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The full-length clone of cucumber green mottle mosaic virus and its application as an expression system for Hepatitis B surface antigen

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Abstract

A cucumber green mosaic mottle virus (CGMMV) full-length clone was developed for the expression of Hepatitis B surface antigen (HBsAg). The expression of the surface displayed HBsAg by the chimeric virus was confirmed through a double antibody sandwich ELISA. Assessment of the coat protein composition of the chimeric virus particles by SDS-PAGE analysis showed that 50% of the coat proteins were fused to the HBsAg. Biological activity of the expressed HBsAg was assessed through the stimulation of in vitro antibody production by cultured peripheral blood mononuclear cells (PBMC). PBMC that were cultured in the presence of the chimeric virus showed up to an approximately three-fold increase in the level of anti HBsAg immunoglobulin thus suggesting the possible use of this new chimeric virus as an effective Hepatitis B vaccine.

Keywords: CGMMV; Infectious transcripts; HBsAg; Tobamovirus; Chimeric virus

1. Introduction

Hepatitis B virus infection is a major global health problem despite the availability of safe and effective vaccines (Tahan et al., 2003). The cost for virus detection and vaccination remains high as the production of Hepatitis B surface antigen (HBsAg) is largely

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restricted to eukaryotic systems due to the requirements of precise post-translational modifications. To date, functional HBsAgs have been successfully expressed in several systems such as transgenic plants (Richter et al., 2000; Gao et al., 2003; Ramirez et al., 2003; Huang et al., 2005), yeast (Valenzuela et al., 1982; Bitter et al., 1988; Li et al., 2004) and chimeric animal viruses (Smith et al., 1983). However, there remain problems such as low-yield and higher production costs involved in transgenic plant as well as other cell culture based systems developed. Safety issues remain unresolved when chimeric animal viruses are used. Therefore, there remains a requirement for a fast, high yielding cost effective yet safe method for the production of functional HBsAgs.

Chimeric plant virus based peptide expression systems have many advantages over conventional expression systems which include aspects of safety, ease of production and scalability (Liu et al., 2005). Tobamoviruses such as tobacco mosaic virus (TMV) and cucumber green mottle mosaic virus (CGMMV) have the added advantage of having monopartite genomes thus making genetic manipulation relatively simple with the introduced peptide displayed in multiple copies on the surface of the virus particles. Chimeric TMVs have been developed for many different uses (Turpen, 1999; Perez Filgueira et al., 2003; Wu et al., 2003; Kang et al., 2004). However, the successful expression of a heterologous sequence is very much dependent on the physiochemical properties of the resulting fusion coat protein (Liu et al., 2005). Chimeric TMV that expresses fusion coat protein along with the expression of native coat protein through the control of a leaky UAG signal have been shown to show greater tolerance toward the variation in the physiochemical properties of the heterologous sequences (Skuzeski et al., 1991; Hamamoto et al., 1993; Sugiyama et al., 1995; Turpen et al., 1995). However the production of the fusion coat protein through such systems were reported to be only around 5–10% of the total coat protein yield (Skuzeski et al., 1991; Hamamoto et al., 1993; Sugiyama et al., 1995; Turpen et al., 1995).

In this study, we report the development of a chimeric CGMMV based HBsAg expression system. The system expresses fusion protein in parallel with a modified clone of the wild type CGMMV coat protein at an approximate ratio of 1:1. The displayed HBsAg is capable of stimulating the production of HBsAg specific antibody in in vitro cultured peripheral blood mononuclear cells (PBMC).

2. Materials and methods

2.1. The antigenic epitope

The “a” determinant of the HBsAg covering amino acid residues Pro111 to Thr141 was used as the heterologous epitope (Tan, 2002).

2.2. Construction of the CGMMV full-length clone

The plasmid pUC19 (Yanisch-Perron et al., 1985) was modified for use as the backbone in the construction of the transcription CGMMV vector by deletion of the *Hin*dIII site from the multiple-cloning site. This was to preserve the uniqueness of the corresponding site in the CGMMV genomic cDNA. The pUC19 plasmid was digested overnight with *Hin*dIII, the 5′ overhang filled with Deep Vent^{TM} DNA polymerase (NEB) and then religated to form circular DNA before transformation into *E. coli* strain DH5α.

The transcription vector itself was based on a previously characterized CGMMV (Ugaki et al., 1991) (Genbank accession no. D12505). The summary of the construction processes is shown in Fig. 1A and B. Viral RNA was isolated from purified CGMMV as previously described (Tan et al., 2000). The purified RNA was used as template in a two step RT-PCR reaction. The synthesis of first strand cDNA was carried out with primer 3UTRSPH 5′-AAAGCATGCTGGGCCCCTACCCGGGGAA-3′ and Superscript^{TM} III, according to the manufacturer’s protocol. The first strand cDNA was then ligated to a shortened T7 promoter using *Taq* ligase and oligonucleotide T7SHfused 5′-TAATACGACTCACTATAGTTTTAATTTTTATAAT-3′ followed by long distance polymerase chain reaction (LD-PCR) with primers 3UTRSPH and T7CG 5′-CCGAGCTCGTAATACGACTCACTATAGTTTTA-3′. Both the products from the LD-PCR were purified through two rounds of phenol–chloroform extraction, followed by ethanol precipitation. The purified DNA and the cloning vector were simultaneously digested with *Sph*I and *Sac*I. The digested DNA fragments from both reactions