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# N-Terminal Amino Acid Sequences of Receptor-Like Proteins that Bind to preS1 of HBV in HepG2 Cells

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**Abstract:** One of the essential functions of virus surface proteins is the recognition of specific receptors on target cell membranes, and cellular receptors play an important role in viral pathogenesis. But the earliest steps of hepatitis B virus (HBV) infection, such as hepatocyte receptor interaction with the virus, are poorly understood. Previous work has suggested an important role of the preS1 region of HBV envelope protein in mediating viral binding to hepatocytes. Although hepatitis B virus (HBV) infection appears to be initiated by specific binding of virions to cell membrane structures via one or potentially several viral surface proteins, data showing the identification or isolation of the HBV receptor (s) are not yet available. The receptor-like proteins on the plasma membrane surface of HepG2 cells that bind to PreS1 were separated and identified using affinity chromatography, and the amino-terminal amino acid sequences of the receptor-like proteins were determined.

**Key words:** hepatitis B virus (HBV), MBP (maltose binding protein)-preS1, receptor.

A virus is generally known to attach to specific receptors on a host cell (Tardieu *et al.*, 1982) and this attachment determines the viral tropism or pathogenesis (Sumuzness *et al.*, 1980). Details of these early events in viral pathogenesis, including the nature of the cellular receptor, remain unclear for hepatitis B virus (HBV). HBV-associated particles were known to bind to the human hepatoma cell line HepG2 (Neurath *et al.*, 1986) and to purified human liver plasma membranes (Pontisso *et al.*, 1989). The proteins of HBV involved in binding to target cells consist of three related proteins of the viral surface designated as small (S), middle (M), and large (L) hepatitis B surface proteins. These are the translation products of 3 overlapping open reading frames with different initiation sites. The large protein, which is present in a small amount, has a 174 amino acid extention over the small protein. This region included the preS1 and preS2 components (Herrman *et al.*, 1984). In this region, the preS1 domain was reported to possess the HBV hepatocyte receptor binding site, located between amino acid residues 21 and 47, suggesting an important role in viral pathogenesis (Sumuzness *et al.*, 1980; Neurath *et al.*, 1986; Petti *et al.*, 1991). However, hepatocyte receptor (s) or receptor-like proteins for HBV are not yet well known. Here we report the identification and amino-terminal sequence analysis of plasma membrane proteins from HepG2 cells binding to the preS1 domain.

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## Materials and Methods

### Preparation of affinity chromatography

Amylose resin was used to prepare an affinity column to separate membrane proteins from HepG2 cells that bind to MBP (Maltose Binding Protein)-preS1. MBP-preS1 (Rhyum *et al.*, 1994) was coupled to the amylose resin (Biolabs, USA) by incubating for 12 h at 4℃ with a continuous orbital mixing in 20 mM Tris-HCl buffer, pH 7.4. The resin was washed with the same buffer and used for separation and identification of receptor (s) or receptor-like proteins.

### Isolation of plasma membrane and separation of proteins that bind to MBP-preS1

HepG2 cells were cultured on 650 ml EZIN flask (Nunc, Denmark) for about 5 days and scraped with a policeman (Nunc, Denmark). The cells were homogenized in 5 volumes of lysis buffer (100 mM KCl, 50 mM HEPES, 5 mM NaCl, 3.5 mM $MgCl_2$, 0.5 mM EGTA, pH 7.2) using a Teflon homogenizer. Cell debris and unbroken cells were removed from the suspension by centrifugation at 1,000 rpm for 10 min. The supernatant was ultracentrifuged at 100,000×*g* for 60 min

(Kim *et al.*, 1995). The pelleted plasma membrane fractions were resuspended in 20 mM Tris-HCl buffer, pH 7.4 containing 1% Triton X-100, 1mM PMSF and 1% sodium deoxycholate. The suspension was mixed with MBP-preS1-amylose resins and incubated for 24 h at 4℃ with constant orbital mixing. The mixture was then washed extensively with 20 mM Tris-HCl buffer, pH 7.4 containing 0.2 M NaCl and 10 mM β-mercaptoethanol. The bound proteins were eluted with 50 mM Triethylamine-HCl buffer, pH 11.5. The eluted protein fractions were neutralized immediately and concentrated using centricon and analyzed by 10% SDS-PAGE, using the Mighty Small II electrophoresis kit (Hoefer, San Francisco, USA).

### Electroblotting and N-terminal Amino acid sequence analysis

Electrophoresed proteins were electroblotted to PVDF (polyvinylidenedifluoride) membrane in CAPS (cyclohexylamino-1-propane sulfonic acid) transfer buffer (pH 11) for 3 h at 100 V. The transferred protein bands were cut with a scalpel and subjected to amino acid sequence analysis using porton-1090 gas phase peptide sequencer.

## Results and Discussion

Among the HBV surface proteins, the preS1 region was known to be involved in the hepatocyte HBV receptor binding (Neurath *et al.*, 1986). The previous reports demonstrated that the L protein expressed in the form of rHBsAg was able to bind in a specific manner to a human liver membrane fraction enriched with plasma membranes (Pontisso *et al.*, 1989, 1990). In the present report, the fusion protein, MBP-preS1, was used in order to identify receptor-like proteins on HepG2 cells that bind to MBP-preS1. Plasma membrane fractions from HepG2 cells were separated by ultracentrifugation and subjected to an affinity chromatography using MBP-preS1 coupled amylose resin. As a result (Fig. 1a and 1b), four protein bands with approximate molecular weights of 68 KD, 66 KD, 51 KD and 45 KD were identified. The proteins were labelled HL1, HL2, HL3 and HL4. This result is similar to the report of Neurath *et al.* (1986) which showed 3~4 different protein bands on HepG2 cells binding to radiolabelled F35.25 monoclonal antibody specific for preS1 (21~47). However, there are conflicting reports on a number of receptors and their molecular weights located in HepG2 cells that bind to preS1 (Petit *et al.*, 1992; Swan *et al.*, 1989; Dash *et al.*, 1992). In order to characterize the identified receptor-like proteins, the protein bands were transferred to PVDF membrane and subjected to amino-terminal amino acid sequence analysis and the result is shown in Table 1. When these sequences were compared with the known protein sequences of human origin, no sequence homology with any protein was found. Therefore, the present results indicate that the above four proteins seemed to be new proteins even though some questions remain to be solved. At present, work leading to separating and identifying individual clones from total chromosomal DNA of HepG2 cells is being carried out.

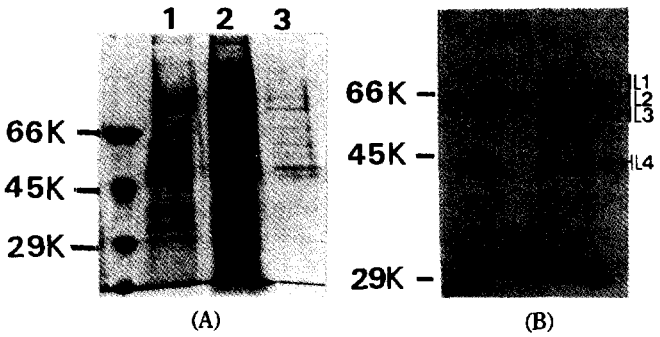


**Fig. 1.** The analysis of partially purified receptor(s) or receptor-like proteins. A. Lane 1 : HepG2 cell homogenate; lane 2 : The supernatant after centrifugation at 1000 rpm; lane 3 : Plasma membrane enrichment fraction after Ultracentrifugation at 100,000 ×*g*. B. Receptor-like proteins of plasma membrane fractions eluted from the MBP-preS1-amylose column.

**Table 1.** N-terminal amino acid sequences of receptor-like proteins

| | Amino acid sequences |
|---|---|
| HL1 | KHIPKH |
| HL2 | STHKREIALYFKNELEXHRP |
| HL3 | KPLXYFTLTLXFLTLXQKLP |
| HL4 | NFRITXLYLXFXLKXPWH |

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