COMMUNICATION

HEPATITIS B VIRUS PRECORE PROTEIN MODELING: AN EXPERIENCE FROM NEW DELHI, INDIA

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ABSTRACT

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Hepatitis B virus (HBV) genome infecting hepatocellular carcinoma (HCC) cases with special emphasis on mutation has been widely studied across the globe. However there is a paucity of information in proteomic level. The current study was aimed to analyze the structure and chemical composition of a core protein of HBV found in a HCC patient from New Delhi using bioinformatics software's. An HCC case was diagnosed as per EASL 2001, guidelines and viral DNA was isolated followed by amplification of the core gene. The amplicon was sequenced commercially from Macrogen Korea and the nucleotide sequence was converted to amino acids using DNA 2.0. The protein sequence was than analysed using RaptorX for its structure and chemical composition. A total of 90 hydrogen bonds, 8 helices and 4 turns were found in this current protein model. However there were no strands in the model. Stable Ramachandran map was viewed. Highest numbers of Leucine were observed in the proposed structure. A stable protein model is successfully proposed in this study.

Keywords: HBV, chronic liver disease, precore protein, modeling, Ramachandran plot

INTRODUCTION

Hepatitis B is a global health problem. In spite of the availability of a successful vaccine, more than 350 million people are infected with HBV worldwide. Chronic hepatitis B (CHB) is the 10th leading cause of mortality worldwide, with more than 1 million deaths annually attributed to CHB-associated complications such as liver cirrhosis and hepatocellular carcinoma (HCC) (Brunetto *et al*., 1993; Grandjacques *et al*., 2000). Hepatitis B virus (HBV) infection is the major risk factor associated with the development of HCC in regions with large populations like China, India and southern Asia because of the high endemicity of the virus in these places (Gasteiger *et al*., 2005). The HBV genome is comprised of 4 gene transcribing 4 different proteins each having a specific role. However, precore gene has been widely studied partly because of the high mutation rate in this region and also confirming the virus genotype. Mutations in the precore (pre-C) gene are more frequent in patients with persistent viremia and severe disease (Kane, 1996). Again the study of precore protein is important as it is associated with many important functions in the viral replication cycle

including RNA packaging and DNA synthesis. Few commonly documented mutations in pore core region are precore stop codon mutation (Lavanchy, 2004), T → V at codon 120 (Lee, 1996), 159 Pro → Thr: 13 Ser → Thr (Perz *et al*., 2006) and A1762T/G1764A double mutation (Yuan *et al*., 2007).

A lot of information in the genomic level of HBV has been generated in the last two decades by extensive research and using advanced technology across the globe as well as in India. However, proteomic and protein modeling data on HBV is scanty especially from India. With this in mind an attempt has been made through this research work to provide some light to a protein model and other associated parameters from a protein sequence submitted to Genebank (protein id: AFD32897) by us. The current finding is an extension of a larger study on whole genome amplification of HBV virus isolated from HCC/chronic hepatitis B cases from three different geographical locations of India (a total of 270 sequences from 75 HCC+ chronic hepatitis B cases).

METHODOLOGY

Enrollment of cases

A case of chronic hepatitis B was enrolled from the OPD of Lok Nayak Hospital, New Delhi, India.

Ethics committee approval

The study was approved by the ethical committee board of Lok Nayak Hospital, New Delhi, India and confronted to the ethical committee guidelines of EASL Helsinki 1975. The study subjects provided written informed consent prior to its enrollment in the study.

Diagnostic criteria

The patient was diagnosed as per AASLD guidelines provided by Lok *et al*., 2001.

DNA extraction/ amplification and sequencing

Viral DNA was isolated using phenol chloroform method and the precore gene was amplified using specific primers under standardized protocol. Nucleotide sequencing was carried out from Macrogen Korea (ABI prism).

Nucleotide sequence was submitted to gene bank (BankIt1600217 (55)) and was then translated into amino acid and aligned using BioEdit v7.0.9.

Bioinformatics software used

The protein sequence thus obtained (protein id: AFD32897) was punched for structure modeling with the help of RaptorX for 3D structure of the protein. Other parameters of the proposed model were evaluated using ProtParam (Yuasa *et al*., 2000) and RasMol.

RESULTS

The protein model designed (Figure 1) in this research work was found with the chemical formulae $C_{626}H_{958}N_{162}O_{181}S_6$ with 1933 atoms and 121 amino acids. The molecular

weight of the protein was found to be 13841.8 while the theoretical pI was calculated to be 5.00.

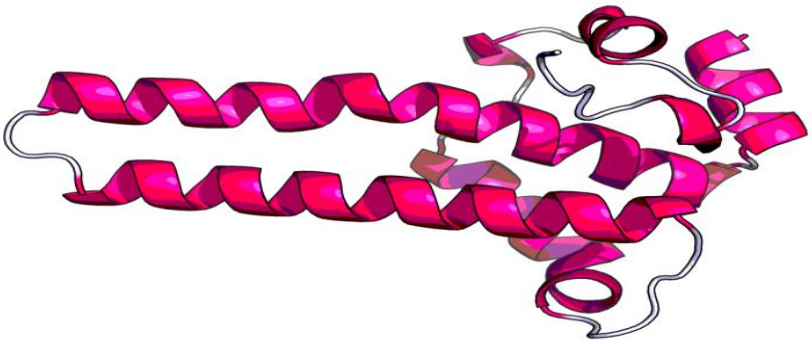

Figure 1. Modeling of HBV precore protein isolated from chronic liver disease patient from New Delhi (protein id: AFD32897).

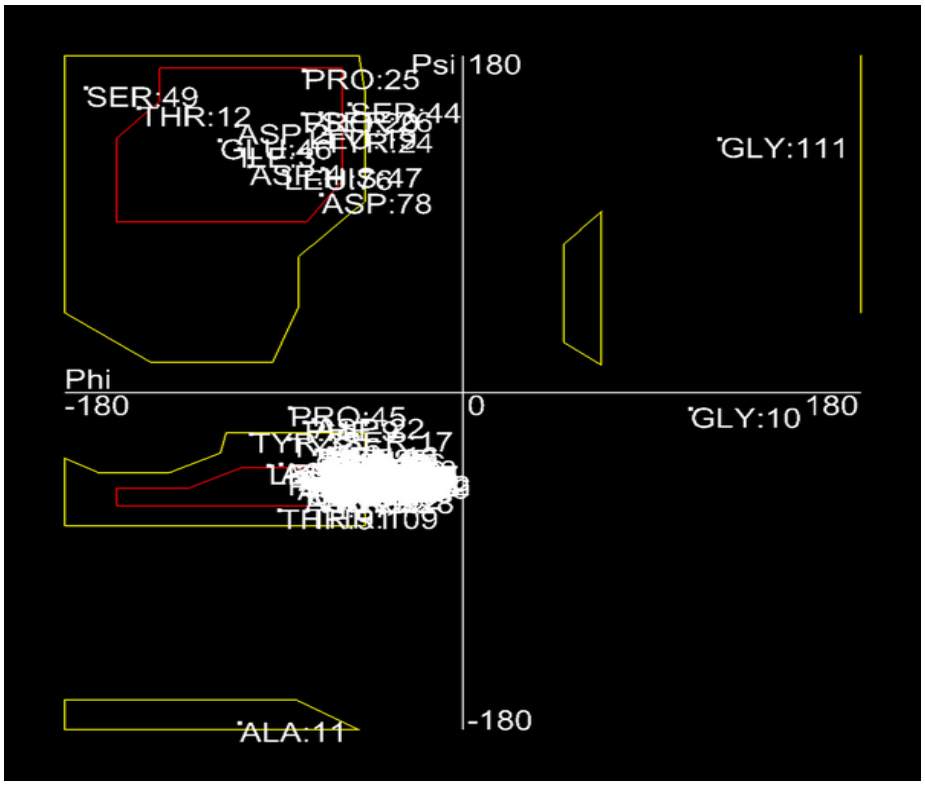

Figure 2. Ramachandran plot for the proposed model of protein.

A total of 90 hydrogen bonds, 8 helices and 4 turns were found in this current protein model. However there were no strands in the model (Table 1). The chemical composition of the amino acids revealed the highest number of Leucine (15.7%) followed by glutamate and alanine (6.6% each) (Table 2). Ramachandran plot was analysed to analyze the stability of the proposed protein model and it was found that all values were within the permissible levels favoring the formation of a stable protein molecule.

TABLE 1

Chemistry of the Proposed Protein Model

Parameters	Numbers
No. of H – Bonds	90
No. of Helices-	08
No. of Strands	0
No. of Turns	4
Atomic composition:	
Carbon C	626
Hydrogen H	958
Nitrogen N	162
Oxygen O	181
Sulfur S	6

TABLE 2

Amino Acid Composition of the Proposed Protein Model

Amino acids present	No. of amino acid	Percentage of amino acids
Ala (A)	8	**6.6%**
Arg I	6	5.0%
Asn (N)	5	4.1%
Asp (D)	7	5.8%
Cys I	3	2.5%
Gln (Q)	3	2.5%
Glu I	8	**6.6%**
Gly (G)	5	4.1%
His (H)	5	4.1%
Ile (I)	4	3.3%
Leu (L)	19	**15.7%**
Lys (K)	2	1.7%
Met (M)	3	2.5%
Phe (F)	5	4.1%

Continued:

Pro (P)	6	5.0%
Trp (W)	3	2.5%
Tyr (Y)	5	4.1%
Val (V)	8	6.6%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%
(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

CONCLUSION

Looking at the stability factors of the proposed model, it can be said that stable precore protein has been rightly proposed in this study. The same needs to be submitted to protein data bank and a database for HBV a viral protein needs to be framed using the huge genomic information achieved by direct and commercial nucleotide sequencing.

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