**Paramecium bursaria Chlorella Virus 1 Encodes Two Enzymes Involved in the Biosynthesis of GDP-L-fucose and GDP-D-rhamnose***

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Michela Tonetti‡§, Davide Zanardi‡, James R. Gurnon¶, Floriana Frusciene‡, Andrea Armiriotti‡, Gianluca Damonte‡, Laura Sturla‡, Antonio De Flora‡, and James L. Van Etten**

From the §Department of Experimental Medicine, Section of Biochemistry and Center of Excellence for Biomedical Research, University of Genova, 16132 Genova, Italy, the ¶Department of Pathology, University of Nebraska, Lincoln, Nebraska 68588-0666, the **Nebraska Center of Virology, University of Nebraska, Lincoln, Nebraska 68588-0666

At least three structural proteins in Paramecium bursaria Chlorella virus (PBCV-1) are glycosylated, including the major capsid protein Vp54. However, unlike other glycoprotein-containing viruses that use host-encoded enzymes in the endoplasmic reticulum-Golgi to glycosylate their proteins, PBCV-1 encodes at least many, if not all, of the glycosyltransferases used to glycosylate its structural proteins. As described here, PBCV-1 also encodes two open reading frames that resemble bacterial and mammalian enzymes involved in de novo GDP-L-fucose biosynthesis. This pathway, starting from GDP-α-mannose, consists of two sequential steps catalyzed by GDP-α-mannose 4,6 dehydratase (GMD) and GDP-4-keto-6-deoxy-α-mannose epimerase/reductase, respectively. The two PBCV-1-encoded genes were expressed in Escherichia coli, and the recombinant proteins had the predicted enzyme activity. However, in addition to the dehydratase activity, PBCV-1 GMD also had a reductase activity, producing GDP-α-rhamnose. In vivo studies established that PBCV-1 GMD and GDP-4-keto-6-deoxy-α-mannose epimerase/reductase are expressed after virus infection and that both GDP-L-fucose and GDP-D-rhamnose are produced in virus-infected cells. Thus, PBCV-1 is the first virus known to encode enzymes involved in nucleotide sugar metabolism. Because fucose and rhamnose are components of the glycans attached to Vp54, the pathway could circumvent a limited supply of GDP sugars by the algal host.

**Experimental Procedures**

Cloning and Expression of Recombinant GMD and GMER—Production of virus PBCV-1 and DNA isolation were performed as described (16, 17). Gene-specific primers were used for PCR amplification of the keto-6-deoxy-α-mannose epimerase/reductase; p.i., postinfection; ORF, open reading frame; GC-MS, gas chromatography-mass spectrometry; HPLC, high pressure liquid chromatography.
protein encoding regions for the putative GMD and GMER enzymes, ORFs A118R and A295L, respectively. The forward primers used to amplify GMD (5'-AATTGGATCCATGGTATCTCAGTTGATAAAATGATCATAGT) and GMER (5'-AATTGGATCCATGGTATCTCAGTTGATAAAATGATCATAGT) contained a BamHI restriction site, whereas the reverse primers for GMD (5'-AATTCTCGAGTTATTTATTTCCAAACGTATCTGA) and GMER (5'-AATTCTCGAGTTATTTATTTCCAAACGTATCTGA) contained a XhoI restriction site. Polymerase chain reactions (50 μl) were performed with 1.5 units of Pfu polymerase (Sigma), in the presence of 2 mM MgSO4, 0.2 mM deoxyribonucleotides triphosphate, and 0.1 μM primers. The PCR products were purified from 1.2% agarose gels using a rapid ligation kit (Roche Applied Science). The resulting plasmids were used to transform E. coli K803. DNA sequencing of positive clones was performed by PRIMM (Firenze, Italy). E. coli K803 cells containing vectors with the correct inserts were grown in 2 ml ofYT medium (1.6% tryptone, 1% yeast extract, 0.5% NaCl, pH 7.0), containing 200 μg/ml ampicillin. Expression of both glutathione S-transferase fusion proteins was induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside. Optimal protein recovery was obtained by induction at 20 °C for 16 h for GMD and at 30 °C for 2 h for GMER. Cell lysis, purification of the fusion proteins, and proteolytic cleavage of the glutathione S-transferase tag were performed as described (18). Protein concentrations were determined by the Bradford assay (Bio-Rad), and protein purity was monitored by SDS-PAGE. Both recombinant proteins were stored at 4 °C in 50 mM Tris/HCl, pH 7.0, 150 mM NaCl, 1 mM EDTA, and 1% dithiothreitol in the presence of 10% glycerol.

In Vivo Expression of PBCV-1 GMD and GMER—Chlorella NC64A was grown in modified Bold's basal medium and infected with PBCV-1 following described procedures (16). Aliquots were withdrawn at various times postinfection (p.i.), and RNA was isolated, electrophoresed under denaturing conditions on 1.5% agarose/formaldehyde gels, stained with ethidium bromide, and transferred to nylon membranes as described (19). The RNA was hybridized with a18R or a295L probes; the probes were labeled with 32P using the random primers DNA labeling system (Invitrogen). After hybridization, radioactivity bound to the probes; the blots were washed; products were monitored by reverse phase and anion exchange HPLC (see below).

To prepare the GMER substrate GDP-4-keto-6-deoxy-D-mannose, 5 mM GDP-D-mannose was incubated with 200 μg/ml of recombinant viral GMD for 30 min. Conversion of GDP-D-mannose to GDP-4-keto-6-deoxy-D-mannose was determined by HPLC (see below). At the end of the reaction GMD was removed from the incubation mixture by ultrafiltration, using Microcon YM-10 microconcentrators (Millipore). Because of its chemical instability, GDP-4-keto-6-deoxy-D-mannose was used immediately after production. Recombinant GMER activity was assayed by monitoring GDP-1-fucose production by reverse phase HPLC. The standard reaction contained 100 μM substrate in 50 mM Tris/HCl, pH 7.0, and 1 mM NADP+; products were monitored by reverse phase and anion exchange HPLC (see below).

Fig. 1. SDS-PAGE of purified recombinant PBCV-1 GMD and GMER. After purification and cleavage by Pre-scission protease, 2 μg each of GMD and GMER were electrophoresed on a 12% SDS-PAGE gel. Both proteins, detected by Coomassie Brilliant Blue staining, have high purity and an electrophoretic mobility comparable with their predicted molecular mass (38.5 kDa for GMD and 35.6 kDa for GMER).

Fig. 2. Reverse phase HPLC analysis of the products from PBCV-1 GMD and GMER enzyme activities. The eluate was monitored by continuous flow scintillation counting. A, GDP-ν-mannose (peak 1) was incubated with GMD and NADP+, leading to the formation of the intermediate compound GDP-4-keto-6-deoxy-ν-mannose (peak 2). B, GDP-4-keto-6-deoxy-ν-mannose (peak 2) incubated with GMER and NADPH, was converted to GDP-ν-fucose (peak 3). D, incubation of GDP-ν-mannose with GMD and both NADP+ and NADPH resulted in the formation of a new compound (peak 4), whose retention time differed from the expected nucleotide sugars. E, incubation of GDP-ν-mannose (peak 1) with both GMD and GMER in the presence of NADP+ and NADPH led to the formation of both GDP-ν-fucose (peak 3) and a new unidentified compound (peak 4).
sugars by comparing HPLC retention times with known standards and
by electrospray mass spectrometry analyses. Monosaccharides obtained
after acid hydrolysis were analyzed by TLC and GC-MS (see below).

Cell extracts from uninfected and virus-infected algae were assayed
for enzyme activity in Tris/HCl, pH 7.0, 100 μM 14C-labeled substrates,
and either 1 mM NADP+ or NADPH at a protein concentration of 5
mg/ml. Products were monitored by both reverse phase and anion
exchange HPLC.

**Chromatographic Analyses of GMD and GMER Products**—Products
formed from both recombinant proteins and cell extracts were compared

![Diagram](image-url)

**Fig. 3.** Electrospray ionization-MS spectra of nucleotide sugars pro-
duced by GMD activity. GDP-D-mannose (A), GDP-4-keto-6-deoxy-D-mannose
produced by the dehydratase activity of recombinant GMD (B), and the new com-
 pound obtained by GMD in the presence of NADPH (C). The m/z of the GDP-4-
ket-6-deoxy-D-mannose is congruent with the loss of a water molecule from
GDP-D-mannose. After the NADPH-
dependent reduction of the intermediate compound by GMD, the m/z of the new compound is increased by two units, sug-
gesting the reduction of the ketone group on C-4, with the consequent formation of
a GDP-6-deoxy-hexose.

![Diagram](image-url)

**Fig. 4.** Anion exchange HPLC analysis of the products from PBCV-1 GMD activity. To improve separation of the products resulting from
GMD activity, a new anion exchange procedure was developed (see “Experimental Procedures”). The eluate was monitored at 260 nm. The peak
numbers are the same as in Fig. 2: peak 1 is GDP-D-mannose, peak 2 is GDP-4-keto-6-deoxy-D-mannose, peak 4 is the new GDP sugar.
with known standards by TLC and HPLC. TLC was performed on the 
\(^{14}\text{C}\)-radioabeled monosaccharides after acid hydrolysis, as described in Ref. 11. To analyze the intermediate compound GDP-4-keto-6-deoxy-D-
mannose, samples were reduced with NaBH\(_4\) prior to hydrolysis (11). The 
radio-label was detected with the Cyclone system (Canberra Packard).

Sample extractions and reverse phase HPLC, using a C18 column (Waters), followed published procedures (11). Better separation of GDP-
mannose, GDP-4-keto-6-deoxy-D-mannose, and GDP-D-rhamnose was 
achieved by anion exchange chromatography. For this analysis, 100-µl 
 aliquots of the extracted samples were injected onto a Wesca Anion/R 
column (Alltech) and eluted with a 100–500 mM NH\(_4\)HCO\(_3\) gradient at 
a flow rate of 2 ml/min. An additional step, using 1 M NaCl, was 
required to elute NADPH, which otherwise remained tightly bound to 
the column. For both reverse phase and anion exchange HPLC, eluates 
were monitored by UV absorbance at 260 nm and by continuous flow 
scintillation counting (Packard Radiomatic 500TR).

Electrospray MS Analysis of GDP Sugars—Electrospray analysis 
was performed on an Agilent 1100 MSD ion trap instrument (Agilent 
Technologies, Palo Alto, CA) by flow injection of the samples with an 
infusion pump (KD Scientific, New Hope, PA). The reaction mixtures 
were diluted 1:19 with water to reduce salt concentration; spectra were 
acquired in the negative ion mode in the mass range of the expected m/z 
ratios. The ion source parameters were set to obtain optimal signal to 
noise ratio for molecules of interest (20).

Monosaccharide Analysis by GC-MS—GDP sugars obtained with 
the recombinant enzymes were purified by anion exchange chromatography 
as described above. The GDP-D-rhamnose standard was kindly pro-
vided by Dr. Paul Messner, Universitat fu¨r Bodenkultur, Wien, Austria (21). 
Samples were derivatized according to Henry et al. (22) and 
Merkle and Poppe (23) with slight modifications. Briefly, the nucleotide 
sugars were hydrolyzed with 2 N HCl for 90 min at 100 °C, dried in a 
Speed-Vac, and the resulting monosaccharides were reduced to their 
alditols with NaBH\(_4\) (10 mg/ml in 1 M ammonia solution). The 
products were acetylated in 1:1 pyridine/acetic anhydride, resus-
cipated in (–2)-octanol, and heated at 120 °C overnight with a catalytic 
amount of trifluoroacetic acid. The solution was dried, acetylated, and 
analyzed as described above. The single ion monitoring chromatograms 
of the 273 ion corresponding to the D- and L-rhamnose standards were 
compared with those of sugar standards: fucose, produced by GMER 
activity (A), and rhamnose, produced by GMD reductase activity (B).

To determine the D or L nature of the rhamnose monosaccharide, 
(S)-octyl glycoside derivatives were obtained from the dried hydrolyzed 
GDP-rhamnose according to Leontein et al. (24). Samples were dis-
olved in (S)-2-octanol and heated at 120 °C overnight with a catalytic 
amount of trifluoroacetic acid. The solution was dried, acetylated, and 
analyzed as described above. The single ion monitoring chromatograms 
of the 273 ion corresponding to the D- and L-rhamnose standards were 
compared with the unknown epimeric form.

 RESULTS

Expression of PBCV-1 Recombinant GMD and GMER En-
zymes—PBCV-1 ORFs A118R (345 amino acids) and A295L 
(317 amino acids) (accession number U42580 for PBCV-1 ge-
nome) encode proteins that resemble GMD and GMER, respec-
tively, from prokaryotic and eukaryotic organisms. Resequenci-
ing both genes indicated that the putative GMD sequence was 
isomeric to that initially reported, whereas GMER differed at 
identical amino acid positions 142 and 143, with AK instead of ER. The 
nucleotide and the deduced amino acid sequences have been deposited in GenBank™, 
accession codes AY225120 for GMD and AY225121 for GMER. No other sequences bearing homol-
gy to enzymes involved in the metabolism of nucleotide sugars 
were detected by either BLAST or TFASTA analyses of the 
PBCV-1 genome.

Recombinant PBCV-1 GMD and GMER proteins with the expected molecular weights were obtained in high yield and 
good purity (~2 mg/liter of initial bacterial culture for GMD 
and 0.8 mg/liter for GMER) (Fig. 1). The lower yield for GMER 
resulted from precipitation of the protein during purification. 

FIG. 5. GC-MS analysis of the monosaccharides in the GDP-6-
deoxy-hexoses produced by GMD and GMER. A and B, the nucle-
otide sugars were purified by anion exchange chromatography, and the 
monosaccharides were released by acid hydrolysis, followed by conver-
sion to alditol acetates and GC-MS analysis. Retention times were 
compared with those of sugar standards: fucose, produced by GMER 
activity (A), and rhamnose, produced by GMD reductase activity (B). C, 
rhamnose obtained by GMD reductase activity was derivatized with a 
chiral compound, (S)-2-octanol. The corresponding (S)-octyl glycoside 
derivative, analyzed by GC-MS, had the same retention time as the 
GDP-D-rhamnose standard, indicating that rhamnose synthesized by 
PBCV-1 GMD has a D configuration. For further details, see "Experi-
mental Procedures."

Characterization of PBCV-1 Recombinant GMD and GMER Enzymes—Enzyme assays established that both proteins have 
the expected catalytic activities. Dehydration of GDP-D-mann-
ose by viral recombinant GMD produced a compound with an 
identical retention time on reverse phase HPLC (Fig. 2A, peak 
2) as the GDP-4-keto-6-deoxy-D-mannose, an intermediate in 
the GDP-6-fucose biosynthetic pathway, produced by human recombinant GMD (18). The identity of this compound was 
confirmed by TLC after NaBH\(_4\) reduction, which led to the 
production of both rhamnose and 6-deoxy-talose (11) (not 
shown), and by electrospray MS, which indicated a m/z of 
586.3, consistent with GDP-D-mannose (m/z 604.3) lacking a 
water molecule (Fig. 3, A and B). GDP-4-keto-6-deoxy-D-mann-
ose (peak 2) incubated with NADPH in the absence of enzyme 
was stable, thus ruling out any spontaneous chemical reduc-
which is consistent with a GDP-6-deoxy-hexose (Fig. 3). Reverse phase HPLC (Fig. 2, D) peak 4, formed by GMD reductase activity), and GDP-L-rhamnose (same as reported in Fig. 2. extracts prepared from virus-infected cells. Peak identity is the analysis performed after acid hydrolysis (without NaBH₄ reduction). 50 μg of protein from the Chlorella extracts were electrophoresed in each lane. ST, 10 ng of the recombinant proteins, used as standards; C, uninfected Chlorella.

Fig. 6. Expression of PBCV-1 GMD and GMER transcripts and proteins at various times after virus infection. A, Northern blot analysis for GMD (ORF A118R) and GMER (ORF A295L). B, Western blot analysis, using polyclonal rabbit antiserum raised against recombinant PBCV-1 GMD and GMER. 50 μg of protein from the Chlorella extracts were electrophoresed in each lane. ST, 10 ng of the recombinant proteins, used as standards; C, uninfected Chlorella.

Fig. 7. Reverse phase HPLC of GMD and GMER products in extracts prepared from virus-infected cells. Peak identity is the same as reported in Fig. 2. A, 14C-labeled GDP-6-mannose (100 μM) was incubated with 1 mM NADP⁺ and an algal extract (5 mg/ml of protein) obtained at 180 min p.i. After incubating for 30 min, the substrate was quantitatively converted to GDP-4-keto-6-deoxy-D-mannose (peak 2). B, 14C-labeled GDP-mannose was incubated under the same conditions as reported above but with the addition of 1 mM NADPH. Both GDP-rhamnose (peak 4, formed by GMER reductase activity), and GDP-L-fucose (peak 3, formed by GMER activity) appeared.

Incubation of recombinant GMER with NADPH and GDP-4-keto-6-deoxy-D-mannose produced a compound with a retention time identical to GDP-L-fucose (Fig. 2C, peak 3). Electrospray ionization-MS spectra indicated a m/z of 588.4, which is compatible with GDP-L-fucose (not shown). After hydrolysis, this monosaccharide migrated identically to fucose on TLC. Thus, recombinant viral GMER is a catalytically active, bifunctional enzyme with both epimerase and reductase activities (11).

Unexpectedly, when GMD was incubated with its substrate GDP-D-mannose and both NADP⁺ and NADPH, instead of NADP⁺ alone, an unidentified compound was detected by reverse phase HPLC (Fig. 2D, peak 4). Electrospray MS analysis of this unidentified GMD product indicated a m/z of 588.3, which is consistent with a GDP-6-deoxy-hexose (Fig. 3C). TLC analysis performed after acid hydrolysis (without NaBH₄ reduction) of this C¹⁴-labeled GDP product indicated a single compound that migrated like the 6-deoxyhexose sugar rhamnose (not shown). This same unidentified compound appeared when purified intermediate GDP-4-keto-6-deoxy-D-mannose was reincubated with GMD and NADPH. Parallel experiments established that formation of the new compound was accompanied by a stoichiometric decrease in NADPH absorbance at 340 nm (not shown). GDP-4-keto-6-deoxy-D-mannose was not converted to any other compounds when GMD or NADPH was omitted from the reaction. These findings indicate that the virus GMD also has a stereospecific NADPH-dependent reductase activity. This activity was confirmed by reconstructing the complete pathway, i.e., by adding GMD and GMER to an incubation mixture containing GDP-D-mannose, NADP⁺, and NADPH. These conditions produced both GDP-L-fucose (peak 2) and the unknown compound (Fig. 2D, peak 4). The amounts of GDP-L-fucose and the new compound depended on the relative amounts of GMD and GMER (i.e. a high GMER/GMD ratio resulted in more GDP-L-fucose formation and vice versa).

To confirm the identity of all the nucleotide sugars formed by GMD and GMER, we developed an anion exchange HPLC procedure that improved separation of the nucleotide sugars (Fig. 4). Spectra analyses indicated that each peak contained a guanine moiety. GC-MS analysis on the monosaccharides, performed after acid hydrolysis of the nucleotide sugars and conversion to the corresponding alditol acetates, confirmed that GMER activity produced GDP-L-fucose (Fig. 5A). GDP reductase activity produced only one compound with the same retention time as rhamnose (Fig. 5B). This finding establishes that GMD also acts stereospecifically on the 4-keto group of the intermediate nucleotide sugar that is generated from GDP-D-mannose. The absolute configuration of rhamnose was determined by a GC-MS procedure that differentiates chiral compounds (23). The rhamnose produced by the GMD reductase activity had a retention time identical to that of the GDP-D-rhamnose standard (21) (Fig. 5C).

The V_max of recombinant GMD in the presence of 1 mM NADP⁺ was 34.5 ± 1.2 μmol/h/mg protein for the dehydratase activity, which is similar to recombinant human GMD (18). However, unlike GMDs from other organisms, which are reported to be very unstable, PBCV-1 GMD retains complete activity when stored at either 4°C or −20°C for several weeks. Interestingly, GMD activity decreased about 3-fold if NADP⁺ was omitted from the reaction; NAD⁺ was less effective in stimulating the enzyme activity. This finding suggests that, unlike

### Table 1

Rates of GDP-L-rhamnose and GDP-L-fucose production in Chlorella extracts prepared at different time points after PBCV-1 infection.

| min postinfection | GDP-L-rhamnose (nmol/h/mg protein) | GDP-L-fucose (nmol/h/mg protein) |
|-------------------|------------------------------------|----------------------------------|
| 0                 | ND                                 | 1.2 ± 0.4                        |
| 90                | 6.9 ± 1.2                          | 6.3 ± 0.5                        |
| 120               | 12.3 ± 0.7                         | 5.0 ± 1.2                        |
| 180               | 28.4 ± 3.1                         |                                  |
| 240               | 10.9 ± 1.4                         |                                  |

Chlorella cell-free extracts (total proteins 2.5 mg/ml) prepared at various times postinfection were incubated in the presence of 100 μM GDP-D-[¹⁴C]mannose and 1 mM NADPH. GDP-D-rhamnose and GDP-L-fucose formation was determined by reverse phase HPLC analysis.

ND, not detectable.
Fig. 8. Scheme of the biosynthesis of GDP-L-fucose and GDP-D-rhamnose. PBCV-1 GMD catalyzes both the dehydration of GDP-D-mannose to the intermediate GDP-4-keto-6-deoxy-D-mannose and the NADPH-dependent reduction of this latter compound to GDP-D-rhamnose. NADP⁺ serves as cofactor for GMD during the internal oxido-reduction reaction involved in the dehydration process. The epimerization and the NADPH-dependent reduction of the 4-keto group leading to GDP-L-fucose are carried out by PBCV-1 GMER.

The results presented here demonstrate that Chlorella virus PBCV-1 encodes the genes for two functionally active enzymes involved in GDP-L-fucose biosynthesis. Enzyme activities were detected with recombinant proteins as well as in extracts from virus-infected cells, i.e., both proteins are expressed and function during viral replication in the host. PBCV-1 GMD catalyzes the dehydration of GDP-D-mannose at the C-4 and -6 positions to form the intermediate metabolite GDP-4-keto-6-deoxy-D-mannose (Fig. 8). The virus GMER behaves as a bifunctional enzyme, with an epimerization activity that leads to a change in the sugar configuration, followed by the NADPH-dependent reduction of the 4-keto group of the intermediate to form GDP-L-fucose. These GMD and GMER activities are identical to those of GMD and GMER enzymes from prokaryotes, plants, and animals (11-15).

However, the virus GMD has an additional unexpected NADPH-dependent, stereo-specific reductase activity on the 4-keto group of the intermediate. This activity creates another nucleotide sugar, GDP-D-rhamnose (Fig. 8). This novel activity indicates that PBCV-1 GMD is a bifunctional enzyme. GMDs from other organisms lack this reductase activity, with the exception of a GMD from Aneurinibacillus thermoacrisophilus (21). However, in this latter case the reductase activity only occurred with recombinant His- or glutathione S-transf erase-tagged GMD and only small amounts of GDP-D-rhamnose appeared. Accordingly, the reductase activity observed with the A. thermoacrisophilus enzyme may not occur in vivo. In fact, the bacterium has another enzyme, GDP-deoxy-D-lyxo-4-hexulose reductase (RMD), for GDP-D-rhamnose production (21). In contrast, GDP-D-rhamnose is formed in vivo after PBCV-1 infection, indicating that GMER reductase activity occurs with wild type enzyme and the activity is not an artifact of the recombinant enzyme (e.g., because of incorrect protein folding). The possibility that GDP-D-rhamnose arose in vivo by another enzyme activity encoded by the virus is excluded because no recognizable RMD homologs exist in the PBCV-1 genome. Moreover, the high reductase activity observed with both wild type and recombinant GMD, as well as its kinetic properties² suggest that GDP-D-rhamnose production by GMD is physiologically relevant.

Because both fucose and rhamnose are components of the glycan portion of PBCV-1 major capsid protein Vp54 (4), the viral GMD and GMER enzymes may be necessary to provide the virus with the appropriate nucleotide sugars. Indeed GDP-D-mannose metabolizing activities leading to GDP-D-rhamnose and GDP-L-fucose were not detected in extracts from uninfected cells. However, rhamnose and trace amounts of fucose are among the seven neutral sugars in uninfected

human GMD (18), NADP⁺ is loosely bound to the virus enzyme. GMD reductase activity (510.3 ± 10.9 μmol/h/mg protein) determined on GDP-4-keto-6-deoxy-D-mannose in the presence of NADPH, was about 10-fold higher than the dehydratase activity. The specific activity of GMER epimerase/reductase activity was 17.9 ± 0.7 μmol/h/mg protein. Unlike GMD, viral GMER was unstable, exhibiting a significant loss of activity due to precipitation from solution when maintained at both 4 °C and −20 °C.

**DISCUSSION**

The specific activity of GMER epimerase/reductase activity was determined on GDP-4-keto-6-deoxy-D-mannose in the presence of NADPH and NADP⁺ serves as cofactor for GMD during the internal oxido-reduction reaction involved in the dehydration process. The epimerization and the NADPH-dependent reduction of the 4-keto group leading to GDP-L-fucose are carried out by PBCV-1 GMER.

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Chlorella NC64A cell walls (27). Presumably the host is unable to supply enough of these two monosaccharides for virus production.

To summarize, the results in this report have three unique aspects. (i) This is the first report of a virus encoding enzymes involved in the synthesis of nucleotide sugars. (ii) Unlike GMD enzymes from other organisms, the virus-encoded GMD is a bifunctional enzyme. (iii) Unlike GMD enzymes from other organisms, the virus recombinant GMD is very stable.

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Michela Tonetti, Davide Zanardi, James R. Gurnon, Floriana Fruscione, Andrea Armirotti, Gianluca Damonte, Laura Sturla, Antonio De Flora and James L. Van Etten

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