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SCIENCE DIRECT

Virology 342 (2005) 102 – 110

VIROLOGY

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Genetic rearrangements on the Chlorovirus genome that switch between hyaluronan synthesis and chitin synthesis

Ali Mohammed Mohammed Ali, Takeru Kawasaki, Takashi Yamada*

Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8530, Japan

Received 13 June 2005; returned to author for revision 13 July 2005; accepted 21 July 2005
Available online 19 August 2005

Abstract

Chlorella viruses or chloroviruses form polysaccharide fibers on the cell wall of host *Chlorella* cells after infection. Such polysaccharides are either hyaluronan synthesized by virus-encoded hyaluronan synthase (HAS) or chitin synthesized by viral chitin synthase (CHS). Some chloroviruses synthesize both hyaluronan (HA) and chitin simultaneously. To understand the relationship between "HA-synthesizing" and "chitin-synthesizing" viruses, we characterized the CVK2 genomic regions, one flanking *chs* and the other corresponding to PBCV-1 *has* and found that on CVK2 DNA, a single ORF (PBCV-1 A330R) was replaced with a 5 kbp region containing *chs*, *ugdh2* (the second gene for UDP-glucose dehydrogenase) and two other ORFs, and that *has* was replaced with another *chs* gene. In some chloroviruses, *ugdh* was lost. These results suggest that chlorovirus types changed from "*has* viruses" to "*chs* viruses" or from "*chs* viruses" to "*has* viruses" by exchanging the genes.

Keywords: Chlorovirus; Chitin synthase; Hyaluronan synthase; UDP-glucose dehydrogenase; Gene rearrangement

Introduction

Chloroviruses, or *Chlorella* viruses, are large icosahedral, double-stranded DNA-containing viruses that infect certain strains of the unicellular green alga *Chlorella* (Van Etten and Meints, 1999; Van Etten et al., 1991). These viruses belong to the Phycodnaviridae family and are ubiquitous in the natural environment (Van Etten et al., 1991; Yamada et al., 1991). Analysis of the 330,740-bp genomic DNA sequence of PBCV-1, the prototype member of Phycodnaviridae, revealed various unexpected genes (Kutish et al., 1996; Li et al., 1995, 1997; Lu et al., 1995, 1996). One of these genes, *a98r*, encoded a functional hyaluronan synthase (HAS) (DeAngelis et al., 1997), and was expressed early in the viral infection and produced hyaluronan polysaccharide on the outside of the host *Chlorella* cell wall (Graves et al., 1999). Hyaluronan is a simple linear polysaccharide chain composed of alternating β-1,4-glucuronic acid (GlcA) and β-1,3-*N*-acetylglucosamine (GlcNAc) moieties (Laurent and Fraser, 1992). HAS adds sugar residues from UDP-GlcA and UDP-GlcNAc. Landstein et al. (1998) revealed that PBCV-1 encoded two other enzymes, glutamine:fructose-6-phosphate amidotransferase (GFAT) and UDP-glucose dehydrogenase (UDP-GlcDH or UGDH), that produced sugar precursors (GlcN-6-P and UDP-GlcA, respectively) required for hyaluronan synthesis. The presence of multiple enzymes involved in the hyaluronan biosynthetic pathway suggests some importance of hyaluronan production in the PBCV-1 infection. However, the biological function of PBCV-1 hyaluronan is largely unknown (DeAngelis et al., 1997; Graves et al., 1999; Landstein et al., 1998). Graves et al. (1999) examined the occurrence of the *has* gene in other chloroviruses isolated from diverse geographical regions, and found that the PBCV-1 *has* gene probe did not hybridize to 9 of 37 DNAs from viruses that infect *Chlorella* NC64A. This result indicated that the *has* gene is not always essential for chlorovirus replication. One

* Corresponding author. Fax: +81 824 24 7752.
E-mail address: tayamad@hiroshima-u.ac.jp (T. Yamada).

0042-6822/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2005.07.026

possible explanation is that those *has*-lacking viruses encode an enzyme, or enzymes, that produces alternative polysaccharides on the external surface of the infected *Chlorella* cells.

Recently, Kawasaki et al. (2002) found that the chlorovirus CVK2 has a gene for a functional chitin synthase (CHS) instead of HAS and produced chitin fibers that surrounded the external surface of virus-infected *Chlorella* cells. Chitin, a β-1,4-linked homopolymer of *N*-acetyl-D-glucosamine (GlcNAc), is the second most abundant polymer in nature, after cellulose. Chitin is usually present in insects, crustaceans, and most fungi. Plants, vertebrates, and prokaryotes do not contain chitin. In addition to the structural facets of chitin polymers, chitin oligosaccharides function as important signals in the developmental processes of plants, for example, as elicitors for defense responses and as nodulation-inducers for leguminous plants. CVK2 CHS resembles to the CHS3-type enzymes of yeasts and fungi; however, its size (516 aa) is significantly smaller than fungal enzymes (1000–1300 aa), and the sequence homology is restricted to the carboxy-terminal region of those enzymes where the conserved catalytic site exists (Nagahashi et al., 1995). The smaller size of the CVK2 CHS protein may reflect its simpler regulatory and processing mechanisms as well as different localization processes in the cell. All chloroviruses isolated in Japan, that lacked the *has* gene, contained the *chs* gene. A few viruses contained both the *has* and *chs* genes and synthesized both hyaluronan and chitin on the surface of infected cells. All chloroviruses, including CVK2, studied so far contained a functional gene for GFAT that produced the sugar precursor GlcNAc-6P required for chitin synthesis. These observations raise the question as to how the genes for *has*, *chs*, *gfat*, and *ugdh* are related to each other on the chloroviral genomes, and how "*has* viruses" and "*chs* viruses" diverged. In this work, we characterized the region flanking *chs* and that corresponding to PBCV-1 *has* on the CVK2 genome and found two new ORFs beside the *chs* region; another UDP-GlcDH and GlcNAc deacetylase. There was another *chs*-related gene and another β-1,3 glucanase gene at the PBCV-1 *has*-corresponding region on CVK2 DNA. These results indicate that chlorovirus types changed from "*has* viruses" to "*chs* viruses" or from "*chs* viruses" to "*has* viruses" by exchanging the genes.

Results

On CVK2 DNA, a single ORF (PBCV-1 A330R) is replaced with a 5 kbp region containing chs, ugdh, and two other ORFs

In our previous work (Kawasaki et al., 2002), the CVK2 *chs* was mapped on a 3.0 kbp *Eco*RI fragment of cosmid 3H6 that corresponded to a position around the central part of the 350 kbp CVK2 genome (Fig. 1A). At the equivalent position on PBCV-1 DNA (330 kbp), there was an ORF A330R encoding an unknown protein, close to the tRNA cluster (Li et al., 1995). When a 24 bp nucleotide sequence positioned 105 bp upstream, and a 24 bp sequence positioned 54 bp downstream of A330R of PBCV-1 were used as PCR primers to amplify the CVK2 *chs* region, a 5.0 kbp fragment was obtained from CVK2 DNA by PCR. When the nucleotide sequence was determined for this fragment (DDBJ accession no.AB218663), it revealed four ORFs, *chs*, and three additional ORFs (ORF1–ORF3). ORF1 (379 aa) and ORF2 (251 aa) had the same orientation as *chs* (516 aa) and ORF3 (369 aa) had an opposite orientation, as shown in Fig. 1B. Surveying through the databases for these ORFs gave similar sequences as follows: ORF1 was highly similar to bacterial UDP-glucose dehydrogenase (UGDH) (UniProt/Swiss-Prott accession no.Q57871, FASTA score 430) with amino acid identity of 35% but showed only 31% of amino acid identity with A609L (389 aa). A609L was already known to encode functional *ugdh* on PBCV-1 DNA (Landstein et al., 1998). Therefore, ORF1 is not a mere copy of A609L but encodes a new UGDH gene (*ugdh2*). ORF2 showed low similarity to *Mycoplasma* methionyl-tRNA synthetase (USSP accession no.P47267, FASTA score 119); 26% amino acid identity was restricted to a 150-aa amino-terminal region of ORF2. ORF3 was similar to *Rhizobium* chitooligosaccharide deacetylase (USSP accession no.P02963, FASTA score 148). Chitooligosaccharide deacetylase (CODA) is related to chitin metabolism and is not encoded on PBCV-1 DNA. These results indicate that the gene cluster ORF1-*chs* displaced a single ORF on CVK2 DNA. At the rearrangement points adjacent to A330R and *chs*, there were highly repeated sequences consisting of a 24 bp unit, 5′AGAAGG-TTTCGGGGAAGGGGAAGG, which might be involved in the gene rearrangement.

This gene arrangement of CVK2 was also found in other chitin-synthesizing viruses, including CVHA1, CVIK1, and CVSA1. This was confirmed by Southern blot hybridization where *Eco*RI fragments of genomic DNA were hybridized with *chs* and *ugdh2* as probes. Results shown in Fig. 2 demonstrate exactly identical hybridization patterns of these viruses and no signal was present in non-chitin-synthesizing (*chs* negative) viruses (Fig. 2A). Recently, the complete genomic sequences of some other chloroviruses are publicly available on the website http://greengene.uml.edu. These sequences represent work in progress and have not been published or deposited in the databases. We found almost the same gene cluster in the viruses NY2A and AR158, both of which are probably chitin-synthesizing viruses. Therefore, this gene rearrangement, including *chs*, might have occurred relatively recently and is conserved in some viral lines. Incidentally, the GC-content of this rearranged region (positions 196,060–200,867) on the NY2A genome was 42.1%, a little larger than the value of 40.7% for the remaining part of the genome.