Carbohydrate Research 373 (2013) 76–81

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

One-pot three-enzyme synthesis of UDP-Glc, UDP-Gal, and their derivatives

Yang Zou [a,†], Mengyang Xue [a,†], Wenjun Wang [b,†], Li Cai [c], Leilei Chen [d], Jun Liu [a], Peng George Wang [a], Jie Shen [b,*], Min Chen [a,*]

[a] The State Key Laboratory of Microbial Technology and National Glycoengineering Research Center, Shandong University, 27# Shanda South Road, Jinan, Shandong 250100, China
[b] College of Pharmacy and the State Key Laboratory of Medicinal Chemical Biology, Nankai University, Tianjin 300071, China
[c] University of South Carolina Salkehatchie, Walterboro, SC 29488, USA
[d] Institute of Agro-Food Science & Technology, Shandong Academy of Agricultural Sciences, Jinan 250100, China

ARTICLE INFO

Article history:
Received 30 December 2012
Received in revised form 26 February 2013
Accepted 6 March 2013
Available online 15 March 2013

Keywords:
One-pot reaction system
Three enzymes
UDP-Glc/Gal derivatives

ABSTRACT

A UTP-glucose-1-phosphate uridylyltransferase (SpGalU) and a galactokinase (SpGalK) were cloned from *Streptococcus pneumoniae* TIGR4 and were successfully used to synthesize UDP-galactose (UDP-Gal), UDP-glucose (UDP-Glc), and their derivatives in an efficient one-pot reaction system. The reaction conditions for the one-pot multi-enzyme synthesis were optimized and nine UDP-Glc/Gal derivatives were synthesized. Using this system, six unnatural UDP-Gal derivatives, including UDP-2-deoxy-Galactose and UDP-$GalN_3$ which were not accepted by other approach, can be synthesized efficiently in a one pot fashion. More interestingly, this is the first time it has been reported that UDP-Glc can be synthesized in a simpler one-pot three-enzyme synthesis reaction system.

1. Introduction

Carbohydrates widely existed in many glycosylated natural products and glycoconjugates. They also participate in many biological processes.[1] Sugar nucleotides are often required as the common glycosyl donor substrates in the carbohydrate biosynthesis pathways.[2,3]

Two important sugar nucleotides, UDP-glucose (UDP-Glc) and UDP-galactose (UDP-Gal), participate in many biological processes, such as the biosynthesis of the cell envelope of *Escherichia coli*,[4] capsular polysaccharide,[5] lipopolysaccharide,[6,7] and other membrane-derived oligosaccharides.[8] Some UDP-Gal derivatives may also play an important role in many biological processes. For example, UDP-2-deoxy-galactose has been shown to compete with natural UDP-galactose for incorporation into glycan chains, preventing the 1→2 fucosylation of glycoproteins which have been implicated in cognitive processes such as long-term memory.[9] Another UDP-Gal derivative, UDP-$GalN_3$, could be potentially used for metabolic oligosaccharide engineering combined with bioorthogonal reactions.[10]

In vitro, monosaccharide nucleotides can be obtained by chemical[11,12] and enzymatic approaches, or by extraction from microbial cells.[13] Various chemical methods have been reviewed and eloquently discussed.[12] Recently, a new method for the chemical synthesis of sugar-NDPs in a one-pot reaction in water was reported.[14] Compared with chemical methods, the enzymatic synthesis of sugar-NDPs is less complex.[1]

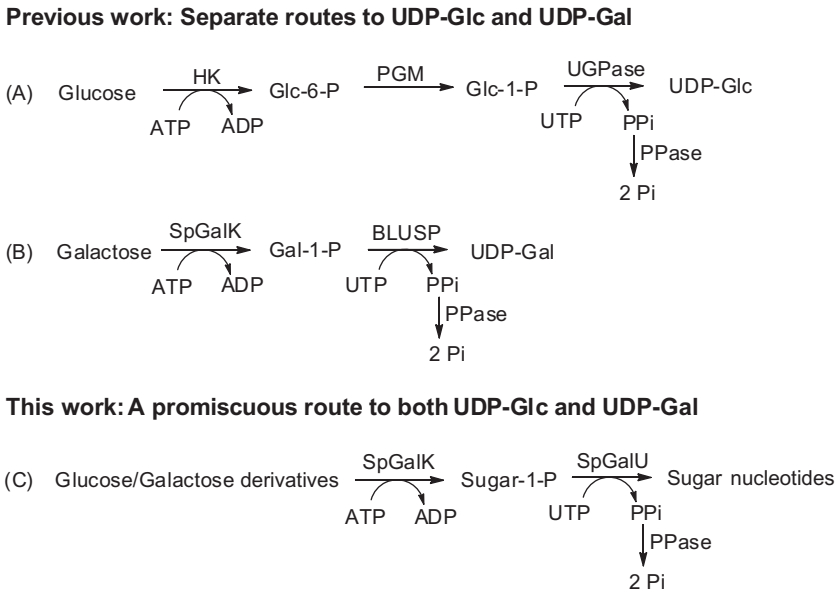
Many enzymatic approaches to synthesize UDP-Glc/Gal have been reported.[15,16] To this point, the simplest approach to obtain UDP-Glc was a four enzyme system in which Glc was converted into UDP-Glc by the action of four enzymes: hexokinase (HK), phosphoglucomutase (Pgm), UDPG pyrophosphorylase (UDPGase), and pyrophosphatase (PPa) (Scheme 1A).[17–19] Unfortunately, the co-factor Mg^{2+}, which was necessary for HK, was an inhibitor of Pgm.[20] On the other hand, however, UDP-Gal has to be prepared from another sugar nucleotide (i.e., UDP-Glc)[21,22] since enzymatic pyrophosphorylation of Gal-1-phosphate was not available until recently. In addition, due to the difficulties involved in the preparative synthesis and purification of UDP-Gal, some researchers have to conjugate the UDP-Gal synthesis to a galactosyltransferase catalyzed reaction.[23,24]

Chen and co-workers reported the simplest route for the formation of UDP-Gal and its derivatives. A promiscuous UDP-sugar pyrophosphorylase (BLUSP) was cloned from *Bifidobacterium longum* and used efficiently in an efficient one-pot three-enzyme system for synthesis of UDP-monosaccharides and their derivatives from simple monosaccharides or derivatives (Scheme 1B).[2]

Virous UDP-galactose pyrophosphorylase activity was identified. However, few natural derivatives of monosaccharide-1-P as

* Corresponding authors. Tel.: +86 531 88366078 (M.C.).
E-mail addresses: jieshen@nankai.edu.cn (J. Shen), chenmin@sdu.edu.cn (M. Chen).
† These authors contributed equally to this work.

0008-6215/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carres.2013.03.005



Scheme 1. (A) One-pot four-enzyme synthesis of UDP-Glc; HK, hexokinase; PGM, phosphoglucomutase; UGPase, uridine-5′-diphosphoglucose pyrophosphorylase; PPase, inorganic pyrophosphatase; (B) One-pot three-enzyme synthesis of UDP-Gal; SpGalK, *Streptococcus pneumoniae* TIGR4 galactokinase; BLUSP, *Bifidobacterium longum* UDP-sugar pyrophosphorylase; PmPpA, *Pasteurella multocida* inorganic pyrophosphatase; (C) efficient one-pot three-enzyme synthesis of UDP-Glc, UDP-Gal, and their derivatives (work in this paper); SpGalK, *Streptococcus pneumoniae* TIGR4 galactokinase; SpGalU, *Streptococcus pneumoniae* TIGR4 UDP-Glc pyrophosphorylase; PPase, inorganic pyrophosphatase.

substrates for USPs have been tested.[2]Given that limited methods were available for the preparation of UDP-Gal, especially its useful derivatives, we introduced herein an efficient one-step three-enzyme catalytic system (Scheme 1C) for the synthesis of UDP-Gal. Using this system, UDP-Glc and six unnatural UDP-Gal derivatives, including UDP-2-deoxy-Galactose and UDP-GalN_3 which were not accepted by Chen's novel pyrophosphorylase (USPs), could be synthesized efficiently in a one pot fashion.

In this optimized system as shown in Scheme 1C, glucose or galactose is first phosphorylated by a galactokinase and the resulting sugar-1-phosphate is subsequently pyrophosphorylated with UTP by a uridine diphosphate glucose pyrophosphorylase. Both reactions are carried out in a one-pot fashion. It is worth noting that both the sugar kinase (GalK, EC 2.7.1.6) and the pyrophosphorylase (GalU, EC 2.7.7.9) are cloned from the same bacteria, *Streptococcus pneumoniae* TIGR4. A commercial inorganic pyrophosphatase (PPase, EC 3.6.1.1) was added to drive the reaction forward by degrading the by-product pyrophosphate (PPi) formed from uridylyltransferase[25] because the accumulation of PPi may inhibit the enzymatic activity of SpGalU.

In our previous work, we fully characterized the pneumococcal galactokinase (GalK),[26] which provided us a library of Glc/Gal-1-phoshate analogs. In addition, some of the biochemical characters of the pneumococcal glucose 1-phosphate uridylyltransferase (GalU) have also been characterized in previous reports.[27]

2. Results and discussion

2.1. Optimization of the reaction conditions

In this study, we first optimized the three-enzyme synthesis reaction conditions in order to improve the yield of UDP-Glc/Gal and their derivatives. Products were purified using the combination of size exclusion chromatography and silica gel chromatography.

Prior to applying the system shown in Scheme 1, SpGalK and SpGalU were also proved to be active by synthesis of Gal-1-P and UDP-Glc, respectively. The effects of different buffer and pH value on the activity of SpGalK and SpGalU were investigated. pH profile study of UDP-Glc revealed that its catalytic activity was optimum in a relatively narrow pH range of 7.0–8.5 and the highest relative activity of these enzymes appeared when the pH value was 8.0 in Tris–HCl buffer. Extremely low activity was observed when the pH of the reaction reached below 6.5 or higher than 8.5 (Fig. 1). For SpGalK, it preferred pH higher than 7.0 for optimum activity, showed optimum activity at pH 8.0 and low activity at pH lower than 7.0 or higher than 8.5.[26] For SpGalU, it had an optimum pH of 8.0–8.5.[28] Therefore, pH 8.0 would be optimum for the one-pot three-enzyme reactions. Interestingly, both of them have the same pH profile for activity in this one pot system as that previously reported.[26,27]

In addition, the effect of temperature on the activity of SpGalK and SpGalU was investigated. It was found that the optimal temperature (Fig. 2) for this one-pot three-enzyme reaction system was 42 °C. For SpGalK, it preferred 40–45 °C for optimum activity.

Both SpGalK and SpGalU need divalent cation Mg^{2+} as co-factor, different concentrations of Mg^{2+} ranging from 0 to 50 mM were added in the reaction mixture and the optimum concentration

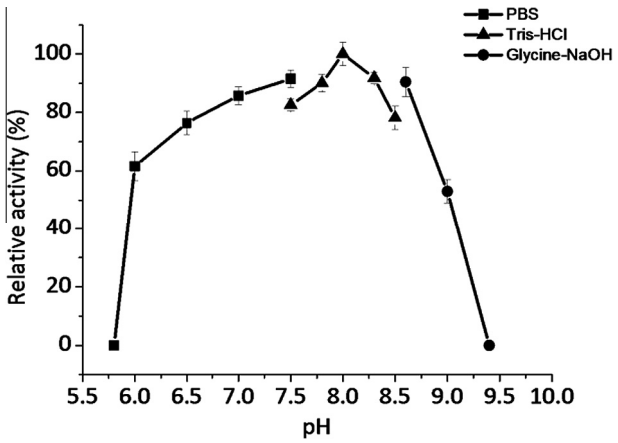


Figure 1. The effects of buffer and pH on one-pot three-enzyme reaction system.

was 8 mM (Fig. 3). As mentioned before, in the formal four enzyme reaction system, Mg^{2+} is an inhibitor of latter enzyme Pgm, thus, our reaction system would be more suitable for one pot reaction.

SpGalK has the similar pH value with SpGalU whose optimum pH value is 8.0–8.5. Considering both of them come from the same bacteria, they might have similar optimum temperature. Compared with BLUSP, the optimum pH value of which is 6.5, SpGalU may be more suitable and easier to get the highest enzyme activity in one pot reaction system with SpGalK.

2.2. Production of UDP-Glc in preparation scale

To demonstrate the application of this one-pot three-enzyme catalytic system in chemo-enzymatic synthesis of monosaccharide nucleotides, UDP-Glc was produced in preparation scale in vitro. The reaction was carried out under optimized conditions and incubated for 24 h. The reaction was terminated when no further formation of UDP-Glc was detected by CE and TLC. The product UDP-Glc was purified by Bio-Gel P2 gel filtration. UDP-Glc was eluted by deionized water via gravity. Fractions containing the UDP-Glc product were collected and lyophilized by freeze-dryer. 7 mg UDP-Glc presented as white foam was obtained from 28.8 mg glucose. The purified UDP-Glc was characterized by mass spectrometry (negative mode) (Fig. S2) and ^{1}H NMR spectrometry (Fig. S3). The exact mass of UDP-Glc is 566.0 and 565.2 $[M-H]^-$ is found in mass spectrometry.

2.3. Synthesis of UDP-Glc/Gal derivatives by one-pot three-enzyme system

To illustrate the application of this one-pot three-enzyme system in chemo-enzymatic synthesis of UDP-Glc/Gal analogs, we chose 14 monosaccharides which could be used by SpGalK as the substrates of one-pot reaction system which contain Tris–HCl buffer (100 mM, pH 8.0), $MgCl_2$ (8 mM), monosaccharide or a derivative (8 mM), ATP (10 mM), UTP (10 mM), 10 μM SpGalK, 10 μM SpGalU, and 0.1 U inorganic yeast pyrophosphatase. The reactions were carried out at 42 °C for 24 h and analyzed by thin layer chromatography (TLC) (Fig. S4) and capillary electrophoresis assays (Fig. S5). Among which, are three natural monosaccharides (Gal/Glc/L-Man), seven Gal analogs with structural modifications at C-2 position, two Gal analogs with structural modifications at C-4 position, and two Gal analogs with structural modifications at C-6 position.

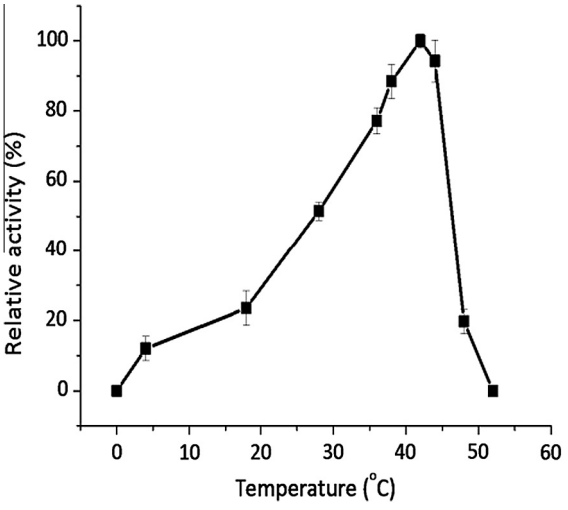


Figure 2. The effect of temperature on one-pot three-enzyme reaction system.

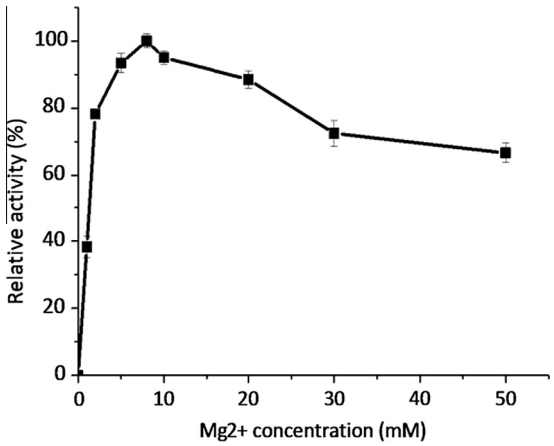


Figure 3. Metal requirement by one-pot three-enzyme reaction system.

As shown in Table 1, nine of 14 compounds were converted into corresponding monosaccharide nucleotides. For the natural monosaccharides, the yield of UDP-Gal could reach 90%, in comparison, the yield of UDP-Glc was only 26%. The relatively low yield of UDP-Glc may be attributed to the less optimal SpGalK activity for Glc as previous reports showed that its recognition ability for Glc was half of Gal, but the approach reported in our paper was the easiest method to produce UDP-Glc by now. L-Man could not be converted into corresponding UDP-L-Man with this one-pot system. This was most likely due to the less optimal activity of SpGalU toward L-Man-1-P.

For the analogs with structural modification at C-2 position, the yield of UDP-2-N_3Gal (**9**) could reach 78%, and the yield of UDP-GalNAc (**3**) was 32%. However, the yield of UDP-2-deoxyGal (**8**) was just 15%. Those sugars were failed to be synthesized in previous reports.[2] The one-pot three-enzyme system reported in our paper was undoubtedly a noticeable supplement. Unfortunately, the synthesis of UDP-Gal derivatives modified at C-2 position with a larger substituent including 2-EtCOHN (**10**), 2-PrCOHN (**11**), 2-PhCOHN (**12**), 2-N_3CH_2COHN (**13**) using this one-pot three-enzyme system failed. SpGalK could use these sugars well in previous report.[27] This may be attributed to the larger steric hindrance for SpGalU. These results demonstrated that, Gal derivatives with relatively smaller substituent at C-2 position were accepted with good conversion yield and SpGalU has poor tolerance at UDP-Gal derivatives modified at C-2 position.

For the analogs with structural modification at C-4 position, the yield of UDP-4-deoxyGal (**7**) reached 76%. On the contrary, the yield of UDP-4-N_3Gal (**6**) was lower than 5%. It was demonstrated that, the percent conversion would be reduced slightly with the gradually increased steric hindrance at C-4 position. For the analogs with structural modification at C-6 position, the yield of UDP-6-N_3Gal (**4**, 44%) and UDP-6-deoxyGal (**5**, 68%) was at similar level. These monosaccharide nucleotides were first synthesized in our one-pot three-enzyme system. This result also showed that the one-pot three-enzyme reaction system in this paper has good tolerance to 6-modified Gal analogs and the yield would be reduced slightly with the gradually increased steric hindrance.

More interestingly, two UDP-Gal derivatives with an azido functionality were successfully synthesized. Both of these UDP-sugars could be further used in the metabolic oligosaccharide engineering combined with bioorthogonal reactions.[29–31] UDP-2-deoxy, 4-deoxyGal could be used in enzymatic synthesis of *N*-acetyllactosamine and its analogs.[32,33] UDP-6-deoxyGal could be transferred to asialo agalacto α_1-acid glycoprotein and exhibited potential application to remodel the *N*-glycans on a glycoprotein.[34] These

Table 1
Yields of the monosaccharide nucleotides synthesized by one-pot three-enzyme system

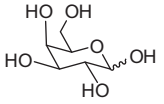
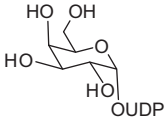
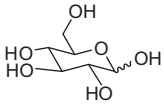
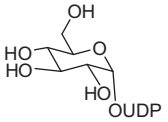
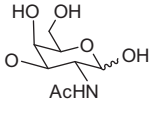
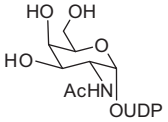
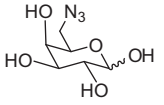
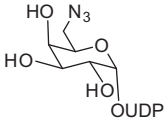
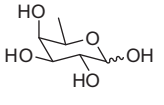
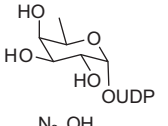
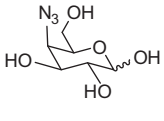
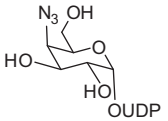
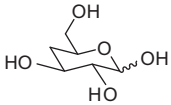
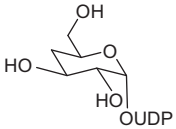
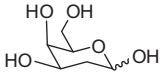
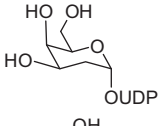
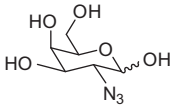
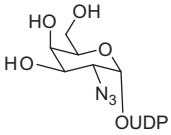
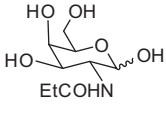
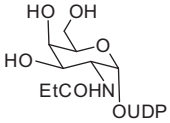
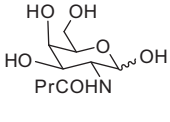
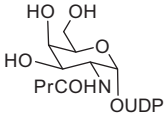
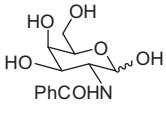
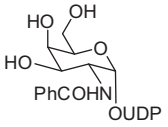
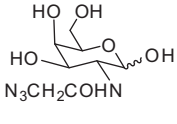
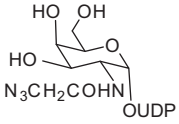
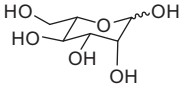
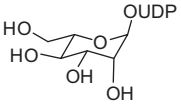
Entry	Substrate structure	Product	TLC	MS	Yields (%)
1			+	+	90%
2			+	+	26%
3			+	+	32%
4			+	+	44%
5			+	+	68%
6			ND	+	<5%
7			+	+	76%
8			ND	+	15%
9			+	+	78%
10			ND	ND	ND
11			ND	ND	ND
12			ND	ND	ND
13			ND	ND	ND

Table 1 (*continued*)

Entry	Substrate structure	Product	TLC	MS	Yields (%)
14			ND	ND	ND

+, Detect; ND, not detect.

unnatural sugar nucleotides synthesized using one-pot three-enzyme reaction system were useful donors for modification of carbohydrate chains using galactosyltransferase.

3. Conclusion

In conclusion, we developed and optimized an effective one-step three-enzyme catalytic system (Scheme 1) to synthesize the UDP-Glc/Gal and their derivatives. Compared with other approaches, in this system, glucose or galactose or both can be phosphorylated and corresponding UDP-sugars could be produced subsequently. The yield of UDP-Gal could reach 90% and the yield of UDP-Glc was 26% using Gal/Glc, ATP, and UTP as substrates. This is the first time it has been reported that UDP-Glc can be synthesized by three-enzymes in one-pot reaction system. By this approach, UDP-Glc could be produced in multiple mg scale in vitro rapidly and efficiently. More interestingly, our system seemed more suitable for synthesizing most UDP-Gal analogs with C-2, -4, and -6 modifications. Using this one-pot reaction system, we synthesized nine UDP-Glc/Gal derivatives. Among which, the yields of UDP-Gal (**1**), UDP-6-deoxyGal (**5**), UDP-2-N_3Gal (**9**), and UDP-4-deoxyGal (**7**) were higher than 50%. Besides, these nine synthesized UDP-Glc/Gal derivatives, could enrich sugar nucleotide analog library and could greatly facilitate our further study into the investigation of carbohydrate metabolic pathways.

4. Material and methods

4.1. Bacterial strains and materials

Recombinant strains of *Escherichia coli* containing gene *galK* and *galU* were from our laboratory. Yeast inorganic pyrophosphatase (PPase, EC 3.6.1.1) and uridine-5′-triphosphate-Na_3 were purchased from Sigma (Deisenhofen, Germany). Adenosine-5′-triphosphate-Na (ATP) was obtained from Solarbio (Beijing, China). Glucose was bought from Sinopharm (Beijing, China). The HisTrap affinity column (5 mL) was from Amersham Pharmacia Biotech (Piscataway, NJ). All reagents and enzymes were used according to the manufacturer's instruction.

4.2. Expression and purification of SpGalK and SpGalU

E. coli BL21 (DE3) strains containing exogenesis recombinant plasmids were cultivated in LB (Luria-Bertani) liquid medium supplemented with ampicillin at 37 °C for overnight, shaking at 220 rpm. A fraction (10 mL) of the cultured cells was transferred into fresh LB medium (1 L) supplemented with ampicillin and the cultivation was continued. When OD_{600} of the incubation media reached 0.6–0.8, IPTG (isopropyl-1-thio-β-D-galactopyranoside) of 0.2 mM was added to induce the expression of each protein at 16 °C for 20 h. Subsequently, the cells were collected by centrifugation at 4 °C, and then washed and suspended in start buffer (500 mM NaCl, 20 mM phosphate buffer, pH 8.0). The cells were

then lysed in an ice bath by sonication for 10 min at 400 W. After centrifugation at 4 °C, 12,000g for 30 min, the supernatant was gathered and loaded onto the HisTrap affinity column, which was previously equilibrated with start buffer. The target His-tagged proteins were eluted with elution buffer (500 mM NaCl, 20 mM phosphate buffer, pH 8.0, 500 mM imidazole). The portions containing the purified proteins were collected and desalted by desalting column with buffer comprising 50 mM Tris–HCl, and then concentrated in Tris–HCl buffer by centrifugation at 3500g for 30 min. The purity and molecular weight was analyzed by 12% SDS–PAGE as described by Laemmli.[35] The concentration of the purified proteins was detected by Bradford method.[36] The purified enzymes were stored in 20% glycerol at −20 °C for further analysis.

4.3. Enzymatic assay of SpGalK and SpGalU

The activity of SpGalK toward Gal was measured by the formation of the product (Gal-1-P). The reactions were carried out in a final volume of 50 μL, containing 100 mM Tris–HCl (pH 8.5), 10 mM $MgCl_2$, 8 mM ATP, 8 mM Gal, and recombinant SpGalK (0.5 mg/mL). After incubated at 45 °C for 180 min, the reactions were terminated by heating in a boiling water bath for 3 min. Subsequently, the reactions were centrifugated at 13,400 rpm for 30 min to remove the precipitation. Then the supernatant was collected and subjected to thin-layer chromatography.[37]

The activity of GalU was analyzed by product (UDP-Glc) formation. The reaction was executed in a 50 μL mixture, comprising 100 mM Tris–HCl (pH 8.5), 10 mM $MgCl_2$, 10 mM Glc-1-P, 10 mM UTP, 1 unit yeast inorganic pyrophosphatase, and recombinant GalU (0.5 mg/mL). After 180 min incubation at 37 °C, the product UDP-Glc was detected by thin-layer chromatography.

4.4. Optimization of the reaction conditions

The one-pot reaction was carried out in a final volume of 50 μL. The reaction mixture contained 100 mM Tris–HCl, 10 mM $MgCl_2$, 10 mM ATP, 10 mM UTP, 10 mM Glc, 1 unit yeast inorganic pyrophosphatase, 0.5 mg/mL SpGalK, and 0.5 mg/mL SpGalU. The optimum conditions of the one-pot reaction were measured with various buffers, at different temperatures and with different concentrations of bivalent cation Mg^{2+}. For the optimal pH experiments, 100 mM of each buffer (PBS/Tris–HCl/Glycine–NaOH) from pH 5.6 to pH 9.3 was added into the reaction system. The optimal temperature assays were executed under standard conditions except that the reactions were incubated under different temperatures (from 0 °C to 52 °C) for 180 min. For the experiments aimed at defining the optimal Mg^{2+} concentration, assays were carried out under standard assay conditions except that different concentrations of Mg^{2+} (from 0 to 50 mM) were added into the reaction system. After incubation, the reactions were quenched by heating at 100 °C for 3 min. The amount of product UDP-Glc was detected by a Bechman Coulter P/ACE MDQ Capillary Electrophoresis (CE) system equipped with a UV detector and a 50 cm capillary tubing (75 μm I.D.). Assays were run at 25 kV with 25 mM sodium borate buffer (pH 9.4) for 20 min. Percent conversions were calculated from peak areas of UDP-sugar and UTP monitored by UV absorbance at 262 nm. All assays were carried out in duplicate.

4.5. Production of UDP-Glc in preparation scale

To demonstrate the application of this one-pot three-enzyme catalytic system in the synthesis of monosaccharide nucleotides, UDP-Glc was produced in preparation scale in vitro. The reaction was performed in 10 mL reaction mixture comprising 100 mM Tris–HCl, 10 mM $MgCl_2$, 20 mM ATP, 20 mM UTP, 16 mM Glc, 50 units of yeast inorganic pyrophosphatase, 1 mg/mL SpGalK, and 1 mg/mL SpGalU. The reaction was incubated at 42 °C for 24 h. When no further formation of UDP-Glc was observed by CE and TLC, the reaction was terminated.

4.6. Purification of UDP-Glc

The reaction was terminated by heating in boiling water bath for 3 min, and subsequently centrifuged at 13,400 rpm at room temperature for 30 min. The supernatant was collected, concentrated and then loaded onto the top of the Bio-Gel P-2 column (2.5 × 100 cm) gel bed, which was preprocessed by degassed deionized water. The product UDP-Glc was eluted by deionized water and collected in 10 mL tubes using a fraction collector.[36] CE was used to detect which fractions contain UDP-Glc. The fractions which only comprise the sugar nucleotide UDP-Glc were gathered in a 50 mL centrifuge tube. The freeze-dryer was used to lyophilize the solution to a white powder. Then the UDP-Glc was characterized by mass spectrometry (negative mode) and ^1H NMR.

4.7. Synthesis of UDP-Glc/Gal derivatives by one-pot three-enzyme system

We chose 14 monosaccharides which could be used by GalK in previous report[36] as substrates for one-pot three-enzyme system to synthesize various sugar nucleotides. The reactions were carried out in the system containing 50 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, various monosaccharides, 10 mM ATP, 10 mM UTP, 0.5 mg/mL SpGalK, 0.5 mg/mL SpGalU, and 0.1 U PPase. The reactions were incubated at 42 °C for 24 h. The reactions were terminated by heating at 100 °C for 5 min, followed by centrifugated at 13,400 rpm at room temperature for 30 min to remove protein precipitation. The products were determined by TLC, CE and MS. For CE analysis, 5 μL of each sample was diluted into 50 μL and subjected to CE analysis as described above.

4.8. ESI-MS Analysis

ESI-MS analysis was carried out using a Shimadzu LCMS-IT-TOF mass spectrometer. The parameters used were as follows: electrospray voltage of 3.5 kV for the negative mode, CDL temperature 200 °C, flow rate of nebulizer gas was 1.5 L/min, and CID MS/MS collision energy of 50%. A mobile phase which contained 50% aqueous acetonitrile and 0.1% formic acid was delivered by a Shimadzu LC-20 AB pump with 50 μL/min flow rate. The sample (5 μg) was injected and analyzed in the negative ion mode.

4.9. 600 MHz ^1H NMR spectroscopy identification

6 mg UDP-Glc was dissolved in 500 μL D_2O at rt for the NMR assay. ^1H NMR spectra was recorded with the Bruker Avance 600 MHz spectrometer. The chemical shifts were measured by reference to internal DHO (δ = 4.700).

Acknowledgements

This work was supported by National Major Scientific and Technological Special Project for 'Significant New Drugs Development' (2012ZX09502001-005), 863 Program (No. 2012AA021504), NSFC (No. 31070824, 31270983, 31100039), and SRF for ROCS, SEM. L.C. acknowledges support from University of South Carolina Salkehatchie.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2013.03.005.

References

1. Bourgeaux, V.; Cadene, M.; Piller, F.; Piller, V. *ChemBioChem* **2007**, *8*, 37–40.
2. Muthana, M. M.; Qu, J.; Li, Y.; Zhang, L.; Yu, H.; Ding, L.; Malekan, H.; Chen, X. *Chem. Commun. (Camb.)* **2012**, *48*, 2728–2730.
3. Guan, W.; Cai, L.; Wang, P. G. *Chemistry* **2010**, *16*, 13343–13345.
4. Weissborn, A. C.; Liu, Q.; Rumley, M. K.; Kennedy, E. P. *J. Bacteriol.* **1994**, *176*, 2611–2618.
5. Lan, W. *Surface Carbohydrates of the Prokaryotic Cell*; Academic Press: Sutherland, 1977.
6. Fukasawa, T.; Jokura, K.; Kurahashi, K. *Biochem. Biophys. Res. Commun.* **1962**, *7*, 121–125.
7. Sundararajan, T. A.; Rapin, A. M.; Kalckar, H. M. *Proc. Natl. Acad. Sci.* **1962**, *48*, 2187–2193.
8. Schulman, H.; Kennedy, E. P. *J. Biol. Chem.* **1977**, *252*, 6299–6303.
9. Murrey, H. E.; Gama, C. I.; Kalovidouris, S. A.; Luo, W. I.; Driggers, E. M.; Porton, B.; Hsieh-Wilson, L. C. *Proc. Natl. Acad. Sci.* **2006**, *103*, 21–26.
10. Boons, G.-J. *Carbohydr. Chem.* **2010**, *36*, 152–167.
11. Ernst, C.; Klaffke, W. *J Org Chem.* **2003**, *68*(14), 5780–5783.
12. Wagner, G. K.; Pesnot, T.; Field, R. A. *Nat. Prod. Rep.* **2009**, *26*, 1172–1194.
13. Ko, J. H.; Shin, H. S.; Kim, Y. S.; Lee, D. S.; Kim, C. H. *Appl. Biochem. Biotechnol.* **1996**, *60*, 41–48.
14. Tanaka, H.; Yoshimura, Y.; Jøgensen, M. R.; Cuesta-Seijo, J. A.; Hindsgaul, O. *Angew. Chem., Int. Ed.* **2012**, *51*, 11531–11534.
15. Bülter, T.; Elling, L. *Glycoconjugate J.* **1999**, *16*, 147–159.
16. Bülter, T.; Elling, L. *J. Mol. Catal. B: Enzym.* **2000**, *8*, 281–284.
17. Ma, X.; Stockigt, J. *Carbohydr. Res.* **2001**, *333*, 159–163.
18. Mao, Z.; Shin, H. D.; Chen, R. R. *Biotechnol. Prog.* **2006**, *22*, 369–374.
19. Wong, C.-H.; Haynie, S. L. *J. Am. Chem. Soc.* **1983**, *105*, 115.
20. Tan, A. W. *Biochim. Biophys. Acta* **1979**, *582*, 543–547.
21. Holden, H. M.; Rayment, I.; Thoden, J. B. *J. Biol. Chem.* **2003**, *278*, 43885–43888.
22. Schulz, J. M.; Watson, A. L.; Sanders, R.; Ross, K. L.; Thoden, J. B.; Holden, H. M.; Fridovich-Keil, J. L. *J. Biol. Chem.* **2004**, *279*, 32796–32803.
23. Wong, C. H.; Ichikawa, Y. *J. Org. Chem.* **1992**, *57*, 4343–4344.
24. Liu, Z.; Zhang, J.; Chen, X.; Wang, P. G. *ChemBioChem* **2002**, *3*, 348–355.
25. Bülter, T.; Elling, L. *Glycoconjugate J.* **1996**, *16*, 147.
26. Chen, M.; Chen, L. L.; Zou, Y.; Xue, M. Y.; Liang, M.; Jin, L.; Guan, W. Y.; Shen, J.; Wang, W.; Wang, L.; Liu, J.; Wang, P. G. *Carbohydr. Res.* **2011**, *346*, 2421–2425.
27. Bonofiglio, L.; Garcia, E.; Mollerach, M. *Curr. Microbiol.* **2005**, *51*, 217–221.
28. Zou, Y.; Wang, W.; Cai, L.; Chen, L.; Xue, M. Y.; Zhang, X.; Shen, J.; Chen, M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3540–3543.
29. Khidekel, N.; Arndt, S.; Lamarre-Vincent, N.; Lippert, A.; Poulin-Kerstien, K. G.; Ramakrishnan, B.; Qasba, P. K.; Hsieh-Wilson, L. C. *J. Am. Chem. Soc.* **2003**, *125*, 16162–16163.
30. Khidekel, N.; Ficarro, S. B.; Peters, E. C.; Hsieh-Wilson, L. C. *Proc. Natl. Acad. Sci.* **2004**, *101*, 13132–13137.
31. Rexach, J. E.; Clark, P. M.; Hsieh-Wilson, L. C. *Nat. Chem. Biol.* **2008**, *4*, 97–106.
32. Uchiyama, T.; Hindsgaul, O. *J. Carbohydr. Chem.* **1998**, *17*, 1181–1190.
33. Srivastava, G.; Hindsgaul, O.; Palcic, M. M. *Carbohydr. Res.* **1993**, *245*, 137–144.
34. Kajihara, Y.; Endo, T.; Ogasawara, H.; Kodama, H.; Hashimoto, H. *Carbohydr. Res.* **1995**, *269*, 273–294.
35. Laemmli, U. K. *Nature* **1970**, *227*, 680–685.
36. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248–254.
37. Zhao, G.; Guan, W.; Cai, L.; Wang, P. G. *Nat. Protoc.* **2010**, *5*, 636–646.