An anti-viral peptide derived from the preS1 surface protein of hepatitis B virus

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The preS1 surface protein of the hepatitis B virus (HBV) is a key factor involved in initial viral entry into hepatocytes. It has been long postulated that an anti-HBV effect should be achievable using peptide fragments of the preS1. Recent reports demonstrated that several preS1-derived lipo-peptides in genotype D HBV exhibit nano to picomolar inhibitory activity against HBV infection. In this study, an acylated analog of a preS1 fragment, a 21-residue lipo-peptide (named 7524 BVS7) with a sequence of palmitoyl-GMGTNLSPNPPLGFFPDHQLDC-NH2 from genotype C HBV was produced based upon a previous structural study and was shown potent inhibits HBV infection with an IC50 of ≈ 20 nM. [BMB reports 2008; 41(9): 640-644]

INTRODUCTION

Despite continued efforts to develop more efficient anti-viral vaccines and better anti-viral pharmaceuticals, fatal viral infections from, e.g., human immunodeficiency virus (HIV) and more recently avian influenza virus (AIV) still remain a huge threat to public health. Hepatitis B, which is caused by a small DNA virus known as hepatitis B virus (HBV), at a quick glance appears much less intimidating than symptoms caused by HIV or AIV. Yet, it is also a serious health threat afflicting chronically as well as acutely more than 300 million people worldwide (1-3). Left untreated hepatitis B often develops into more serious diseases such as cirrhosis, hepatocellular carcinoma, or eventual liver failure (4). Vaccination against HBV has been largely successful and has contributed to alleviating problems associated with HBV infection except for lingering problems associated with non-responsiveness, low immunogenicity or emergence of escape mutants (5-7). Recent discoveries of monoclonal antibodies with broad anti-HBV specificity may provide a potential immunoprophylaxis against HBV (8).

Therapeutic remedies for hepatitis B other than preventive measures utilizing anti-HBV vaccines typically include anti-viral agents such as lamivudine or adefovir. However, these treatments are not HBV specific and often cause side effects or exhibiting decreased efficacy due to drug resistance (9). In addition, attempts to discover new anti-HBV agents from natural products (10, 11) have not yet resulted in any new anti-HBV agents. The successful development of a peptide derived from the gp41 surface protein of HIV into a potent HIV-inhibitor Fuzeon (12) and the fact that neuraminidase inhibitors (13) are effective against influenza virus convincingly illustrates that blocking initial viral attachment to host cells can be a highly effective measure against viral infections. In the last two decades there has been an accumulation of evidence, which clearly indicates that the HBV preS1 surface proteins are involved in the initial viral attack of hepatocytes. This conclusion strongly suggests that preS1-derived peptides hold great promise as an effective HBV-blocking agent (14-19). Several studies, including an early study that proposed the residues 21-47 in preS1 are important for HBV binding to a putative hepatocyte receptor (20), have demonstrated that the N-terminal half of the preS1 is vital for viral attachment to host cells (14-19). Nevertheless results from different studies failed to provide a consensus preS1 sequence that would be effective against HBV infection. This is partly due to the fact that a detailed three-dimensional structure of the preS1 protein is currently not available. In the case of the anti-HIV Fuzeon peptide knowledge of helical structural of the peptide greatly helped investigators to synthesize a peptide that corresponded to the trimeric helical bundle portion of gp41 which mediates binding of HIV to host cells (21).

The preS1 is an envelope protein that constitutes the outermost part of the so-called large surface protein in HBV (22). In the case of genotype C, the most prevalent type of HBV in Asian populations, it consists of 119 amino acid residues. The corresponding surface protein in Genotype D, which is the dominant form in European HBV carriers, is slightly shorter consisting of only 109 amino acid residues. Other than CD data, which indicated that the long polypeptide representing the preS1-domain of the L-protein was unstructured (23), no other
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Fig. 1. Anti-core immunofluorescence of HepaRG cells infected by HBV in the presence of preS1 derived peptides. HepaRG cells were infected in the presence of different concentrations of preS1 derived peptides. (A) 7524 BVS7 and (B) a control peptide 6524 BVS7. The cells were fixed 11 days after infection and stained with a polyclonal rabbit antiserum against native HBV capsids.

Results and Discussion
Several strategies can be and have been used for prevention or cure of viral infection (5-7, 12-14), where the oldest known one is anti-viral vaccines (5-7). Anti-HBV vaccines became available two decades ago and were proven to be effective. Another highly effective preventive measure against viral infection is to curb the virus at the initial attachment stage to the host cells or to prevent viral fusion as has been elegantly proven in the case of anti-HIV drug Fuzeon (12) or anti-influenza agent Tamiflu or Relenza (13). Even though the former utilizes a peptide fragment and the latter a small chemical compounds they both rely on the same strategy, blocking initial viral entry. In order to develop an anti-HBV agent following a similar strategy several investigators attempted to map the regions(s) of HBV surface proteins that would be crucial for the initial binding of HBV to a putative hepatocyte receptor (14-20). An early study which suggested that the preS2 portion of HBV might mediate binding of viral particles to hepatocytes (32) was determined to be false. Even though it has been well established that the preS1 is most critical for HBV infection there has not been a consensus on which region(s) of the preS1 is most critical for infection (14-20). In addition to the absence of structural knowledge on the preS1 domain another, perhaps more serious, bottleneck in delineating effective preS1 domains responsible for HBV attachment was the lack of an efficient cell culture assay system. Recently this issue has been overcome by the availability of HBV-susceptible HepaRG cell lines (33, 34).

Fig. 1 shows fluorescence images of secreted HBV particles when cells were treated with an inhibitory peptide 7524 BVS7 and a control peptide 6524 BVS7 (21P-47P; PLGGFPDHHQK- DPAFGANSNPDWDFNP-NH2). Fig. 2 shows a concentration dependence of HBV infection inhibition by 7524 BVS7. From this treatment peptide 7524 BVS7 was shown to significantly inhibit HBV infection with an IC50 of \( \sim 20 \mu M \). The amino acid sequence of 7524 BVS7 is palmitoyl-GMGTNLSVPNPLGF- FPDHQDC-NH2, which differs at 5 locations from the corresponding sequence of the preS1 in genotype D HBV. Thus our results confirm the hypothesis (14) that preS1-derived peptides from one genotype may also inhibit viral infections by other HBV genotypes. An interesting observation is that 6524 BVS7, which correspond to the preS1 residues 21-47 and was previously proposed to be responsible for hepatocyte receptor binding (20), is inactive against HBV infection up to 2 \( \mu M \). Since myristoylation at the N-terminus of preS1 is known to be important for viral infection it is plausible that the inability of
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Fig. 2. HBsAg secretion of HepaRG cells infected by HBV in the presence of preS1 derived peptides. HepaRG cells were infected in the presence of different concentrations of preS1 derived peptides. The supernatants between day 7 to 11 after infection were collected for analysis by an HBsAg assay (Abbott).

6524 BS7 to prevent HBV infection may be due to the lack of an N-terminal acylation. Yet, such a possibility can be excluded since in a previous observation the acylated analog of preS1 peptides, similar to 6524 BS7, showed only weak inhibitory activity (14).

The current work along with previous investigations (12-14) unequivocally confirms that the blocking of viral attachment to host cells is valid approach for developing effective HBV inhibitors. Such a strategy might also be useful for battling against the recent epidemic of SARS. The preS1 fragments that displayed inhibitory effects against HBV infection may also be useful in the development of more effect anti-HBV vaccine. Our results show that the efficient preventive strategy is possible even without completely eradicating HBV. NMR structural studies are under way in order to map the key structural features within the preS1-derived peptides that are important for receptor binding. The precise nature of the putative HBV receptor on hepatocytes is still unknown (35-37). Once the receptor is unambiguously identified, a rational design of small molecule HBV inhibitors based upon the receptor-bound structure of anti-HBV preS1 peptides may be possible.

MATERIALS AND METHODS

Peptide preparation
Peptides were synthesized by a solid phase method with a multiple peptide synthesizer, APEX 348Ω (Advanced Chemtech, Louisville, KY). The C-termini of the synthesized peptide were then amidated. In the case of 7524 BS7 with the sequence of palmitoyl-GMTNLVLPNLGGFQDHC- NH2 (pal-11G-31D-Cys of genotype C HBV preS1) the palmitic acid was coupled to the N-terminal glycine of the synthesized peptide at the last step of synthesis. Peptides were purified by reverse phase HPLC using Vydac C18 columns and the peptide mass was confirmed by MALDI-TOF mass spectrometry. The control peptide (6524 BS7) corresponds to the residues 21∼47 of the preS1 (21P∼47P; PLGFFPDQYNPDWP-NH2) in genotype C HBV.

Immunofluorescence assay of HepaRG cells
Differentiated HepaRG cells were pre-incubated for 30 min with or without preS1 derived peptides followed by a co-incubation of cells with concentrated HBV and varying concentrations of peptides for 20h at 37°C. The cells were washed three times with PBS and maintained media containing 2% DMSO. The cells were fixed 11 days after infection and stained with a polyclonal rabbit antiserum against native HBV capsids.

HBsAg assay of HepaRG cells
Differentiated HepaRG cells were pre-incubated for 30 min with or without preS1 derived peptides followed by a co-incubation of cells with concentrated HBV and varying concentrations of the peptide for 20h at 37°C. Cells were washed three times with PBS and maintained in the presence of 2% DMSO. The supernatants between day 7 to 11 after infection were collected for HBsAg assay (Abbott).

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