by liquid scintillation counting.