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Biochemical characterization of GDP-L-fucose *de novo* synthesis pathway in fungus *Mortierella alpina*

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ABSTRACT

Mortierella alpina is a filamentous fungus commonly found in soil, which is able to produce large amount of polyunsaturated fatty acids. L-Fucose is an important sugar found in a diverse range of organisms, playing a variety of biological roles. In this study, we characterized the *de novo* biosynthetic pathway of GDP-L-fucose (the nucleotide-activated form of L-fucose) in *M. alpina*. Genes encoding GDP-D-mannose 4,6-dehydratase (GMD) and GDP-keto-6-deoxymannose 3,5-epimerase/4-reductase (GMER) were expressed heterologously in *Escherichia coli*. The recombinant enzymes were produced as His-tagged fusion proteins. Conversion of GDP-mannose to GDP-4-keto-6-deoxy mannose by GMD and GDP-4-keto-6-deoxy mannose to GDP-L-fucose by GMER were analyzed by capillary electrophoresis, electro-spray ionization-mass spectrometry, and nuclear magnetic resonance spectroscopy. The k_m values of GMD for GDP-mannose and GMER for GDP-4-keto-6-deoxy mannose were determined to be 0.77 mM and 1.047 mM, respectively. Both NADH and NADPH may be used by GMER as the coenzyme. The optimum temperature and pH were determined to be 37 °C and pH 9.0 (GMD) or pH 7.0 (GMER). Divalent cations are not required for GMD and GMER activity, and the activities of both enzymes may be enhanced by DTT. To our knowledge this is the first report on the characterization of GDP-L-fucose biosynthetic pathway in fungi.

Introduction

L-Fucose (6-deoxy-L-galactose) is an important monosaccharide found in both prokaryotes and eukaryotes. It is a sugar component of bacterial lipopolysaccharides [1], and cell wall polysaccharides in plants [2] and some fungi of specific taxa [3–5]. L-Fucose is also a common component of sugar moiety in glycoconjugates and frequently exists as a terminal glycosylation residue [6]. Glycoconjugates including glycoproteins and glycolipids are known to play many important biological roles such as recognition and signaling, protein folding and conformation, and membrane stabilization [7–8].

Fucosylation is carried out by fucosyltransferases, which require the activated nucleotide form of L-fucose (GDP-L-fucose) as the substrate. GDP-L-fucose may be synthesized from GDP-D-mannose via a *de novo* pathway or from L-fucose in a salvage pathway. The salvage pathway, operated by fucose kinase (FUK) and GDP fucose pyrophosphorylase (GFPP), is mostly found in animals and plants, and is mainly used for the recycling of L-fucose derived from cellular fucose-containing macromolecules [6]. In the *de novo* pathway (Fig. 1), GDP-D-mannose is converted to GDP-4-keto-6-deoxy-D-mannose by GDP-D-mannose 4,6-dehydratase (GMD) and then to GDP-L-fucose by GDP-keto-6-deoxymannose 3,5-epimerase/4-reductase (GMER), a bifunctional enzyme with epimerase and reductase activities [6]. The function of GMD and GMER from *Homo sapiens* [9–10], *Helicobacter pylori* [11], *Escherichia coli* [12–13], *Caenorhabditis elegans* and *Drosophila melanogaster* [14], *Paramecium bursaria Chlorella Virus* [15] and *Arabidopsis thaliana* [16] have been characterized *in vitro*.

Mortierella alpina is a well-known polyunsaturated fatty acids (PUFA)-producing oleaginous fungus commonly found in soil, and is one of industrial species for PUFA production [17]. Recently, we have sequenced the whole genome of *M. alpina* (ATCC 32222) (separate study). Sequence analysis reveals the presence of the *de novo* pathway for the synthesis of GDP-L-fucose in *M. alpina*. In this study, we characterized the function of GMD and GMER from *M.*

Abbreviations: GMD, GDP-D-mannose 4,6-dehydratase; GMER, GDP-keto-6-deoxymannose 3,5-epimerase/4-reductase; COSY, correlation spectroscopy; HSQC, heteronuclear single-quantum coherence; HMQC, heteronuclear multi-quantum coherence; TOCSY, total correlation spectroscopy

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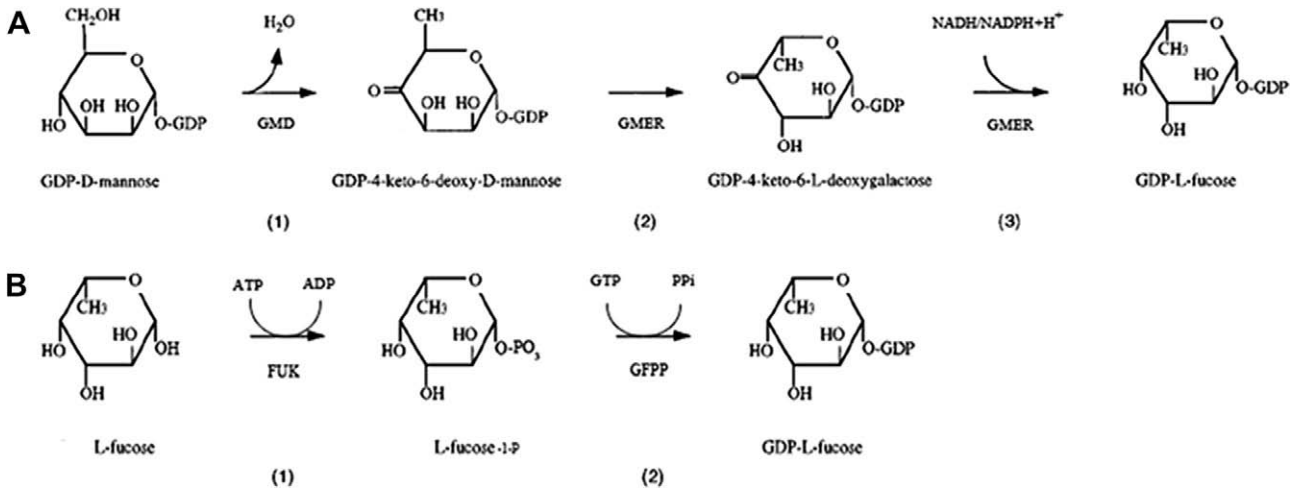

Fig. 1. *De novo* (A) and salvage (B) synthesis pathways of GDP-L-fucose.

alpina, in vitro. Kinetic parameters and some other properties of GMD and GMER were examined. Comparative analyses of the *M. alpina* GMD and GMER proteins with other homologous proteins were carried out, and physiological role of L-fucose in *M. alpina* are discussed.

Materials and methods

Strains and growth conditions. Mortierella alpina (American Type Culture Collection Catalog No. 32222) was cultured on potato dextrose agar at 25 °C for 5–7 days. When the growth was visible, the culture was inoculated into 200 ml of the medium containing 100 g/L glucose, 5 g/L yeast extract, 3 g/L $NaNO_3$, 1 g/L KH_2PO_4, and 0.5 g/L $MgSO_4{\cdot}7H_2O$, and incubated at 25 °C for 4 days with shaking. The mycelia were collected by filtration through 10 layers of sterile cheesecloth, and immediately frozen into liquid nitrogen.

Cloning and plasmid construction. Total RNA was isolated using Trizol reagent (Invitrogen). The genes encoding GMD and GMER were cloned by RT-PCR. Reverse-transcription of mRNA into cDNA was carried out with oligo(dT) primer using Superscript II reverse transcriptase (Invitrogen). PCR was performed with the primer pair GCGAATTCATGTCTTCTCCTATTGAG-3′/5′-CTGAAGCTTTTAGTTGAAGACATCG-3′ (GMD) or 5′-CAGAATTCATGTCTCCCTCAAAGTC-3′/5′-CATAAGCTTTTACTTGCGGATGGT-3′ (GMER) (restriction sites underlined), and the PCR condition used was: denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s and extension at 72 °C for 2 min, 25 cycles, in a final volume of 50 μl. The amplified products were cloned into pET28a$^+$ to construct pLW1534 (containing GMD) or pLW1535 (containing GMER). Presence of the inserts in the plasmids was confirmed by sequencing using an ABI 3730 Sequencer.

Protein expression and purification. Escherichia coli BL21 (DE3) carrying pLW1534 or pLW1535 was grown in LB medium containing 50 μg/ml kanamycin overnight at 37 °C with shaking. The overnight culture was inoculated into 500 ml of fresh medium and grown to OD_{600} 0.6. Expression of GMD was induced by 1 mM IPTG at 25 °C for 4 h, and expression of GMER was induced by 0.05 mM IPTG at 16 °C for 5 h. After the IPTG induction, cells were harvested by centrifugation, washed with binding buffer (50 mM Tris–HCl, pH 8.0, 300 mM NaCl, and 10 mM imidazole), resuspended in the same buffer containing 1 mM phenylmethanesulfonyl fluoride and 1 mg/ml lysozyme, and sonicated (Ultraschallprozessor UP200S). The cell debris was removed by centrifugation, and the supernatant containing soluble proteins was collected. The His_6-tagged fusion proteins were purified by nickel ion affinity chromatography with a Chelating Sepharose Fast Flow column (GE Healthcare) according to the manufacturer's instruction. Unbound proteins were washed out with 100 ml of wash buffer (50 mM Tris–HCl, pH 8.0, 300 mM NaCl, and 25 mM imidazole). Fusion proteins were eluted with 3 ml of elution buffer (50 mM Tris–HCl, pH 8.0, 300 mM NaCl, and 250 mM imidazole), and dialyzed overnight against 50 mM Tris–HCl buffer containing 20% glycerol (pH 7.4) at 4 °C. Protein concentration was determined by the Bradford method. Purified proteins were stored at −80 °C.

Enzyme activity assays. The reaction mixture for GMD contained 50 mM Tris–HCl (pH 8.0), 1 mM GDP-D-mannose (Sigma–Aldrich), 1.5 mM NAD$^+$ or NADP$^+$ (Sigma–Aldrich), 1 μM of purified GMD, in a total volume of 10 μl. The reaction mixture for GMER contained 10 μl of the GMD reaction mixture to supply the substrate for GMER, 50 mM Tris–HCl (pH 8.0), 2.5 mM NADH or NADPH (Sigma–Aldrich), 0.15 μM of purified GMER, in a total volume of 20 μl. Reactions were carried out at 37 °C for 30 min unless otherwise specified before terminated by adding equal volume of chloroform. GMD and GMER products were collected by centrifugation for 5 min at 12000 rpm on an Eppendorf Centrifuge 5810R, and subjected to CE, ESI-MS and NMR analysis. Enzyme activity was indicated by the conversion of the substrate to the product.

CE analysis. Capillary electrophoresis (CE) was performed using a Beckman Coulter P/ACE MDQ Capillary Electrophoresis System equipped with a PDA detector (Beckman Coulter, CA). The capillary was bare silica of 75 μm × 57 cm, with the detector at 50 cm. The capillary was conditioned before each run by washing with 0.1 M NaOH first, and then 25 mM borate-sodium hydroxide (pH 10.0) (used as the mobile phase) for 5 min each. Samples were loaded by pressure injection at 0.5 p.s.i. for 10 s, and electrophoresis was carried out at 20 kV. Peak integration and trace alignment were performed with the Beckman P/ACE station software (32 Karat versions 5.0). Conversion ratio was calculated from peak areas of substrate and product.

Reverse-phase HPLC and ESI-MS analysis. The GMD and GMER reaction product were purified by reverse-phase HPLC using a BioCAD 700E Perfusion Chromatography Workstation (Applied Biosystems, CA) with a Venusil MP-C18 column (5 μm particle, 4.6 mm × 250 mm) (Agela Technologies). The mobile phase used was composed of 4% of acetonitrile and 96% of 50 mM triethylamineacetatic acid (pH 6.8). The flow rate was 0.6 ml/min. Fractions containing expected products were collected, lyophilized and re-dissolved in methanol before injecting into a Finnigan LCQ Advantage MAX ion trap mass spectrometer (Thermo Electron, CA) at negative mode (4.5 kV, 250 °C) for ESI-MS analysis. For MS2

and MS3 analyses, nitrogen was used as collision gas and helium as auxiliary gas and collision energies used were typically 20–30 eV.

NMR spectroscopy and chemical analysis. A sample of purified GDP-β-L-Fucp (0.2 mg) was deuterium-exchanged by freeze-drying from 99.9% ^2H_2O, dissolved in 99.96% ^2H_2O (150 μL) and examined using a Shigemi (Japan) microtube. NMR spectra were recorded on a Avance 600 NMR spectrometer (Bruker, Germany) at 30 °C using internal sodium trimethylsilyl-[2,2,3,3–2H4] propanoate (δH 0.00), acetone (δC 31.45) and external aqueous 85% H_3PO_4 (δP 0) as references. Two-dimensional NMR spectra were obtained using standard pulse sequences from the manufacturer, and TopSpin program (Bruker) was used to acquire and process the NMR data.

GDP-β-L-Fucp was hydrolyzed with 2 M trifluoroacetic acid (100 °C, 2 h) and L-fucose was identified by GLC of the acetylated (*S*)-2-octyl glycosides [18] using a Hewlett–Packard 5880 instrument equipped with an Ultra-2 column (0.20 mm × 25 m) and a temperature program of 160 (3 min) to 290 °C at 3 °C/min.

Measurement of kinetic parameters. To measure the k_m and V_{max} value of GMD for GDP-mannose, reactions were carried out with various concentrations of GDP-mannose (0.4–1.5 mM), and fixed concentration of NAD^+ (2 mM) and GMD (1.13 μM). To measure the k_m and V_{max} value of GMER for GDP-4-keto-6-deoxy mannose, reactions were carried out with various concentrations of GDP-4-keto-6-deoxy mannose (0.5–1.5 mM), and fixed concentration of NADPH (2.5 mM) and GMER (0.10 μM). To measure the k_m and V_{max} values of GMER for NADH and NADPH, reactions were carried out with various concentrations of NADH or NADPH (0.3–2 mM) and fixed concentration of GDP-4-keto-6-deoxy mannose (1 mM) and GMER (0.17 μM). All reactions were carried out at 37 °C for 5 min in a final volume of 20 μl. Conversion of GDP-mannose to GDP-4-keto-6-deoxy mannose, and GDP-4-keto-6-deoxy mannose to GDP-L-fucose was monitored by CE analysis. k_m and V_{max} values were calculated based on the Michaelis–Menten equation.

Determination of temperature and pH optima, divalent cations requirements and protein reducing reagent effect. To determine the temperature optima for GMD and GMER, reactions were carried out at different temperatures (15, 25, 37, 50, and 55 °C), pH 8.0, for 15 min. To determine the pH optima, reactions were carried out at different pH values (pH 5–12), 37 °C, for 15 min. To test the effects of divalent cations and protein reducing reagent on enzyme activity, reactions were carried out in the presence of 5 mM of $MgCl_2$, $MnCl_2$, $FeCl_2$, $CuCl_2$, $CaCl_2$, $CoCl_2$, or DTT at 37 °C, pH 8.0, for 10 min.

Nucleotide sequence accession numbers. The complete gene sequences of GDP-D-mannose 4,6-dehydratase (GMD) and GDP-keto-6-deoxymannose 3,5-epimerase/4-reductase (GMER) have been deposited at GenBank under the accession numbers GU299800 and GU299801.

Results

Expression and purification of GMD and GMER

GMD and GMER from *Mortierella alpina* were expressed as His-tagged fusion proteins in *E. coli* BL21 by IPTG induction, and purified to near homogeneity by nickel ion affinity chromatography (Sup. Fig. 1A). The molecular masses estimated by SDS–PAGE analyses were 45 kDa for GMD and 40 kDa for GMER, corresponding

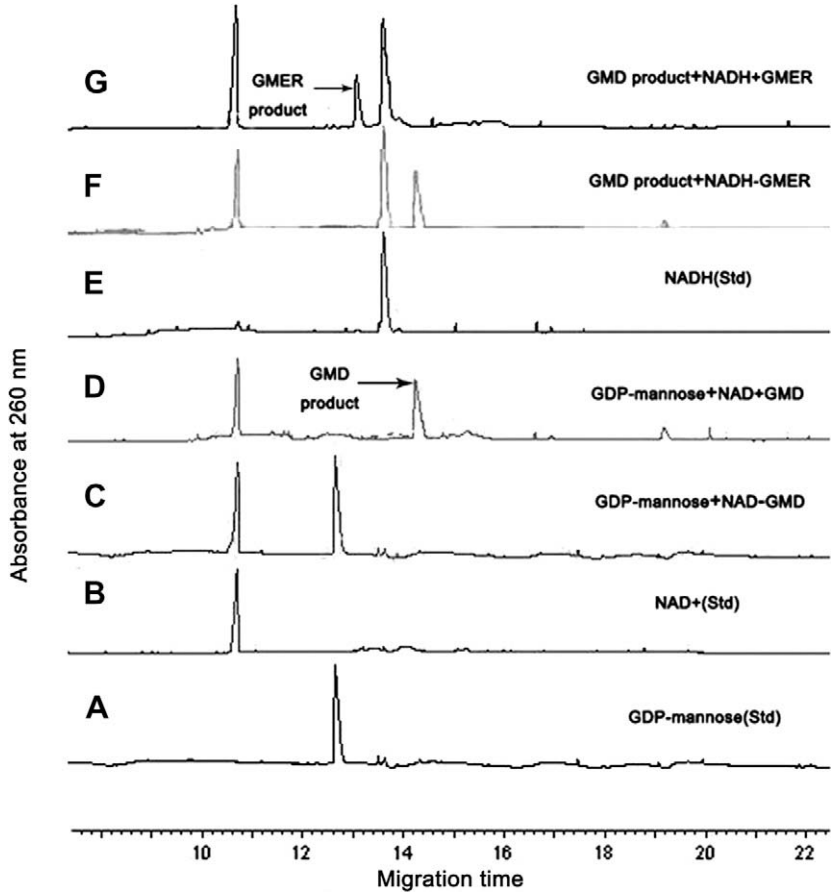

Fig. 2. CE chromatographs of GMD and GMER reaction products. Shown are GDP-D-mannose standard (A); NAD^+ standard (B); GMD reaction without GMD (C); GMD reaction with GMD (D); NADH standard (E); GMER reaction without GMER (F); and GMER reaction with GMER (G).

well to the calculated masses (41.2 kDa and 36.4 kDa plus His tag). The molecular masses estimated by native-PAGE were 140 Kb for GMD and 232 Kb for GMER, indicative of a trimeric and a hexameric protein, respectively (Sup. Fig. 1B).

CE analysis of GMD and GMER products

As shown in CE chromatographs (Fig. 2), GDP-mannose identified as the peak eluted at 12.8 min was converted to a peak eluted at 14.1 min in the reaction catalyzed by GMD in the presence of NAD^+ as the electrons and hydrogen ions carrier (Fig. 2). The same result was obtained when $NADP^+$ was used instead of NAD^+ (data not shown). In the reaction catalyzed by GMER, the 14.1 min peak (GMD product) was converted to a new peak eluted at 13.2 min, accompanied by the conversion of NADH to NAD^+ as judged by increased NAD^+/NADH ratio (Fig. 2), and the same was observed when NADPH was used instead of NADH (data not shown). Therefore, GMER possesses NAD(P)H-dependent reductase activity. In either reaction catalyzed by GMD or GMER, 100% of the conversation rate could be achieved.

Table 1
1H and 13C NMR chemical shifts of GDP-β-L-Fucp (δ, ppm). The H8/C8 correlation of guanine is at δ 8.1/138.5.

Sugar residue	Nucleus	Atom					
		1	2	3	4	5	6
β-Fucp	^{1}H	4.92	3.56	3.65	3.70	3.76	1.23
	^{13}C	98.8	72.4	73.9	72.5	72.8	16.8
β-Ribf	^{1}H	5.93	4.81	4.54	4.35	4.21 (2H)	
	^{13}C	88.1	74.7	71.9	84.2	66.7	

Analysis of GMD and GMER products by electro-spray ionization–mass spectrometry

The GMD (the 14.1 min peak) and GMER (the 13.2 min peak) products were purified by reverse-phase HPLC (data not shown), and analyzed by ESI-MS. Ion peaks at *m/z* 586.10 from the GMD product and *m/z* 588.19 from the GMER product were obtained, corresponding well to the masses for GDP-4-keto-6-deoxymannose (*m/z* 586) and GDP-fucose (*m/z* 588), respectively [16]. MS/MS (MS2) analysis of the two ion peaks (586.10 and 588.19) and the follow-up MS/MS/MS (MS3) analysis of selected MS2 ion peak (442.0 and 442.06) resulted in the detection of ion peaks matching the fragments derived from GDP-4-keto-6-deoxy mannose and GDP-fucose, respectively (Sup. Fig. 2A). Fragments corresponding to each peak are depicted in Sup. Table 1.

Analysis of GMER product by NMR

The identity of the GMER product as GDP-L-fucose was structurally confirmed by NMR analysis. Fucose was identified and its L-configuration was confirmed by GLC analysis of the acetylated (*S*)-2-octyl glycosides, which were derived after full acid hydrolysis of GDP-β-L-Fucp.

A sample of GDP-β-L-Fuc was studied by two-dimensional shift-correlated 1H, 1H, 1H, 13C and 1H, 31P NMR spectroscopy. The 1H NMR chemical shifts were assigned using 1H, 1H COSY, and TOCSY experiments (Table 1). In the TOCSY spectrum there were correlations of H1 with H2–H4 and H5 with H6 of fucopyranose; H1' with H2'–H4' and H3' with H4' and H5' of ribofuranose. Coupling constant values determined from the 1H NMR spectrum, including J1,2 7.8 and J4,5 < 1 Hz, confirmed the β-galacto configuration of

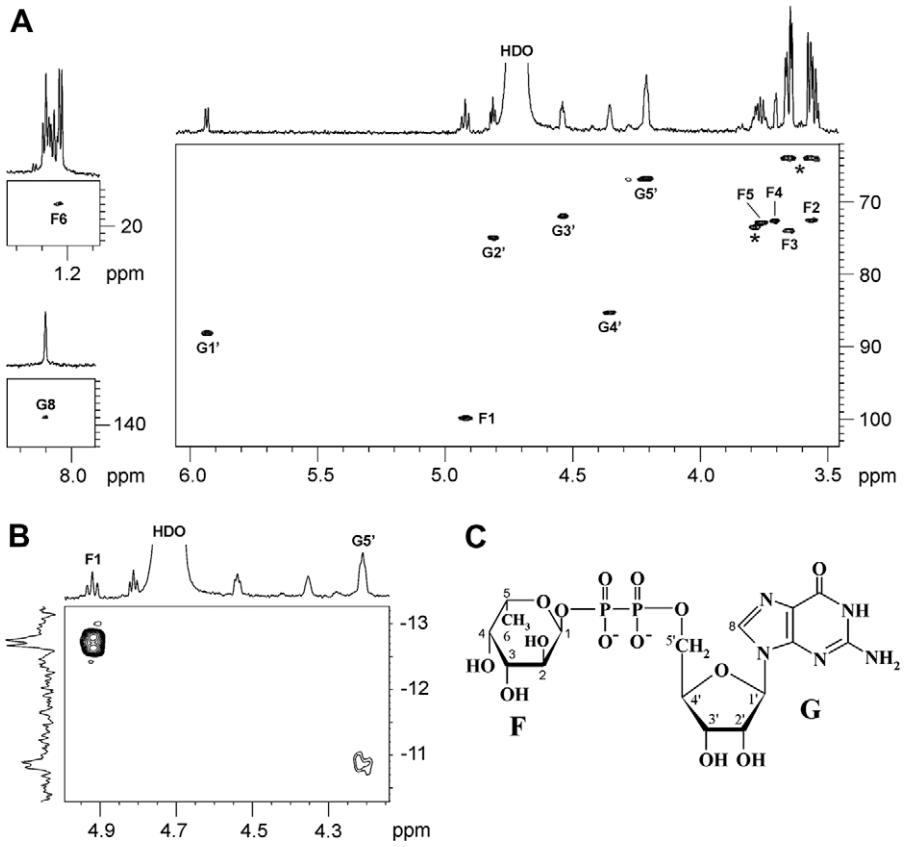

Fig. 3. (A) Parts of a 1H,13C HSQC spectrum, (B) 1H,31P HMQC spectrum, and (C) structure of GDP-β-L-Fucp. Arabic numerals refer to atoms in Fuc and GDP designated as F and G, respectively. Signals of contaminating substances from HPLC buffer are marked with asterisk.

the pyranose sugar, which is thus β-Fucp. A 1H,13C HSQC experiment (Fig. 3A) revealed proton-to-carbon correlations for Fuc, Rib and guanine, and the assigned 13C NMR chemical shifts (Table 1) were in good agreement with published data for GDP [19] and β-Fucp [20]. The 31P NMR spectrum contained signals for a diphosphate group at -10.9 and -12.7, which showed correlations with H5' of GDP at -10.9/5.93 and H1 of β-Fucp at -12.7/4.92 in the 1H,31P HMQC spectrum (Fig. 3B). These data together confirmed finally the structure of GDP-β-L-Fucp as depicted in (Fig. 3C).

Kinetic parameters of GMD and GMER

Kinetic parameters of GMD (for GDP-mannose) and GMER (for GDP-4-keto-6-deoxy mannose, NADH and NADPH) were measured. The initial velocities were determined and used for the calculation of kinetic parameters. The kinetics of the reactions catalyzed by both GMD and GMER fit well to the Michaelis–Menten model, and the kinetic parameters are listed in Table 2. GMER showed similar affinities to NADH and NADPH, but the Vmax value was higher for NADPH (0.024 versus 0.012 mM/min). Therefore, NADPH is a better coenzyme for GMER.

Effects of temperature, pH, divalent cations and protein reducing reagent on the activity of GMD and GMER

GMD and GMER activities were detected at 15 to 55 °C and pH 5.0 to 11.0 (Fig. 4A & B). The optimum temperature was 37 °C for both enzymes, and the optimum pH was pH 9.0 for GMD or pH 7.0 for GMER. GMD activity was significantly enhanced by Mg^{2+}, strongly inhibited by Fe^{2+}, Cu^{2+} and Co^{2+}, but not affected by Ca^{2+}

Table 2
Kinetics parameters. The data shown are the average from three independent experiments.

Enzyme	Substrate	K_m (mM)	V_{max} (mM/min)	K_{cat}/min
GMD	GDP-D-mannose	0. 77 ± 0.007	0.041 ± 0.002	357.71 ± 18.73
GMER	GDP-4-keto-6-deoxy-D-mannose	1.047 ± 0.015	0.033 ± 0.002	1664.72 ± 106.08
GMER	NADPH	1.025 ± 0.054	0.024 ± 0.002	698.89 ± 58.24
GMER	NADH	1.157 ± 0.089	0.012 ± 0.002	334.89 ± 61.77

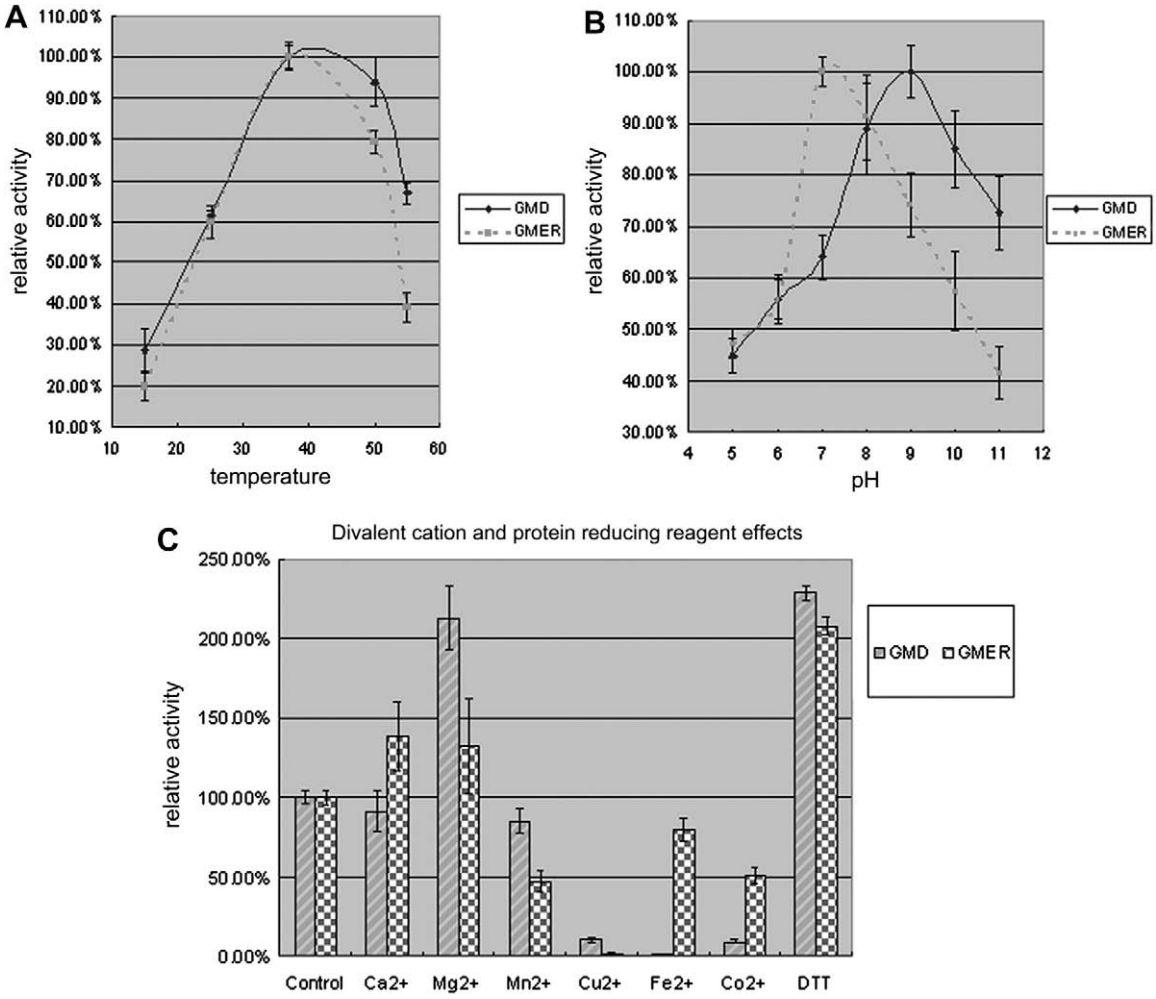

Fig. 4. Effects of temperature (A), pH (B), divalent cations and DTT (C) on the activities of GMD and GMER. 100% GMD activity represents 0.59 (A), or 0.78 (B), or 0.58 enzyme unit (C). 100% GMER activity represents 0.98 (A), 1.15 (B), or 0.81 enzyme unit (C). One enzyme unit is defined as the amount of purified enzyme (mg) required for the conversion of 100 μmol GDP-mannose to GDP-4-keto-6-deoxymannose (GMD), or for the conversion of 200 μmol GDP-4-keto-6-deoxymannose to GDP-fucose (GMER) per hour. The data shown are the average from three independent experiments.

and Mn^{2+}. GMER activity was slightly enhanced by Ca^{2+} and Mg^{2+}, completely inhibited by Cu^{2+}, and inhibited to different degrees by Co^{2+}, Fe^{2+}, and Mn^{2+} (Fig. 4C). DTT enhanced GMD activity by 2.3 folds and GMER activity by 2.1 folds (Fig. 4C). Enhancement of GMD and GMER activity by DTT was also reported for other characterized homologous proteins such as *Helicobacter pylori* enzymes [11].

Discussion

GMD shares 54 to 72% identities to other characterized GMD proteins, and is most similar to *Homo sapiens* GMD. Multiple sequence alignment of *M. alpina* GMD and other characterized GMD proteins reveals the presence of all conserved motifs and residues described for GMD in *M. alpina* GMD (Sup. Fig. 3A), including a Gly-X-X-Gly-X-X-Gly motif at the N terminus involved in co-factor binding [21], 7 conserved residues at C terminus (Asn201, Val212, Lys215, Arg240, Leu233, Val274, and Tyr315) involved in sugar-nucleotide binding [21], Thr148-Tyr172-Lys176 catalytic triad, and Glu150 at active-site [21]. GMER shares 27 to 61% identity to other characterized GMER proteins, and is most similar to *D. melanogaster* GMER. Multiple sequence alignment of the GMER proteins reveals the presence of all conserved motifs and residues described for GMER in *M. alpina* GMER (Sup. Fig. 3B), including Gly-X-X-Gly-X-X-Gly motif at the N terminus for co-factor binding [22], residues (Asn173, Val 188, Leu192, Lys195, Arg209, and Lys216) involved in sugar-nucleotide binding and residues (Asn80, Ser115, Thr116, Cys117, Asn141, Tyr144, His187) involved in 4-keto-sugar interaction [22], and Ser115-Tyr144-Lys148 catalytic triad and other residues (Ser186, Cys117, and His187) involving in catalysis [22]. Therefore, both proteins are well conserved in diverse array of organisms from mammals, invertebrate animals, plants, bacteria, and fungi.

Apart from GMD and GMER genes for the *de novo* pathway, genes encoding fucose kinase (FUK) and GDP fucose pyrophosphorylase (GFPP) required for free fucose-dependent salvage pathway, and a putative fucosyltransferase gene (FUT) responsible for the attachment of L-fucose to other molecules were also found in the genome of *M. alpina*. Based on information from Kyoto Encyclopedia of Genes and Genomes [23], the salvage pathway is rarely found in microorganisms, and the only reported such case is from human symbionts *Bacterioides* [24]. None of the fungal species was found to contain both pathways. Therefore, the presence of both the *de novo* and the salvage pathway in *M. alpina* is of interests, indicating important roles played by L-fucose in this fungus.

In contrast to the presence of D-glucose, *N*-acetylglucosamine, and D-mannose as the major cell wall polysaccharide sugars in all fungi, fucose is only found in a limited fungi species at very low amount as backbone chain modification, and is mostly found in Zygomycetes composed of members from Mortierellales and several other orders [3]. Although not directed proven experimentally, as a member of Mortierella, *M. alpina* cell wall polysaccharide is expected to contain L-fucose [3].

Protein fucosylation is a common type of post-translational glycosylation present in both eukaryotic and prokaryotic organisms [25]. Fucosylation of *cis*-9, *trans*-11-octadecadienoate reductase enabled the protein to be anchored properly into the membrane to function in lipid metabolism in bacterium *Butyrivibrio fibrisolvens* [26]. Fucosylation of spore coat proteins such as SP96 might convey a cell signaling function during spore formation and germination in fungus *Phycomyces blakesleeanus* [4] and *Dictyostelium discoideum* [27]. On the other hand, L-fucose is one of the major sugars found in glycolipids [8], and the presence of this sugar on lipid bodies was observed in ascomycete *Ascocalyx abietina* [28]. *M. alpina* is noteworthy for the accumulation of lipids and the production of glycolipids such as cerebrosides [29]. It is speculated that fucosylation of proteins and/or lipids may also occur in *M. alpina*, and play important cellular roles, especially in lipid metabolism.

To the best of our knowledge, this is the first report on the characterization of the GDP-L-fucose biosynthesis pathway in fungi. The role of L-fucose in *M. alpina* is worthy to be further investigated.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.12.116.

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