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A highly efficient galactokinase from *Bifidobacterium infantis* with broad substrate specificity

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ABSTRACT

Galactokinase (GalK), particularly GalK from *Escherichia coli*, has been widely employed for the synthesis of sugar-1-phosphates. In this study, a GalK from *Bifidobacterium infantis* ATCC 15697 (BiGalK) was cloned and over-expressed with a yield of over 80 mg/L cell cultures. The k_{cat}/K_m value of recombinant BiGalK toward galactose (164 s^{-1} mM^{-1}) is 296 times higher than that of GalK from *E. coli*, indicating that BiGalK is much more efficient in the phosphorylation of galactose. The enzyme also exhibits activity toward galacturonic acid, which has never been observed on other wild type GalKs. Further activity assays showed that BiGalK has broad substrate specificity toward both sugars and phosphate donors. These features make BiGalK an attractive candidate for the large scale preparation of galactose-1-phosphate and derivatives.

1. Introduction

Galactose (Gal) is a ubiquitous monosaccharide found in glycan structures of most organisms. The assembly of Gal-containing glycans requires UDP-Gal and galactosyltransferases. Two pathways were found for the biosynthesis of UDP-Gal: The Leloir pathway and the salvage pathway (Fig. 1). The galactokinase (EC 2.7.1.6, ATP: D-galactose-1-phosphototransferase, GalK) catalyzed reaction, ATP-dependent phosphorylation of Gal to form galactose-1-phospahte (Gal-1-P), is shared by both pathways. Over the past five decades, GalK has attracted significant research attention due to its important role in sugar metabolism.[1,2] Other than the biological significance, GalKs (particularly GalK from *Escherichia coli*, EcGalK) have been extensively investigated and employed for the in vitro preparation of sugar-1-phosphates for glycorandomization.[3,4]

GalKs from a variety of organisms including human, yeast, plants, and bacteria were isolated, expressed, and characterized.[5–8] These enzymes exhibit distinct specific activities. For instance, EcGalK has a k_{cat}/K_m value of 0.552 s^{-1} mM^{-1},[9] whereas GalK form *Saccharomyces cerevisiae* (ScGalK) has a k_{cat}/K_m value of 92.7 s^{-1} mM^{-1} (Table 1), suggesting that ScGalK is over 150 times more efficient than EcGalK. However, the application of ScGalK in the synthesis of sugar-1-phosphates was limited by the low expression level.[8] Substrate specificity studies revealed that GalK could tolerate modifications of Gal at C-2 and C-6. For example, other than Gal, 2-deoxygalactose (Gal2D), galactosamine (GalN), and 6-deoxygalactose (Gal6D, also known as D-fucose) are possible substrates of EcGalK[9] and ScGalK.[10] Recently, structure-based engineering to further broaden the substrate specificity of EcGalK greatly improved the in vitro synthesis of natural and unnatural sugar-1-phosphates. As a milestone, Thorson and co-workers achieved the preparation of 28 sugar-1-phosphates via using a novel mutant (Y371H/M173L) of the enzyme.[3] Nevertheless, large scale synthesis of Gal-1-P and derivatives still partly suffers from the relative low activity of EcGalK. On the other hand, in spite of extensive studies on sugar promiscuity, the specificity of GalK toward phosphate donors has hardly been explored.[11,12]

In this study, we described the over-expression (80 mg/L cell culture) and biochemical characterization of a highly efficient GalK from *Bifidobacterium infantis* ATCC 15675 (BiGalK). Detailed substrate specificity of the enzyme toward both sugars and phosphate donors was also investigated.

Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; CTP, cytidine triphosphate; dATP, deoxyadenosine triphosphate; dCTP, deoxycytidine triphosphate; dGTP, deoxyguanosine triphosphate; dTTP, deoxythymidine triphosphate; dUTP, deoxyuridine triphosphate; Gal, galactose; Gal-1-P, galactose-1-phosphate; Gal2D, 2-deoxygalactose; Gal6D, 6-deoxygalactose; GalA, galacturonic acid; GalK, galactokinase; GalN, galactosamine; GalNAc, *N*-acetylgalactosamine; GDP, guanosine diphosphate; Glc, glucose; GTP, guanosine triphosphate; IPTG, isopropyl-β-D-1-thiogalactopyranoside; ITP, inosine triphosphate; TLC, thin layer chromatography; UDP-Gal, uridine 5′-diphosphogalactose; UTP, uridine triphosphate.

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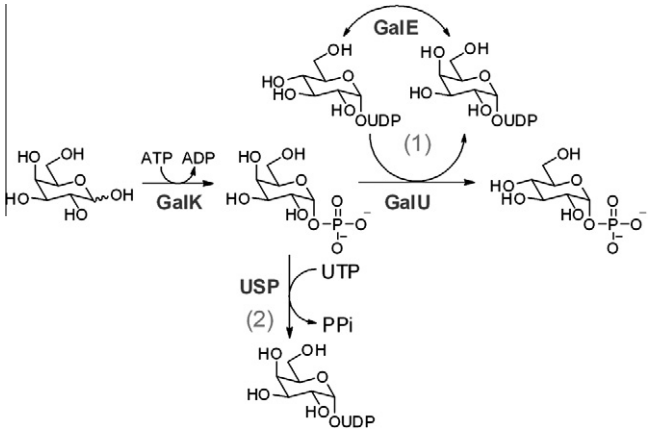

Figure 1. The Leloir pathway (1) and salvage pathway (2) of UDP-Gal biosynthesis. GalK, galactokinase; GalU, galactose-1-phosphate uridyltransferase; GalE, UDP-Gal 4′-epimerase; USP, UDP-sugar pyrophosphorylase.

2. Materials and methods

2.1. Materials

All chemical reagents unless otherwise stated were purchased from Sigma. Ni-NTA agarose was from Qiagen. The chromosome DNA of *Bifidobacterium infantis* ATCC 15697 was purchased from American Type Culture Collection. *E. coli* competent cells BL21(DE3) and expression vector pET22b were from Novagen Inc.

2.2. Molecular cloning

The *BiGalK* (GeneBank No.: 213690928) gene was amplified by PCR using *B. infantis* ATCC 15697 genomic DNA as the template. Primers with restriction sites underlined were as follows: 2062F: 5′-TGACATATGACTGCTGTTGAATTCATTG-3′ (*Nde* I)/2062R: 5′-TATAAGCTTGGCCTCGCGGCTCGCGGAAG-3′ (*Hind* III). Yielded DNA fragments were digested with *Nde* I and *Hind* III, followed by ligation with pET22b vector linearized by same restriction enzymes. The recombinant plasmid was then verified by DNA sequencing and transformed into *E. coli* BL21(DE3) for over-expression.

2.3. Protein over-expression and purification

E. coli BL21(DE3) cells harboring the aforementioned plasmid were grown in LB medium at 37 °C till the $O.D._{600}$ reached 0.8, followed by the addition of isopropyl-β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.2 mM. Expression was allowed to proceed for 20 h at 16 °C with rigorous shaking at 220 rpm. Recombinant BiGalK was purified by affinity chromatography using Ni-NTA agarose via standard procedure. Briefly, cells were collected by centrifugation at 4 °C, 6000×*g*, and re-suspended in buffer A (50 mM Tris–HCl pH 8, 300 mM NaCl, 20 mM imidazole). After brief sonication on ice, insoluble materials were removed by centrifugation at 4 °C, 10,000×*g* for 30 min. Protein purification was performed by loading the supernatant onto a Ni-NTA agarose (2 mL) column pre-equilibrated with buffer A. The loaded column was then washed with 200 mL of buffer A, and the His6-tagged BiGalK was eluted with 10 mL of buffer B (50 mM Tris–HCl pH 7.5, 300 mM NaCl, 250 mM imidazole). After SDS–PAGE analysis, protein containing fractions were concentrated and desalted using PD-10 column (GE Life Science, USA) against buffer C (50 mM Tris–HCl, pH 8.0, 10% glycerol) for long-term storage at 4 °C. The protein concentration was determined in a 96-well plate via using Bradford method.

2.4. Enzymatic activity assay

The kinase activity assay was performed in a reaction system (100 μl) containing: 8 mM of Gal, 10 mM of ATP, 5 mM of $MgCl_2$, 100 mM of Tris–HCl (pH 8.0), and 12 μg of purified BiGalK. The reaction was incubated at 37 °C for 20 min, then analyzed by thin-layer chromatography (TLC) using silica gel TLC plates [*i*-PrOH/NH_4OH/H_2O = 7:3:2 (v/v/v)] and the DNS method described previously[9] (The absorbance at 575 nm was recorded by a Varioskan Flash Multimode Reader, Thermo Scientific). The compounds on TLC plates were stained with anisaldehyde/acetic acid/H_2SO_4/H_2O = 7:3:10:27 (v/v/v/v), and visualized by brief heating.

2.5. Determination of physicochemical properties

Temperature experiments were carried out as described above, except that 100 ng of BiGalK was used and reactions were incubated in different temperatures (4, 16, 25, 30, 37, 45, 55, 65 °C). To determine the dependence of BiGalK on metal ions, reactions were performed at 37 °C with different metal ion salts ($MgCl_2$, $MnCl_2$, $CaCl_2$, $BaCl_2$, $NiSO_4$) at a concentration of 5 mM. The effect of ETDA was tested in presence of 5 mM of EDTA. Other conditions are same as in temperature experiments. pH dependence experiments were performed at 37 °C in 100 mM different buffers (sodium acetate, pH 4.0, 5.0, and 6.0; HEPES, pH 7.0; Tris–HCl, pH 7.5, 8.0, and 8.5; glycine–NaOH, pH 9.0, 10.0, and 11.0) in a total volume of 100 μL, containing 8 mM of Gal, 10 mM of ATP, 5 mM of $MgCl_2$ and 100 ng of BiGalK. All reactions were allowed to proceed for 20 min and analyzed by the DNS method.[9]

2.6. Determination of kinetic parameters

Reactions were carried out at 37 °C for 5 min in a total volume of 50 μL in 100 mM Tris–HCl (pH 8.0), containing: ATP, Gal, 5 mM of $MgCl_2$, and 50 ng of BiGalK. Apparent kinetic parameters were obtained by varying the concentration of Gal (1, 2, 4, 6, 8 mM) at

Table 1
Kinetics of GalKs from different species

Substrate	Enzyme	K_m (mM)	V_{max} (μmol min^{-1} mg^{-1})	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} mM^{-1})	Expression level (mg L^{-1})	References
Gal	BiGalK	0.895	191	146	164	>80	This work
	EcGalK	2.1	1.65	1.16	0.552	25[a]	9
	ScGalK	0.6	55.8	55.8	92.7	1.6[b]	5,8
	HsGalK	0.97		8.7	8.97	2	14
ATP	BiGalK	2.46	191	146	59.5		This work
	EcGalK	2.5	1.65	1.16	0.464		9
	ScGalK	0.15	55.8	55.8	372		5
	HsGalK	0.034		8.7	256		14

[a] Unpublished data.
[b] 1.6 mg ScGalK protein was obtained from 10 g of recombinant yeast cells.

a fixed concentration of ATP (10 mM), or varying the concentration of ATP (0.5, 1, 2, 4, 6 mM) at a fixed concentration of Gal (8 mM). The average value of triplicate assays using the DNS method was employed to generate Lineweaver–Burk plots, and K_m, V_{max}, k_{cat} values were calculated accordingly.

2.7. Determination of specific activities toward sugars and phosphate donors

Specific activities were determined with 8 mM of various sugars (Gal, GalN, Gal2D, Gal6D, galacturonic acid (GalA), Glc, or *N*-acetylgalactosamine (GalNAc)) using 10 mM of ATP as phosphate donor, or with 10 mM of various nucleotides (ATP, dATP, CTP, dCTP, GTP, dGTP, UTP, dUTP, dTTP, ITP, ADP, or GDP) by fixing Gal at the concentration of 8 mM. Other conditions are same as in enzymatic activity assay, except that varied amounts of BiGalK (30 ng–30 μg) were used to make sure the conversion of sugars reached from 15% to 30%. The average value of triplicate assays using the DNS method was employed to calculate specific activities. Same reactions were also carried out at 37 °C for 3 h for TLC analysis.

2.8. Synthesis of Gal-1-P and derivatives

Reaction was performed in a 15 mL tube with 5 mL of 100 mM Tris–HCl (pH 8.0) containing 44 mM of Gal or derivatives (GalN, Gal2D, Gal6D, GalA), 40 mM of ATP, 10 mM of $MgCl_2$, and varied amounts of BiGalK (0.2–2 mg). The reaction mixture was incubated at 37 °C for 4–24 h till ATP completely converted into ADP (analyzed by TLC). After quenching by the addition of 5 mL of ice-cold ethanol, the reaction was kept at 4 °C for 1 h, followed by centrifugation at 4 °C, 6000×*g* for 30 min to remove insoluble materials. The supernatant was then concentrated and subjected to P-2 (Bio-rad, USA) gel filtration chromatography (90 × 2.5 cm) with 50 mM of NH_4HCO_3 as the mobile phase. Product containing fractions were collected and concentrated under vacuum in 45 °C water bath. The yielded product was dissolved in 25 mL of distilled water and concentrated again to remove remanent NH_4HCO_3. NMR and HRMS were used to identify the products. NMR spectra were recorded in D_2O with a Bruker AV 400 spectrometer (^1H NMR at 400 MHz, ^{13}C NMR at 100 MHz, and ^{31}P at 160 MHz). Chemical shifts were reported in δ (ppm) from an internal standard of TMS (δ 0.00). Coupling constants are reported in hertz. High-resolution electrospray-ionization mass spectra (HRESIMS) were obtained on a Varian QFT-ESI mass spectrometer.

3. Results and discussion

3.1. Over-expression and purification of BiGalK

The deduced protein sequence of *Blon_2062* from *B. infantis* ATCC 15697 shares significant amino acid similarities with EcGalK (51%) and other characterized GalKs. The protein also contains characteristic signature motifs of the GHMP superfamily,[13] a large family including galactokinases, homoserine kinases, mevalonate kinases, and phosphomevalonate kinase. We thus assigned the protein as a GalK (named BiGalK). To demonstrate its function, the gene was cloned into pET22b vector, and transformed into *E. coli* BL21(DE3) for over-expression. The protein was expressed with a C-terminal His6-tag under the induction of 0.2 mM IPTG, mainly in soluble form (Fig. 2, lane 4). It was purified to 95% homogeneity (SDS–PAGE analysis) by one-step Ni-NTA affinity chromatography (Fig. 2, lane 6). Typically, over 80 mg of pure BiGalK could be obtained from 1 L of cell cultures, higher than the expression levels of EcGalK, ScGalK[8], and GalK from Human (HsGalK)[14] (Table 1). BiGalK has an apparent molecular weight of 45 kDa on SDS–PAGE, consistent with the calculated value. The protein can be stored at 4 °C in 50 mM Tris–HCl (pH 8.0), 10% glycerol for up to 4 months without significant loss of activity.

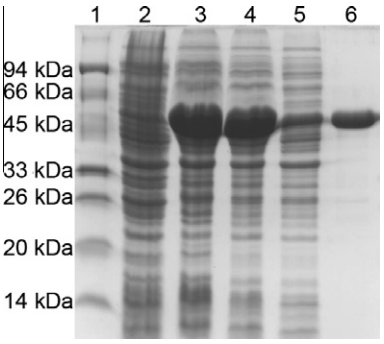

Figure 2. SDS–PAGE analysis of BiGalK over-expression and purification. Lanes: (1) protein standards (molecular weights are indicated in kDa); (2) total cells before induction; (3) total cells after induction by IPTG; (4) supernatant; (5) flow-through from Ni-column; (6) elution from Ni-column.

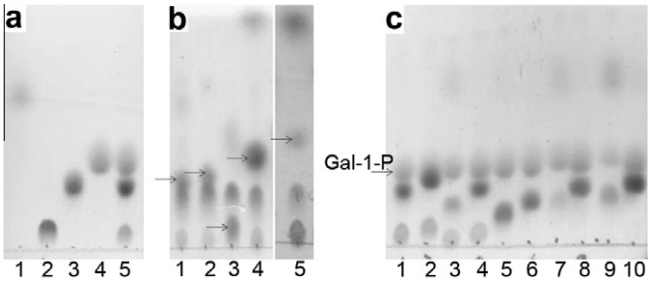

Figure 3. TLC analysis of BiGalK activity assay (a) and substrate specificity assays (b and c). (a) Lanes: (1) Gal; (2) ATP; (3) ADP; (4) Gal-1-P; (5) BiGalK catalyzed reaction. (b) Sugar substrate specificity, reactions containing: (1) Gal + ATP; (2) GalN + ATP; (3) GalA + ATP; (4) Gal2D + ATP; (5) Gal6D + ATP. (c) Phosphate donor specificity, reactions containing: (1) Gal + ATP; (2) Gal + dATP; (3) Gal + CTP; (4) Gal + dCTP; (5) Gal + GTP; (6) Gal + dGTP; (7) Gal + UTP; (8) Gal + dUTP; (9) Gal + ITP; (10) Gal + dTTP. Sugar-1-phosphates were indicated by arrows.

3.2. BiGalK catalyzes the formation of Gal-1-P with high efficiency

TLC was used to analyze the enzymatic activity of BiGalK. As shown in Figure 3a, after reaction, the spot on thin layer plate corresponding to Gal disappeared (also confirmed by the DNS method[9]), while a new spot corresponding to Gal-1-P was detected (Fig. 3a, lanes 1, 4, 5), accompanying with the conversion of ATP to ADP (Fig. 3a, lanes 2, 4, 5). HRMS and ^1H NMR analysis of the product further confirmed the generation of Gal-1-P (Supplementary data). These results demonstrated that BiGalK catalyzes the phosphorylation of Gal to form Gal-1-P in an ATP-dependent manner.

It has been reported that a GalK isoenzyme isolated from another Bifidobacterium strain (*B. bifidum*) exhibited high specific activity toward Gal (318 μmol/min/mg).[6,15] To ascertain whether our recombinant BiGalK possesses such a high activity, kinetic studies were performed. As listed in Table 1, BiGalK has K_m values of 0.895 mM and 2.46 mM toward Gal and ATP, respectively, comparable to that of EcGalK, ScGalK, and HsGalK. The calculated k_{cat}/K_m value (164 s^{-1} mM^{-1}) toward Gal, however, is 296 times higher than that of EcGalK (0.552 s^{-1} mM^{-1})[9] (Table 1), indicating that BiGalK is more efficient in the phosphorylation of Gal. Taken together with the high expression level, BiGalK becomes a more

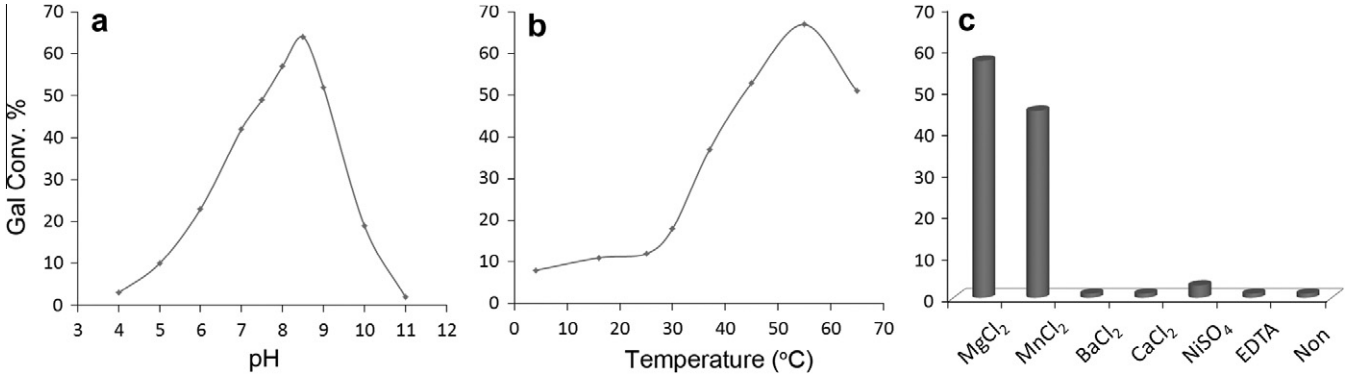

Figure 4. Physicochemical properties of BiGalK. (a) pH dependence; (b) temperature dependence; (c) metal ion dependence. All assays were performed in triplicates with the average yields reported.

attractive potential candidate in the large-scale synthesis of Gal-1-P and derivatives.

3.3. The physicochemical property of BiGalK

Effects of pH, temperature, and metal ions on BiGalK activity were investigated subsequently (Fig. 4). BiGalK has an optimum pH at 8.5, and keeps over 60% of maximal activity at pH between 7.0 and 9.0 (Fig. 4a). Temperature dependence experiments showed that BiGalK exhibited the highest activity at 55 °C (Fig. 4b), slightly higher than that of GalKs from *B. bifidum* (45 °C),[15] *Tetrahymena thermophile* (41 °C)[12], and *Streptococcus pneumoniae* (37 °C).[16] It is noted BiGalK exhibits over 76% maximum activity at 65 °C, while only possesses approximately 20% of maximal activity between 4 °C and 30 °C (Fig. 4b). As expected, BiGalK requires Mg^{2+} as cofactors during phosphorylation, like other enzymes of the GHMP superfamily. Among tested bivalent metal cations, only Mn^{2+} can partly substitute Mg^{2+}, with the reduction of the activity by about 21%.

3.4. BiGalK exhibits broad specificity toward sugar substrates

Substrate specificity studies revealed that GalKs usually could tolerate modifications of Gal at C-2 and C-6 positions, as long as modified groups are not bulkier than the hydroxyl group. For example, they are moderately active with Gal2D and GalN (less than 10-fold decrease in activity compared to Gal),[5,7,9,12] and weakly active toward Gal6D (over 20-fold decrease in activity compared to Gal).[9] Gal derivatives with bulkier groups or modifications at C-4 position, such as GalA, GalNAc, Glc, are not substrates of GalK.[9] However, GalK from *Streptococcus pneumoniae* was found to be an exception, which could use either Glc or GalNAc as substrate.[16]

To investigate whether BiGalK possesses similar sugar substrate specificity, specific activities toward a series of Gal derivatives were determined (Table 2). Results showed that BiGalK has high activity toward Gal (158 μmol min^{-1} mg^{-1}) and Gal2D (99.7 μmol min^{-1} mg^{-1}), moderate activity toward GalN (12.5 μmol min^{-1} mg^{-1}), and weak activity toward Gal6D (0.0781 μmol min^{-1} mg^{-1}). Glc is not a substrate of the enzyme, confirming the vital role of C-4 hydroxyl group in the substrate binding. As expected, GalNAc is not a substrate of BiGalK because of the bulky C-2 acetamido group.

Surprisingly, it was found that BiGalK can also catalyze the phosphorylation of GalA to form GalA-1-phosphate, with a specific activity of 0.0906 μmol min^{-1} mg^{-1}. This is the first wild type GalK found to possess such an activity. It has been reported that a Y371H mutation enables EcGalK to accept GalA and 6-amino-galactose,[17] possibly due to the reduction of stereo-hindrance effect, which may enable the accommodation of Gal derivatives with bulky C-6 groups. Y371 is conserved among GalKs from different species. Yet in BiGalK the equivalent residue of Y371 is L404, thus possibly explaining its activity toward GalA.

The formation of Gal-1-P and four derivatives was further confirmed by TLC (Fig. 3b), HRMS, and NMR analysis (Supplementary data).

Table 2
Specific activity of BiGalK toward sugars and phosphate donors

Substrates	Specific activity (μmol min^{-1} mg^{-1})
Gal	158
Gal2D	99.7
GalN	12.5
Gal6D	0.0781
GalA	0.0906
Glc	ND[a]
GalNAc	ND[a]
ATP	137
dATP	138
CTP	0.551
dCTP	0.931
GTP	28.5
dGTP	31.9
UTP	0.52
dUTP	1.50
ITP	15.7
dTTP	2.52
ADP	ND[a]
GDP	ND[a]

[a] ND, not detected.

3.5. BiGalK utilizes a variety of nucleoside triphosphates as phosphate donors

Specific activities toward a variety of nucleotides were also determined to reveal the specificity of BiGalK toward phosphate donors (Table 2). Interestingly, BiGalK could utilize all of the tested nucleoside triphosphates, with ATP (137 μmol min^{-1} mg^{-1}) and dATP (138 μmol min^{-1} mg^{-1}) as best phosphate donors. In principle, nucleoside triphosphates with purine structures (e.g., ATP, GTP, ITP) serve as better phosphate donors than those with pyrimidine structures (e.g., CTP, UTP, dTTP). For example, the specific activities of BiGalK toward GTP and ITP (28.5 μmol min^{-1} mg^{-1} and 15.7 μmol min^{-1} mg^{-1}) are 50 and 27 times higher than that of CTP (0.551 μmol min^{-1} mg^{-1}), respectively. Similar phenomenon was found as to *N*-acetylhexosamine 1-kinase from *B. longum* JCM1217.[18] On the other hand, BiGalK exhibited nearly 12% higher activity toward dGTP than GTP, and almost twofold higher activi-

ties toward dUTP than UTP (Table 2), suggesting that the enzyme prefers 2′-deoxy nucleoside triphosphates. Nucleoside diphosphates (e.g., ADP, GDP) are not phosphate donors for BiGalK as expected.

To further confirm these results, reactions containing Gal and different nucleoside triphosphates were allowed to proceed for 3 h, followed by TLC analysis. As showed in Figure 3c, Gal-1-P was generated in all reactions systems with over 50% yield, further demonstrating that all 10 nucleoside triphosphates serve as suitable phosphate donors in the BiGalK-catalyzed reactions.

3.6. Preparation and characterization of Gal-1-P and derivatives

Gal-1-P and four derivatives were synthesized in 40 mg scale using purified BiGalK as described in Section 2. Since P-2 gel filtration using distilled water failed to obtain pure target products, 50 mM of NH_4CO_3 was tested as the mobile phase. The approach enabled reliable separation of sugar-1-phosphates and ADP. The reproducibility of the modified approach was also verified by purification of mannose-1-phosphate, 2-deoxyglucose-1-phosphate, *N*-acetylglucosamine-1-phosphate, and *N*-acetylgalactosamine-1-phosphate (unpublished data).

3.6.1. α-D-Galactopyranosyl phosphate (Gal-1-P)

^{1}H NMR (400 MHz, D_2O) δ 5.40 (dd, J = 7.2, 3.6 Hz, 1H), 4.09–4.02 (m, 1H), 3.93–3.87 (m, J = 2.8 Hz, 1H), 3.80 (dd, J = 10.2, 3.1 Hz, 1H), 3.71–3.60 (m, 3H). HRESIMS: $C_6H_{14}O_9P$ ($M+H^+$), calcd 261.0370, found 261.0372.

3.6.2. 2-Amino-2-deoxy-α-D-galactopyranosyl phosphate (GalN-1-P)

^{1}H NMR (400 MHz, D_2O) δ 5.58 (dd, J = 7.3, 3.4 Hz, 1H), 4.11 (dd, J = 7.0, 5.2 Hz, 1H), 4.01 (dd, J = 10.9, 3.2 Hz, 1H), 3.93 (d, J = 3.0 Hz, 1H), 3.68–3.61 (m, 4H), 3.40 (dd, J = 10.8, 2.3 Hz, 1H). HRESIMS: $C_6H_{15}NO_8P$ ($M+H^+$), calcd 260.0530, found 260.0535.

3.6.3. 2-Deoxy-α-D-galactopyranosyl phosphate (Gal2D-1-P)

^{1}H NMR (400 MHz, D_2O): δ 5.55 (dd, $J_{1,P}$ = 7.2 Hz, 1H, H-1), 4.09 (m, 1H, H-5), 4.04 (m, 1H, H-5), 3.82 (dd, 1H, H-4), 3.72–3.63 (m, 2H, H_2-6), 1.89 (m, 1H, H-2a), 1.87 (m, 1H, H-2b); ^{13}C NMR (100 MHz, D_2O): δ 93.5 (d, J = 4.5 Hz, C-1), 71.6 (C-5), 67.7 (C-4), 64.5 (C-3), 61.7 (C-6), 32.5 (d, J = 6.3 Hz, C-2); ^{31}P NMR (162 MHz, D_2O): δ −0.039 (s). HRESIMS: $C_6H_{13}O_8PNa$ ($M+Na^+$), calcd 267.0240, found 267.0238.

3.6.4. 6-Deoxy-α-D-galactopyranosyl phosphate (Gal6D-1-P)

^{1}H NMR (400 MHz, D_2O): δ 5.37 (dd, $J_{1,2}$ = 3.2 Hz, $J_{1,P}$ = 14 Hz, 1H, H-1), 4.16 (m, 1H, H-5), 3.87–3.66 (m, 3H, H-2,3,4), 1.12 (d, 3H, H_3-6); ^{13}C NMR (100 MHz, D_2O): δ 94.6 (C-1), 71.8 (C-3), 69.3 (C-5), 68.2 (C-4), 67.2 (d, J = 3.4 Hz, C-2), 15.3 (C-6); ^{31}P NMR (162 MHz, D_2O): δ −0.376 (s). HRESIMS: $C_6H_{14}O_8P$ ($M+H^+$), calcd 245.0421, found 245.0423.

3.6.5. α-D-Galactopyranosyluronic acid phosphate (GalA-1-P)

^{1}H NMR (400 MHz, D_2O): δ 5.49 (dd, $J_{1,2}$ = 3.6 Hz, $J_{1,P}$ = 6.4 Hz, 1H, H-1), 4.40 (d, 1H, H-5), 4.25 (dd, 1H, H-4), 3.90 (dd, $J_{3,4}$ = 2.8 Hz, $J_{2,3}$ = 10.0 Hz, H-3), 3.77 (dd, 1H, H-2); ^{13}C NMR (100 MHz, D_2O): δ 175.8 (C=O), 94.6 (d, J = 6.2 Hz, C-1), 72.3 (C-5), 70.6 (C-3), 68.3 (C-4), 68.0 (d, J = 7.8 Hz, C-2); ^{31}P NMR (162 MHz, D_2O): δ −0.280 (s). HRESIMS: $C_6H_{12}O_{10}P$ ($M+H^+$), calcd 275.0163, found 275.0170.

4. Conclusion

We have identified and biochemically characterized a highly efficient galactokinase from *B. infantis* with a k_{cat}/K_m value of 164 s^{-1} mM^{-1} toward Gal. Over 80 mg of BiGalK could be obtained from 1 L of cell cultures when over-expressed in *E. coli* BL21(DE3). BiGalK is also active toward Gal2D, GalN, and D-Fuc, and most interestingly, exhibits activity toward GalA, which has never been found on any other wild type GalKs. Furthermore, the enzyme could utilize a variety of nucleoside triphosphates as phosphate donors other than ATP. These features make BiGalK an attractive candidate for large scale synthesis of sugar-1-phosphates.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2012.04.022.

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