

Cite this: *Chem. Commun.*, 2012, **48**, 2728–2730

www.rsc.org/chemcomm

## COMMUNICATION

Efficient one-pot multienzyme synthesis of UDP-sugars using a promiscuous UDP-sugar pyrophosphorylase from *Bifidobacterium longum* (BLUSP)<sup>†</sup>Musleh M. Muthana,<sup>‡a</sup> Jingyao Qu,<sup>‡a</sup> Yanhong Li,<sup>a</sup> Lei Zhang,<sup>a</sup> Hai Yu,<sup>a</sup> Li Ding,<sup>ab</sup>  
Hamed Malekan<sup>a</sup> and Xi Chen<sup>\*a</sup>

Received 5th December 2011, Accepted 9th January 2012

DOI: 10.1039/c2cc17577k

**A promiscuous UDP-sugar pyrophosphorylase (BLUSP) was cloned from *Bifidobacterium longum* strain ATCC55813 and used efficiently with a *Pasteurella multocida* inorganic pyrophosphatase (PmPpA) with or without a monosaccharide 1-kinase for one-pot multienzyme synthesis of UDP-galactose, UDP-glucose, UDP-mannose, and their derivatives. Further chemical diversification of a UDP-mannose derivative resulted in the formation of UDP-*N*-acetylmannosamine.**

Carbohydrates are widespread in nature and play pivotal roles in biological systems. The key enzymes for the formation of glycosidic bonds in carbohydrates are glycosyltransferases. Most glycosyltransferases require monosaccharide nucleotides as the common activated donor substrates. Among monosaccharide nucleotides used by mammalian glycosyltransferases, many are uridine 5'-diphosphate (UDP)-monosaccharides such as UDP-glucose (UDP-Glc), UDP-galactose (UDP-Gal), UDP-glucuronic acid (UDP-GlcA), UDP-*N*-acetylglucosamine (UDP-GlcNAc), UDP-*N*-acetylglactosamine (UDP-GalNAc), and UDP-xylose (UDP-Xyl).<sup>1</sup> In addition, UDP-mannose (UDP-Man) has been isolated from *Mycobacterium smegmatis* and proposed to be an intermediate in the biosynthesis of mycobacterial polysaccharides.<sup>2</sup> Furthermore, UDP-*N*-acetylmannosamine (UDP-ManNAc) and UDP-*N*-acetylmannosaminuronic acid (UDP-ManNAcA) have been used by some bacteria for producing capsular polysaccharides containing ManNAc<sup>3</sup> or ManNAcA<sup>4,5</sup> residues or forming ManNAcβ1-4GlcNAc-PP-undecaprenol (lipid II) for the biosynthesis of cell wall teichoic acids of Gram-positive bacteria.<sup>6–10</sup>

The simplest biosynthetic route for obtaining monosaccharide nucleotides such as UDP-monosaccharides usually involves the formation of a monosaccharide-1-phosphate catalyzed by a monosaccharide-1-phosphate kinase followed by the formation of monosaccharide nucleotides catalyzed by a nucleotidyltransferase (or pyrophosphorylase).<sup>11,12</sup> However, the simplest route has not been applied routinely for the formation of UDP-Gal due to

the less common access to UTP:galactose-1-phosphate uridylyltransferases or UDP-Gal pyrophosphorylases (EC 2.7.7.10) for direct formation of UDP-Gal from Gal-1-phosphate and UTP. For example, UDP-Gal used in galactosyltransferase-catalyzed enzymatic synthesis of galactosides has been more frequently obtained from UDP-Glc by reactions catalyzed by UDP-Gal 4-epimerases<sup>13–16</sup> or UDP-glucose:galactose-1-phosphate uridylyltransferases (EC 2.7.7.12, GalT or GalPUT) in the Leloir pathway.<sup>17,18</sup>

Nevertheless, UDP-galactose pyrophosphorylase activity was identified from yeast *Saccharomyces fragilis*,<sup>19</sup> pigeon liver, and mammalian livers.<sup>20</sup> The enzyme was purified from bovine liver<sup>20</sup> and Gram-positive bacterium *Bifidobacterium bifidum*.<sup>21</sup> Recently, promiscuous UDP-sugar pyrophosphorylases (USPs) (EC 2.7.7.64) that can use various monosaccharide 1-phosphates in the presence of UTP for direct synthesis of UDP-monosaccharides including UDP-Glc, UDP-Gal, UDP-GlcA, *etc.* were cloned from plants such as pea (*Pisum sativum* L.) sprouts (PsUSP)<sup>22</sup> and *Arabidopsis thaliana* (AtUSP).<sup>23</sup> Enzymes which share sequence homology to plant USPs were also cloned from *Leishmania major* and *Trypanosoma cruzi*, two trypanosomatid protozoan parasites, and were shown to have good activity towards Gal-1-P and Glc-1-P and weaker activity towards xylose-1-phosphate and GlcA-1-P.<sup>24,25</sup> A USP with broad substrate specificity and optimal activity at 99 °C was also cloned from a hyperthermophile archaea *Pyrococcus furiosus* DSM 3638 for which Glc-1-P, Man-1-P, Gal-1-P, Fuc-1-P, GlcNH<sub>2</sub>-1-P, GalNH<sub>2</sub>-1-P, and GlcNAc-1-P were all shown to be tolerable substrates, and both UTP and dTTP could be used as nucleotide triphosphate substrates by the enzyme.<sup>26</sup> Nevertheless, none of these enzymes has been used in preparative-scale or large-scale synthesis of sugar nucleotides and non-natural derivatives of monosaccharide-1-P have not been tested as substrates for USPs.

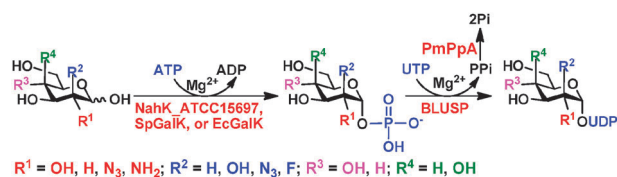
Here we report the cloning of a promiscuous USP from a probiotic *Bifidobacterium longum* strain ATCC55813 and its application in an efficient one-pot three-enzyme system for preparative-scale synthesis of UDP-monosaccharides and their derivatives from simple monosaccharides or derivatives (except for UDP-Glc which was synthesized from Glc-1-P in a one-pot two-enzyme system as discussed below). These compounds will be tested as potential donor substrates for various glycosyltransferases.

<sup>a</sup> Department of Chemistry, University of California, One Shields Avenue, Davis, California 95616, USA.  
E-mail: chen@chem.ucdavis.edu; Fax: +1 530-752-8995;  
Tel: +1 530-754-6037

<sup>b</sup> College of Science, Northwest A&F University, Yangling, Shaanxi, China

<sup>†</sup> Electronic supplementary information (ESI) available: Experimental details for cloning, expression, and purification of BLUSP, chemical and enzymatic synthesis, NMR and HRMS data. See DOI: 10.1039/c2cc17577k

<sup>‡</sup> These authors contributed equally.



**Scheme 1** One-pot three-enzyme system for the synthesis of UDP-monosaccharides and derivatives. Enzymes used: NahK\_ATCC15697, *Bifidobacterium infantis* strain ATCC15697 *N*-acetylhexosamine 1-kinase;<sup>28</sup> SpGalK, *Streptococcus pneumoniae* TIGR4 galactokinase;<sup>27</sup> EcGalK, *E. coli* galactokinase;<sup>17</sup> BLUSP, *Bifidobacterium longum* UDP-sugar pyrophosphorylase; PmPpA, *Pasteurella multocida* inorganic pyrophosphatase.<sup>29</sup>

In general, three enzymes were used in one-pot to synthesize UDP-monosaccharides including UDP-Gal, UDP-Glc, UDP-Man, and their derivatives. As shown in Scheme 1, the first enzyme was a monosaccharide 1-kinase selected from a galactokinase cloned from *Escherichia coli* K-12 (EcGalK),<sup>17</sup> a galactokinase cloned from *Streptococcus pneumoniae* TIGR4 (SpGalK),<sup>27</sup> or an *N*-acetylhexosamine 1-kinase cloned from *Bifidobacterium infantis* strain ATCC15697 (NahK\_ATCC15697).<sup>28</sup> The second enzyme was a promiscuous USP that we cloned from *Bifidobacterium longum* ATCC55813 (BLUSP) (see ESI† for details about cloning, expression, and purification). It catalyzes the reversible formation of UDP-monosaccharide and pyrophosphate from UTP and monosaccharide-1-phosphate ( $\text{UTP} + \text{monosaccharide-1-phosphate} \rightleftharpoons \text{UDP-monosaccharide} + \text{PPi}$ ) with tolerance of some substrate modifications. The third enzyme was an inorganic pyrophosphatase that we cloned from *Pasteurella multocida* strain P-1059 (ATCC15742) (PmPpA)<sup>29</sup> for hydrolyzing the pyrophosphate by-product formed by BLUSP to drive the reaction towards the formation of UDP-monosaccharides and derivatives.

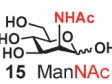
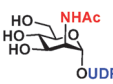
Prior to applying the one-pot three-enzyme system shown in Scheme 1 to the preparative-scale synthesis of the UDP-sugars, galactose (**1**) and its C2-derivatives (**2–5**), glucose and its C2-derivatives (**7–10**), as well as mannose (**11**) and its C2-derivatives (**12–15**) (Table 1) were tested as substrates for various monosaccharide-1-kinases including SpGalK, EcGalK, and NahK in small-scale reactions containing Tris-HCl buffer (100 mM, pH 8.0), monosaccharide or a derivative (15 mM),  $\text{MgCl}_2$  (10 mM), ATP (18 mM), and a kinase (0.2 mg  $\text{mL}^{-1}$ ). The reactions were carried out at 37 °C for 1 h, 4 h, and 24 h and analyzed by thin layer chromatography (TLC) and capillary electrophoresis assays. We were able to identify at least one of these kinases for each monosaccharide that gave a yield higher than 58% for the formation of the corresponding monosaccharide-1-phosphate. The observed results from thin-layer chromatography (TLC) and capillary electrophoresis (CE) (Table S1 and Fig. S4, ESI†) confirmed the previously reported activities of NahK, SpGalK, and EcGalK toward their respective substrates except for mannosamine (**13**), a NahK substrate which was not previously tested.<sup>27,28,30</sup>

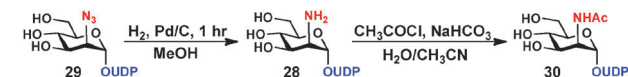
As the one-pot three-enzyme system was not able to synthesize UDP-Glc from Glc efficiently in small-scale assays (<20% yield was observed), inexpensive and commercially available Glc-1-P (**6**) was used in a two-enzyme system containing BLUSP and PmPpA for the high-efficient synthesis of UDP-Glc (**21**) in 99% yield.

**Table 1** Synthesis of UDP-monosaccharides using the one-pot three-enzyme system shown in Scheme 1. ND, not detected

Substrate	Kinase	Product	Yield (%)
<b>1</b> Gal	EcGalK or SpGalK	<b>16</b> UDP-Gal	86
<b>2</b> 2-deoxyGal	EcGalK or SpGalK	<b>17</b> UDP-2-deoxyGal	ND
<b>3</b> GalNH <sub>2</sub>	EcGalK	<b>18</b> UDP-GalNH <sub>2</sub>	ND
<b>4</b> GalN <sub>3</sub>	SpGalK	<b>19</b> UDP-GalN <sub>3</sub>	ND
<b>5</b> GalNAc	SpGalK	<b>20</b> UDP-GalNAc	ND
<b>6</b> Glc-1-P	None	<b>21</b> UDP-Glc	99
<b>7</b> 2-deoxyGlc	NahK	<b>22</b> UDP-2-deoxyGlc	56
<b>8</b> GlcNH <sub>2</sub>	NahK	<b>23</b> UDP-GlcNH <sub>2</sub>	43
<b>9</b> GlcN <sub>3</sub>	NahK	<b>24</b> UDP-GlcN <sub>3</sub>	61
<b>10</b> GlcNAc	NahK	<b>25</b> UDP-GlcNAc	ND
<b>11</b> Man	NahK	<b>26</b> UDP-Man	60
<b>12</b> ManF	NahK	<b>27</b> UDP-ManF	92
<b>13</b> ManNH <sub>2</sub>	NahK	<b>28</b> UDP-ManNH <sub>2</sub>	ND
<b>14</b> ManN <sub>3</sub>	NahK	<b>29</b> UDP-ManN <sub>3</sub>	90

Table 1 (continued)

Substrate	Kinase	Product	Yield (%)
 15 ManNAc	NahK	 30 UDP-ManNAc	ND



**Scheme 2** Synthesis of UDP-ManNAc (**30**) from UDP-ManN<sub>3</sub> (**29**) in 79% yield *via* the formation of UDP-ManNH<sub>2</sub> (**28**) by catalytic hydrogenation followed by acetylation.

The synthesis of all other UDP-sugars listed in Table 1 was carried out using the one-pot three-enzyme system shown in Scheme 1. As shown in Table 1, the one-pot three-enzyme system provided excellent yields for the formation of UDP-Gal (**16**, 86%), UDP-ManF (**27**, 92%), and UDP-ManN<sub>3</sub> (**29**, 90%) from the corresponding monosaccharides Gal (**1**), ManF (**12**), and ManN<sub>3</sub> (**14**), respectively. Three of the derivatives of UDP-Glc including UDP-2-deoxyGlc (**22**), UDP-GlcNH<sub>2</sub> (**23**), and UDP-GlcN<sub>3</sub> (**24**) were obtained from 2-deoxyGlc (**7**), glucosamine (GlcNH<sub>2</sub>, **8**) and GlcN<sub>3</sub> (**9**) in 56%, 43%, and 61% yields, respectively. The moderate yields for these three compounds may be attributed by less optimal NahK kinase activity for GlcNH<sub>2</sub> (**8**) and GlcN<sub>3</sub> (**9**), and the less optimal BLUSP activity for 2-deoxyGlc (**7**). UDP-Man (**26**) was synthesized from Man (**11**) in moderate 60% yield using the one-pot three-enzyme system and the moderate yield was most likely due to the less optimal activity of BLUSP towards Man-1-P. The synthesis of four UDP-Gal derivatives including its 2-deoxy, 2-deoxy-2-amido-, 2-deoxy-2-azido-, and 2-deoxy-2-acetamido-derivatives (**17–20**) using the one-pot three-enzyme system was not successful. In addition, UDP-GlcNAc (**25**), UDP-ManNH<sub>2</sub> (**28**), and UDP-ManNAc (**30**) could not be produced from the corresponding monosaccharides (**10**, **13**, and **15**) using the one-pot three-enzyme system. These were most likely due to the substrate restriction of BLUSP instead of the kinases used.

Although UDP-ManNH<sub>2</sub> (**28**) and UDP-ManNAc (**30**) were not directly available from ManNH<sub>2</sub> (**13**) and ManNAc (**15**), respectively, *via* the one-pot three-enzyme system shown in Scheme 1, they can be readily prepared *via* simple chemical modification reactions from UDP-ManN<sub>3</sub> (**29**) obtained from the one-pot three-enzyme system. As shown in Scheme 2, a simple one-step catalytic hydrogenation of UDP-ManN<sub>3</sub> (**29**) produced UDP-ManNH<sub>2</sub> (**28**). Acetylation of the amino group in UDP-ManNH<sub>2</sub> (**28**) provided an easy access of UDP-ManNAc (**30**). A similar chemical acylation of UDP-ManNH<sub>2</sub> can be used to synthesize other acyl derivatives of UDP-ManNAc.<sup>31,32</sup>

In conclusion, taking advantage of the substrate promiscuity of several monosaccharide-1-phosphate kinases and a *Bifidobacterium longum* UDP-sugar pyrophosphorylase (BLUSP), we developed an efficient one-pot multienzyme system to quickly obtain structurally defined UDP-Gal, UDP-Glc, UDP-Man, and their derivatives in preparative scales. With additional simple chemical modifications of the UDP-ManN<sub>3</sub> produced by the one-pot three-enzyme reaction, biosynthetically useful UDP-ManNAc was readily obtained. The acquired UDP-sugar and derivatives

are excellent probes for testing the donor substrate specificity of diverse glycosyltransferases.

This work was supported by an NIH grant R01HD065122 and an NSF grant CHE-1012511. X. Chen is a Camille Dreyfus Teacher-Scholar and a UC-Davis Chancellor's Fellow. The authors would like to thank Professor Min Chen of Shandong University in China and Professor Peng George Wang of Georgia State University for providing the plasmid of SpGalK.

## Notes and references

- 1 *Essentials of Glycobiology*, ed. A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart and M. E. Etzler, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2nd edn, 2008, pp. 47–73.
- 2 S. Singh and S. E. Hogan, *Microbios*, 1994, **77**, 217–222.
- 3 J. S. Swartley, L. J. Liu, Y. K. Miller, L. E. Martin, S. Edupuganti and D. S. Stephens, *J. Bacteriol.*, 1998, **180**, 1533–1539.
- 4 L. J. McKerral and R. Y. Lo, *Infect. Immun.*, 2002, **70**, 2622–2629.
- 5 M. Portoles, K. B. Kiser, N. Bhasin, K. H. Chan and J. C. Lee, *Infect. Immun.*, 2001, **69**, 917–923.
- 6 N. Murazumi, K. Kumita, Y. Araki and E. Ito, *J. Biochem.*, 1988, **104**, 980–984.
- 7 Y. H. Zhang, C. Ginsberg, Y. Yuan and S. Walker, *Biochemistry*, 2006, **45**, 10895–10904.
- 8 K. Yokoyama, H. Mizuguchi, Y. Araki, S. Kaya and E. Ito, *J. Bacteriol.*, 1989, **171**, 940–946.
- 9 N. Murazumi, Y. Araki and E. Ito, *Eur. J. Biochem.*, 1986, **161**, 51–59.
- 10 C. R. Harrington and J. Baddiley, *Eur. J. Biochem.*, 1985, **153**, 639–645.
- 11 X. Chen, *ACS Chem. Biol.*, 2011, **6**, 14–17.
- 12 J. Yang, X. Fu, J. Liao, L. Liu and J. S. Thorson, *Chem. Biol.*, 2005, **12**, 657–664.
- 13 X. Chen, J. Zhang, P. Kowal, Z. Liu, P. R. Andreana, Y. Lu and P. G. Wang, *J. Am. Chem. Soc.*, 2001, **123**, 8866–8867.
- 14 J. W. Fang, J. Li, X. Chen, Y. N. Zhang, J. Q. Wang, Z. M. Guo, W. Zhang, L. B. Yu, K. Brew and P. G. Wang, *J. Am. Chem. Soc.*, 1998, **120**, 6635–6638.
- 15 Y. Ichikawa, Y.-C. Lin, D. P. Dumas, G.-J. Shen, E. Garcia-Junceda, M. A. Williams, R. Bayer, C. Ketcham, L. E. Walker, J. C. Paulson and C.-H. Wong, *J. Am. Chem. Soc.*, 1992, **114**, 9283–9288.
- 16 A. Zervos and L. Elling, *J. Am. Chem. Soc.*, 1996, **118**, 1836–1840.
- 17 X. Chen, J. W. Fang, J. B. Zhang, Z. Y. Liu, J. Shao, P. Kowal, P. Andreana and P. G. Wang, *J. Am. Chem. Soc.*, 2001, **123**, 2081–2082.
- 18 T. Endo, S. Koizumi, K. Tabata, S. Kakita and A. Ozaki, *Carbohydr. Res.*, 1999, **316**, 179–183.
- 19 H. M. Kalcker, B. Branganca and A. Munch-Peterson, *Nature*, 1953, **172**, 1038.
- 20 K. J. Isselbacher, *J. Biol. Chem.*, 1958, **232**, 429–444.
- 21 L. Lee, A. Kimura and T. Tochikura, *J. Biochem.*, 1979, **86**, 923–928.
- 22 T. Kotake, D. Yamaguchi, H. Ohzono, S. Hojo, S. Kaneko, H. K. Ishida and Y. Tsumuraya, *J. Biol. Chem.*, 2004, **279**, 45728–45736.
- 23 L. A. Litterer, J. A. Schnurr, K. L. Plaisance, K. K. Storey, J. W. Gronwald and D. A. Somers, *Plant Physiol. Biochem.*, 2006, **44**, 171–180.
- 24 S. Damerow, A. C. Lamerz, T. Haselhorst, J. Fuhring, P. Zarnovican, M. von Itzstein and F. H. Routier, *J. Biol. Chem.*, 2010, **285**, 878–887.
- 25 T. Yang and M. Bar-Peled, *Biochem. J.*, 2010, **429**, 533–543.
- 26 R. M. Mizanur, C. J. Zea and N. L. Pohl, *J. Am. Chem. Soc.*, 2004, **126**, 15993–15998.
- 27 Y. Li, H. Yu, Y. Chen, K. Lau, L. Cai, H. Cao, V. K. Tiwari, J. Qu, V. Thon, P. G. Wang and X. Chen, *Molecules*, 2011, **16**, 6396–6407.
- 28 M. Chen, L. L. Chen, Y. Zou, M. Xue, M. Liang, L. Jin, W. Y. Guan, J. Shen, W. Wang, L. Wang, J. Liu and P. G. Wang, *Carbohydr. Res.*, 2011, **346**, 2421–2425.
- 29 K. Lau, V. Thon, H. Yu, L. Ding, Y. Chen, M. M. Muthana, D. Wong, R. Huang and X. Chen, *Chem. Commun.*, 2010, **46**, 6066–6068.
- 30 J. Yang, X. Fu, Q. Jia, J. Shen, J. B. Biggins, J. Jiang, J. Zhao, J. J. Schmidt, P. G. Wang and J. S. Thorson, *Org. Lett.*, 2003, **5**, 2223–2226.
- 31 H. Cao, S. Muthana, Y. Li, J. Cheng and X. Chen, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 5869–5871.
- 32 Y. Chen, V. Thon, Y. Li, H. Yu, L. Ding, K. Lau, J. Qu, L. Hie and X. Chen, *Chem. Commun.*, 2011, **47**, 10815–10817.