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Substitution of the catalytic acid–base Glu^{237} by Gln suppresses hydrolysis during glucosylation of phenolic acceptors catalyzed by *Leuconostoc mesenteroides* sucrose phosphorylase

Johanna Wiesbauer [a], Christiane Goedl [a], Alexandra Schwarz [a], Lothar Brecker [b], Bernd Nidetzky [a,*]

[a] Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Petersgasse 12, A-8010 Graz, Austria
[b] Institute of Organic Chemistry, University of Vienna, Währingerstraße 38, A-1090 Vienna, Austria

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ABSTRACT

Stereoselective glycosylation of a phenolic hydroxyl is a key transformation in the (bio)synthesis of natural products. Biocatalytic transglycosylation usually provides the desired glycosidic product in exquisite anomeric purity. However, loss of substrate and product to hydrolysis often limits application of the method. Kinetic studies and *in situ* proton NMR analysis of reaction time courses were used here to characterize glucosylation of substituted phenol acceptors by *Leuconostoc mesenteroides* sucrose phosphorylase in the presence of α-D-glucose 1-phosphate (αG1P) as donor substrate. In the wild-type enzyme, hydrolysis of the sugar 1-phosphate strongly prevailed (about 10-fold, ∼1.6 U/mg) over glucosyl transfer to the 2,6-difluorophenol acceptor (∼0.17 U/mg) used. A mutated phosphorylase in which the catalytic acid–base Glu^{237} had been replaced by Gln (E237Q) did not display hydrolase activity under transglucosylation conditions and therefore provided substantial (∼7-fold) enhancement of transfer yield. Utilization of the donor substrate was however slowed down (about 400-fold, ∼0.004 U/mg) in E237Q as compared to wild-type enzyme (∼1.6 U/mg). In a series of mono- and disubstituted phenols differing in hydroxyl pK_a between 7.02 and 8.71, the transferase activity of E237Q was found to be dependent on steric rather than electronic properties of the acceptor used. Both wild-type and mutated enzyme employed 4-nitrophenyl-α-D-glucopyranoside (4-NPG) as a slow artificial substrate for phosphorolysis and hydrolysis (native: ∼0.12 U/mg; E237Q: ∼0.02 U/mg).

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1. Introduction

Glycosylation is a useful method to increase the water solubility of bioactive polyphenols which hence become more suitable for application in functional foods and cosmetics [1–9]. Sucrose phosphorylase was shown to catalyze glucosyl transfer to phenolic hydroxy groups in a range of acceptor substrates including various catechin compounds [5–9]. This enzyme naturally utilizes sucrose for the glucosylation of phosphate, according to the reversible reaction shown in Eq. (1).

$$\text{Sucrose} + HPO_4^{2-} \leftrightarrow \alpha\text{-D-glucose1-}PO_4^{2-} + \text{D-fructose} \quad (1)$$

Abbreviations: αG1P, α-D-glucose 1-phosphate; 2-CP, 2-chlorophenol; 2,6-DCP, 2,6-dichlorophenol; 2,6-DFP, 2,6-difluorophenol; E237Q, $Glu^{237} \rightarrow$ Gln mutated sucrose phosphorylase; 2-FP, 2-fluorophenol; MES, 2-(N-morpholino)ethane sulphonic acid; 4-NPG, 4-nitrophenyl-α-D-glucopyranoside.

* Corresponding author. Tel.: +43 316 873 8400; fax: +43 316 873 8434.
E-mail address: bernd.nidetzky@tugraz.at (B. Nidetzky).

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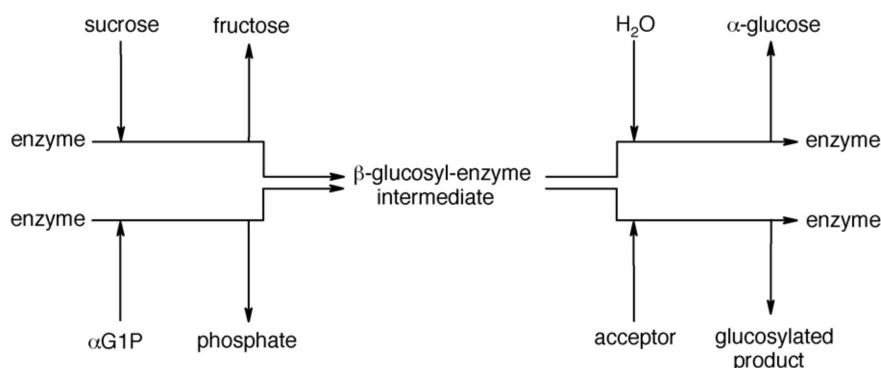
The catalytic mechanism of sucrose phosphorylase follows a two-step double displacement-like reaction that involves formation of a covalent β-glucosyl enzyme intermediate [10–13], as shown in Scheme 1. In the absence of phosphate, the intermediate reacts with water to give the hydrolysis product α-D-glucose or under conditions in which a suitable external glucosyl acceptor is present, it can be intercepted to yield a new α-glucosidic product [5–9,14,15]. Hydrolysis and glucosyl transfer are therefore competing reactions of the glucosylated enzyme (Scheme 1). Atom-efficient utilization of the donor substrate demands that hydrolysis be suppressed to the maximum extent possible. Using wild-type enzyme, the hydrolysis path is unfortunately often the one that predominates [14]. Hydrolysis is a general problem in biocatalytic transglycosylation processes [16,17].

Scheme 2 shows that during glucosylation of sucrose phosphorylase by sucrose, departure of the fructose leaving group is assisted by Brønsted catalysis from a conserved glutamic acid in the active site. While enzyme deglucosylation to phosphate is expected to have a low requirement for general base catalytic facilitation, addition of water under hydrolysis conditions arguably necessitates such participation from the now anionic Glu. We have recently



Scheme 1. Kinetic mechanism for transglucosylation to external acceptors catalyzed by sucrose phosphorylase under conditions in which the natural acceptor substrate phosphate is absent.

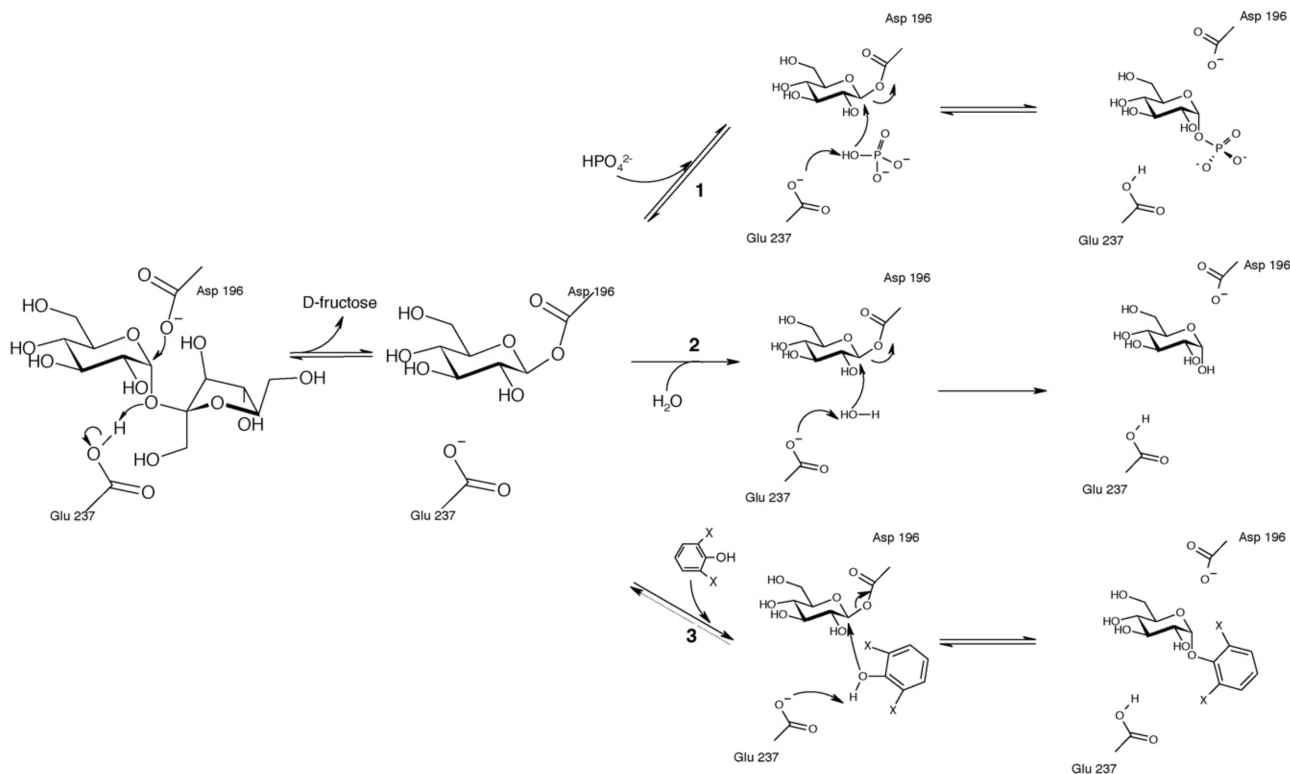
shown that site-directed substitution of the relevant Glu^{237} in *Leuconostoc mesenteroides* sucrose phosphorylase by a catalytically incompetent Gln results in almost complete loss of activity towards sucrose [13]. However, glucosylation of the $Glu^{237} \rightarrow$ Gln mutated enzyme (E237Q) by α-D-glucose 1-phosphate (αG1P) proceeded with an efficiency comparable to that of glucosylation of wild-type sucrose phosphorylase using the same donor substrate. Obviously, departure of a phosphate leaving group does not require facilitation from a catalytic general acid. The covalent intermediate of E237Q underwent catalytic glucosyl transfer to anionic nucleophiles (azide, acetate, formate) whereas the wild-type enzyme was transferase-incompetent and gave only hydrolysis product under otherwise identical reaction conditions [13].

Acceptor group pK_a is expected to determine the intrinsic chemical reactivity of a substrate to become glucosylated. Due to their relatively low pK_a (≤10), phenolic hydroxy groups might therefore undergo enzymatic glucosylation by E237Q despite the absence of base catalytic assistance from the mutated phosphorylase to the reaction. If additionally E237Q was relatively lower in hydrolase activity than the wild-type enzyme, a notably improved transglucosidase might be obtained. We report here on a comparison of wild-type sucrose phosphorylase and E237Q as catalysts for glucosylation of different phenolic acceptors using αG1P as donor substrate. Substantial enhancement of transfer compared to hydrolysis efficiency was found for the mutated phosphorylase.

2. Experimental

2.1. Chemicals

2,6-Difluorophenol (2,6-DFP; $pK_a = 7.45$), 2-fluorophenol (2-FP; $pK_a = 8.71$), 2-chlorophenol (2-CP; $pK_a = 8.50$), 2,6-dichlorophenol



Scheme 2. Catalytic mechanism of sucrose phosphorylase from *Leuconostoc mesenteroides* utilized for phosphorolysis of sucrose (path 1), hydrolysis (path 2), and transfer to acceptors (path 3). The requirement for base catalytic facilitation by Glu^{237} during deglucosylation of the enzyme will depend on the acceptor used. Acceptors displaying high intrinsic reactivity (e.g. substituted phenols having low pK_a) could become glucosylated in the absence of base catalysis and might be useful substrates for the $Glu^{237} \rightarrow$ Gln mutated enzyme that shows low hydrolase activity [13].

(2,6-DCP; pK_a = 7.02), and 4-nitrophenol (pK_a = 7.08) were from Sigma–Aldrich. 4-Nitrophenyl-α-D-glucopyranoside (4-NPG) was from TCI Europe (Eschborn, Germany). All other chemicals, reagents, and analytical enzymes were described elsewhere [12–14].

2.2. Enzymes

Wild-type sucrose phosphorylase from *L. mesenteroides* was recombinantly produced in *E. coli* DH10B using a pQE30 expression vector encoding the enzyme harboring an N-terminal oligohistidine tag [14]. A mutated phosphorylase in which the catalytic acid–base Glu^{237} was substituted by Gln was prepared as described recently in *E. coli* JM109 cells [12,13]. Both enzymes were isolated using reported protocols [12–14] and were used as preparations giving a single protein band in SDS PAGE.

2.3. Assays and analytical methods

Protein was determined using the Bio-Rad dye binding method calibrated against BSA in the concentration range 0.1–1.0 mg/mL. The activity of the phosphorylase preparations used was measured at 30 °C using assays that monitored either the phosphorolysis of sucrose (wild-type enzyme) or the arsenolysis of αG1P (wild-type enzyme; E237Q). A coupled enzymatic assay described elsewhere in detail [18] was employed to determine initial rates of phosphorolysis. Arsenolysis was performed by incubating the enzyme (wild-type: ~2 μg/mL; E237Q: ~0.02 mg/mL) in the presence of 100 mM αG1P and 75 mM sodium arsenate dissolved in 50 mM MES buffer, pH 7.0. Incubation was done in 2-mL plastic tubes for about 15–20 min using an agitation rate of 550 rpm (Thermomixer comfort, Eppendorf). The glucose released was measured using the "hexokinase" method described below. The reaction rate was calculated as [glucose]/time.

Phosphate produced upon conversion of αG1P was measured using a colorimetric test [19] referenced against known phosphate concentrations in the range ≤1.00 mM. Glucose was determined enzymatically using coupled assays based on the enzyme pair hexokinase/glucose 6-phosphate dehydrogenase or modified glucose oxidase/peroxidase [20,21,15]. Alternatively, HPLC was used for glucose determination. A Merck-Hitachi LaChrome LC system was employed that was further equipped with a L-7250 autosampler, a L-7400 UV detector, and a L-7490 RI detector. Two Aminex HPX-87C and HPX-87H columns (i.d. 300 mm × 7.8 mm) from Bio-Rad were operated at 65 and 30 °C respectively using water and 5 mM H_2SO_4 respectively as eluent (0.6 mL/min).

2.4. Transglucosylation – kinetic studies

Reactions were performed in 50 mM MES buffer, pH 7.0, in a total volume of 1–2 mL. A constant concentration of αG1P (10 mM) was used. Acceptor phenol was added in concentrations between 5.0 and 50 mM. Wild-type enzyme was used in a concentration of ~2 μg/mL whereas E237Q was present at ~0.2 mg/mL. Incubations were performed at 30 °C using an agitation rate of 550 rpm (Thermomixer comfort). Relevant control reactions lacking the enzyme or one of the substrates were carried out, and the reported values are corrected for blank readings.

Samples (~50 μL) taken from the reaction mixture at certain times up to 24 h were analyzed for phosphate and glucose as well as by TLC. Plates were employed and a mixture of n-butanol, 2-propanol, ethanol, and water (4:3:2:1, by volume) was used as eluent. Spots were visualized using a solution of thymol (0.5%, w/v) in ethanol containing 5% (by volume) H_2SO_4.

2.5. Transglucosylation – in situ proton NMR

NMR spectra were recorded on a DRX-600 Avance spectrometer (Bruker, Rheinstetten, Germany), equipped with a *xyz*-gradient inverse probe. ^{1}H irradiation and measurement frequency was 600.13 MHz, and the sample temperature was 298.1 K. All spectra were processed with the Topspin 1.3 software. Proton spectra were recorded with an acquisition of 32,768 data points, a relaxation delay of 1.0 s, and between 16 and 128 scans. After zero filling to 65,536 data points the free induction decays were directly Fourier transformed to spectra with 6000 Hz (^{1}H). All spectra were recorded in D_2O and referenced on external acetone (δ ^{1}H: 2.225 ppm). The overwhelming HDO signal was suppressed by presaturation.

For *in situ* NMR, all enzymatic transformations were performed directly in the NMR sample tube (0.65–0.70 mL) at 25 °C using 50 mM MES buffer in D_2O (pD 6.65). αG1P (10 mM) and 2,6-DFP (25 mM) were dissolved in buffer and the pD was adjusted. Enzyme was added from an aqueous stock solution. The concentration of wild-type enzyme and E237Q was 2 μg/mL and 0.2 mg/mL, respectively. Measurements were performed in regular intervals over 14 h. Between 16 and 64 proton spectra were directly taken in each enzymatic reaction.

2.6. Enzymatic conversion of 4-nitrophenyl-α-D-glucopyranoside

A constant concentration of 5.0 mM 4-NPG was used. Reactions were carried out at 30 °C in 50 mM MES buffer, pH 7.0, containing (*phosphorolysis*) or lacking (*hydrolysis*) 5.0 mM potassium phosphate. Reactions were started by adding wild-type (0.2 mg/mL) or mutated enzyme (0.2 mg/mL). Release of 4-nitrophenol was measured at 405 nm using a Beckmann DU-800 spectrophotometer. The molar extinction coefficient of 4-nitrophenol in MES buffer was determined as 8.82 mM^{-1} cm^{-1} under the conditions used.

3. Results and discussion

3.1. Selection of suitable analytical methods

Kinetic analysis of glucosyl transfer from αG1P to phenolic acceptors was based on the mechanism shown in Scheme 1. In this mechanism, the covalent β-glucosyl enzyme intermediate partitions between reaction with the acceptor (*glucosyl transfer*) and reaction with water (*hydrolysis*). Under hydrolysis-only conditions when a suitable acceptor is absent, the initial rates of formation of phosphate ($V_{phosphate}$) and glucose ($V_{glucose}$) are identical. A rate ratio $V_{phosphate}/V_{glucose}$ significantly greater than unity is therefore indicative of glucosyl transfer to the acceptor. From the mass balance of the reaction (Scheme 1), the difference in the concentrations of phosphate and glucose yields the concentration of transfer product.

While determination of $V_{phosphate}$ was straightforward under all reaction conditions used, we encountered problems using enzymatic assays for measuring the concentration of glucose in samples containing phenolic acceptor. Using 2,6-DFP (25 mM), the colorimetric response of the glucose oxidase/peroxidase assay was strongly perturbed with respect to maximum wavelength of absorbance (405 → 585 nm) and sensitivity (~5-fold decrease). Because each acceptor would have required a separate calibration for every single concentration employed in the reactions, the "glucose oxidase" assay was not further used. Applying the hexokinase/glucose 6-phosphate dehydrogenase method, we observed that glucose determination in samples from transglucosylation experiments was compromised by a poorly defined end-point of the coupled enzymatic reaction, arguably caused by

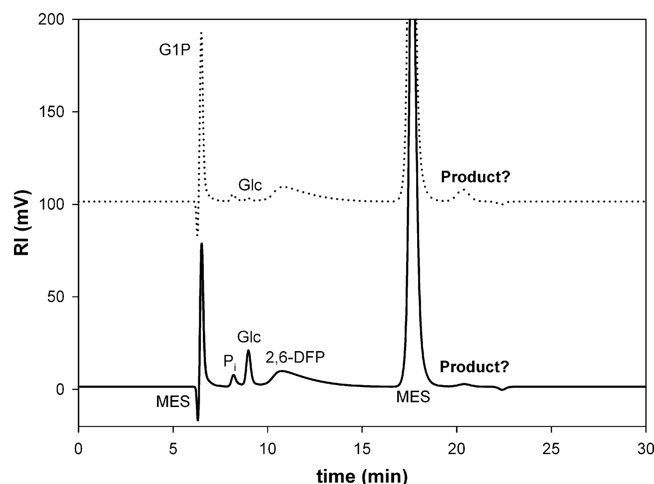


Fig. 1. Transglucosylation monitored by HPLC (HPX-87H column). Samples were taken from conversions of 10 mM αG1P catalyzed by wild-type enzyme (solid line) and E237Q (dashed line, shifted by 100 mV) under conditions when 2,6-DFP acceptor was added in a concentration of 25 mM. The reaction time was 24 h.

instability of the transfer product under assay conditions. HPLC was therefore used for measurement of glucose release.

Fig. 1 shows HPLC chromatograms of samples taken from 24 h-long conversions of αG1P catalyzed by wild-type enzyme and E237Q under conditions when 2,6-DFP acceptor was added in concentrations of 25 mM. Formation of glucose was hardly affected by 2,6-DFP in the reaction of wild-type sucrose phosphorylase while it was clearly suppressed in the reaction of E237Q as compared to the relevant controls that lacked the external acceptor. The conversion of αG1P was not altered (wild-type enzyme) or enhanced (E237Q) in the presence of 2,6-DFP.

3.2. Kinetic studies of transglucosylation

Fig. 2A shows time courses of phosphate release from αG1P catalyzed by E237Q under conditions of a varied concentration of 2,6-DFP acceptor. The linear relationship between [phosphate] and time was used to calculate the phosphorolysis rate, $V_{phosphate}$. Addition of 2,6-DFP caused a marked (~8-fold) increase of $V_{phosphate}$ for E237Q as compared to phosphate-release rate under hydrolysis-only conditions (Fig. 2B). At high acceptor concentrations (≥25 mM), however, $V_{phosphate}$ decreased due to inhibitory effects that were not further pursued. $V_{phosphate}$ for the wild-type enzyme was not affected by added 2,6-DFP in concentrations of up to 50 mM (data not shown). The dependence of $V_{phosphate}$ for E237Q on the concentration of 2,6-DFP was fitted with Eq. (2) where k_{cat} is the turnover number (s^{-1}), E is the molar enzyme concentration (3.6 μM; calculated from the protein concentration of 0.2 mg/mL and the enzyme molecular mass of 55.7 kDa), K_m is an apparent Michaelis constant for the acceptor (mM), and V_0 is the hydrolysis rate in the absence of 2,6-DFP.

$$V_{phosphate} = \frac{k_{cat}E[2,6\text{-DFP}]}{K_m + [2,6\text{-DFP}]} + V_0 \quad (2)$$

Data for [2,6-DFP] in the range 5–25 mM were fitted assuming a constant value of 9 $\mu M\,h^{-1}$ for V_0. An estimate of 20 ± 3 mM was obtained for K_m. The k_{cat} of $9.4 \times 10^{-3}\,s^{-1}$ is higher than the k_{cat} of $6.3 \times 10^{-4}\,s^{-1}$ E237Q in the synthesis of sucrose (from αG1P and fructose). However, it is much lower than the k_{cat} of 21 s^{-1} for the arsenolysis of αG1P by E237Q.

Rate enhancement for E237Q by the external acceptor is explicable on account of a kinetic mechanism where in the absence of 2,6-DFP, reaction of the covalent glucosyl enzyme intermediate is rate-limiting for the overall conversion catalyzed by the mutated phosphorylase. Interception of the glucosylated enzyme by the acceptor results in a faster regeneration of the free enzyme for reaction.

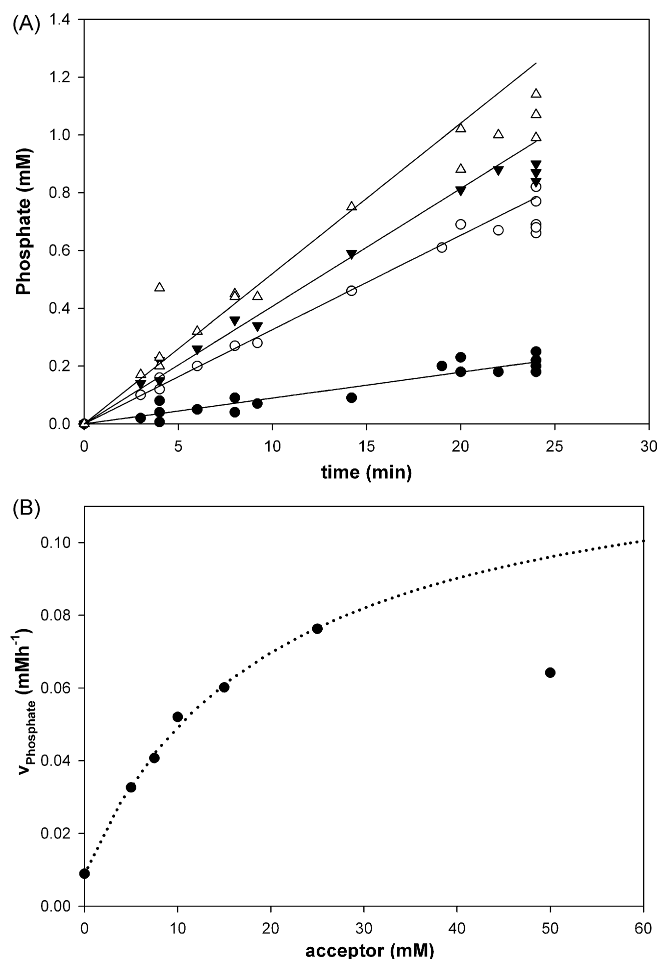


Fig. 2. Glucosylation of 2,6-DFP catalyzed by E237Q. (A) Time course analysis. Hydrolysis (closed circles), 5 mM 2,6-DFP (open circles), 7.5 mM 2,6-DFP (closed triangles), and 10 mM 2,6-DFP (open triangles). Higher concentrations are not shown in this figure. (B) Enhancement of $V_{phosphate}$ by 2,6-DFP. The dotted line shows a nonlinear fit of equation 1 to the data. Data at 50 mM were not used in the fit because the decrease in activity could be the result of enzyme inhibition, inactivation or a combination of the two effects. The concentration of E237Q was 0.2 mg/mL.

3.3. Reaction time courses monitored by in situ proton NMR

Using transglucosylation conditions (25 mM 2,6-DFP), the conversion of αG1P by wild-type enzyme and E237Q was analyzed directly in the NMR sample tube. Fig. 3 shows *in situ* recorded ^{1}H-NMR spectra at different incubation times. The reaction of the wild-type enzyme yielded mainly glucose and only tiny amounts of glucosyl transfer product (Fig. 3A). The first observed formation of α-glucose upon hydrolysis of αG1P is consistent with the double displacement-like catalytic mechanism of sucrose phosphorylase in which the anomeric configuration of the donor substrate is retained in the reaction product. However, mutarotation leads to mixture of the α-anomer and the β-anomer, which converges after extended reaction times to the dynamic equilibrium with an excess of the β-anomer. Using E237Q, hydrolysis of donor substrate was suppressed below detection limit and only formation of the transfer product was observed (Fig. 3B).

Fig. 4 shows time course of product formation by wild-type and mutant enzyme. Considering the difference in enzyme concentration, conversion of donor substrate was about 400-fold

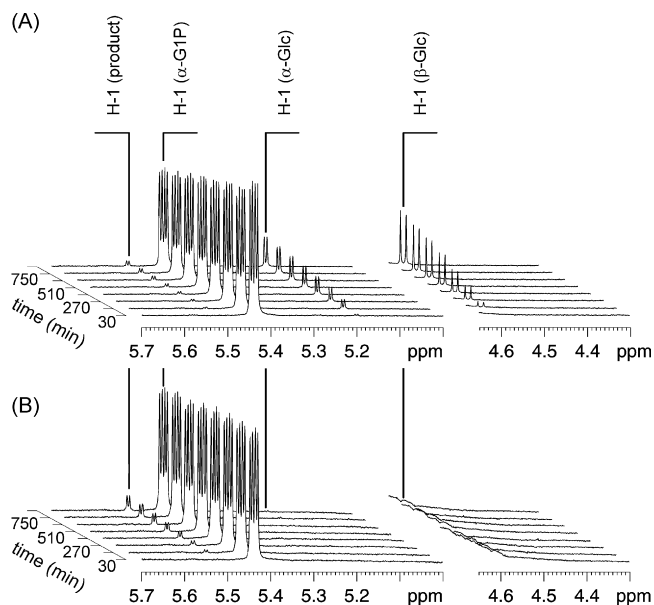


Fig. 3. Stack plot of *in situ* proton NMR measurements of transglucosylation catalyzed by wild-type sucrose phosphorylase (A) and E237Q (B). Shown is the region of anomeric protons with decreasing H-1 signal of substrate αG1P and the increasing H-1 signals of the formed transglucosylation product as well as of α-Glc and β-Glc.

faster by wild-type sucrose phosphorylase (∼1.6 U/mg) than E237Q (∼0.004 U/mg). The β-glucosyl enzyme intermediate formation in the wild-type preferred reaction with water (∼1.6 U/mg) was ∼10-fold over intermediate of the wild-type enzyme preferred reaction with water (∼1.6 U/mg) about 10-fold over reaction with the 2,6-DFP acceptor (∼0.17 U/mg). For E237Q, however, selectivity of the glucosylated enzyme for reaction with acceptor was absolute, as no glucose could be detected. Based on the same amount of substrate converted, E237Q provided a ∼7-fold enhancement in trangluco-sylation yield as compared to the wild-type enzyme. HPLC was employed to monitor the progress of the same reactions in aqueous buffer (pH 7.0) at 30 °C keeping all other conditions identical to those used in the NMR measurements. Results fully fitted to NMR-

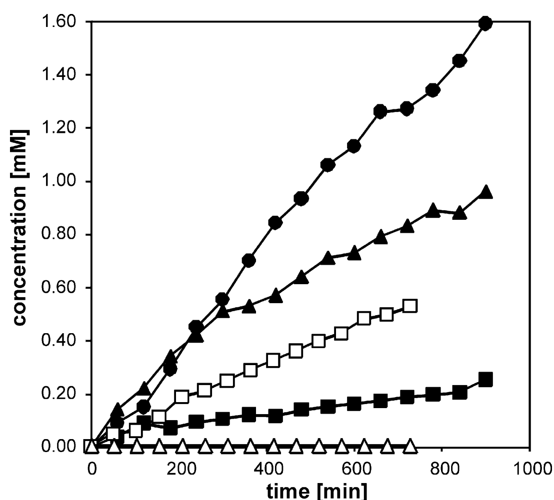


Fig. 4. Time course of product formation using data from *in situ* proton NMR measurement (Fig. 3). Transformation using the wild-type enzyme leads to transglycosylation product (closed squares) and α-Glc (closed triangles), which is partly converted to β-Glc (closed circles) by mutarotation. In comparison, E237Q-catalyzed reaction provides exclusively the transglycosylation product (open squares), while no detectable amounts of α-Glc and β-Glc (summed, open triangle) have been formed.

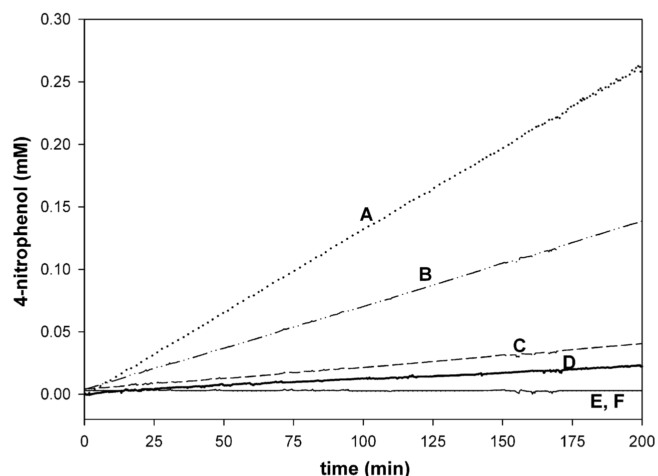


Fig. 5. Time courses of phosphorolysis and hydrolysis of 4-NPG catalyzed by wild-type sucrose phosphorylase and E237Q. Each reaction was monitored by release of 4-NP. Hydrolysis by wild-type enzyme (A) and E237Q (D); phosphorolysis by wild-type enzyme (B) and E237Q (C). Control reactions lacking enzyme in the absence (F) and presence of P_i (E).

derived selectivities indicating that kinetic isotope effects did not influence glucosyl transfer and hydrolysis.

Kitao, Sekine and colleagues reported on the use of wild-type sucrose phosphorylase from *L. mesenteroides* for glucosylation of the phenolic hydroxy group in a series of aryl acceptors such as phenol, benzene diol and triols, hydroxy benzylalcohols and hydroxy benzoic acids [6]. They used sucrose as donor substrate, typically in 30- to 50-fold excess (by mass) over acceptor (1–2% w/v) and chose pH 7.5. Among the compounds tested, benzene-1,2-diol was most efficiently glucosylated [6]. Unfortunately, the extent to which hydrolysis competed with glucosyl transfer under these conditions cannot be inferred from the papers.

3.4. Structure–activity relationship analysis for glucosyl transfer to phenolic acceptors by E237Q

In the absence of catalytic assistance from a Brønsted base, interception of the β-glucosyl enzyme intermediate of E237Q by phenolic nucleophiles might depend on the pK_a of the reactive hydroxy group on the acceptor substrate. Compounds having a relatively low pK_a would be expected to undergo glucosyl transfer efficiently. We therefore examined 2-FP, 2-CP, 2,6-DFP, and 2,6-DCP each at a constant concentration of 10 mM, comparing $V_{\text{phosphate}}$ and V_{glucose} observed under transglucosylation conditions with the corresponding control rates. Judging by a rate ratio $V_{\text{phosphate}}/V_{\text{glucose}}$ that was not significantly different from unity, no glucosyl transfer occurred to 2-CP and 2,6-DCP. Like 2,6-DFP ($V_{\text{phosphate}}/V_{\text{glucose}} \approx 4.0$), 2-FP served as acceptor ($V_{\text{phosphate}}/V_{\text{glucose}} \approx 2.7$) for glucosyl transfer from E237Q. The data suggest that steric properties of the acceptor phenol (F → Cl) are considerably more important for determining the efficiency of enzymatic transglucosylation by E237Q than intrinsic electronic properties of the reactive hydroxyl measured in the value of pK_a.

3.5. Enzymatic conversion of 4-nitrophenyl-α-D-glucopyranoside

To further examine the role of acid–base catalysis in the reaction of sucrose phosphorylase with phenolic leaving groups/nucleophiles, we measured phosphorolysis (Fig. 5) and hydrolysis of 4-NPG by wild-type enzyme and E237Q. The wild-type enzyme converted 4-NPG with a specific activity of ∼0.1 U/mg (apparent $k_{cat} \approx 0.1\ s^{-1}$), irrespective of the presence or absence of phosphate. The result implies that enzyme glucosylation from

4-NPG is the rate-determining step in the reaction of wild-type phosphorylase. It is known from literature data on this enzyme that breakdown of the covalent intermediate occurs with rate constant of >100 s^{-1} [13] in phosphorolysis and ∼2 s^{-1} [12] in hydrolysis. The mutant E237Q catalyzed the phosphorolysis of 4-NPG with a specific activity of $\sim 2 \times 10^{-2}$ U/mg. The corresponding specific activity of the mutated enzyme for hydrolysis was about half that for phosphorolysis.

The comparison of wild-type enzyme and E237Q regarding conversion of 4-NPG reveals the apparent absence of general acid catalysis from Glu^{237} to the conversion of the aryl-α-D-glucopyranoside substrate. The loss of specific activity towards 4-NPG resulting from the substitution of Glu^{237} by Gln was just about 5-fold. It can be compared with the 12.4-fold decrease in the k_{cat} of E237Q for arsenolysis of αG1P (∼21 s^{-1}) as compared to the corresponding k_{cat} of the wild-type enzyme (∼261 s^{-1}) [13]. It was shown previously that arsenolysis of αG1P proceeds without substantial acid–base catalytic assistance from Glu^{237} [13]. Phosphorolysis and synthesis of sucrose, by contrast, require catalytic facilitation from Glu^{237} acting as a general acid and base, respectively. E237Q is a $\sim 10^5$-fold poorer catalyst for these reactions than is the wild-type enzyme.

The low activity of both wild-type and mutated sucrose phosphorylase towards 4-NPG suggests that in contrast to αG1P, the non-natural substrate cannot be brought into an enzyme-bound conformation that would support efficient glucosyl transfer to the catalytic nucleophile Asp^{196}. It would seem that sucrose phosphorylase has a problem in productively accommodating the 4-nitrophenyl leaving group at sub-site +1 of the enzyme. Sub-site +1 binds the fructosyl moiety and phosphate during the natural conversion of sucrose. 4-NPG is clearly not a good substrate to measure the activity of sucrose phosphorylase [22]. Difficult positioning of substituted phenols at sub-site +1 would be consistent with the low activity of E237Q for glucosyl transfer to these acceptors. It also provides a plausible explanation for why in the wild-type enzyme, hydrolysis is the by far preponderant reaction under transglucosylation conditions.

4. Conclusions

Substitution of the catalytic acid–base Glu^{237} of *L. mesenteroides* sucrose phosphorylase by a Gln generated a variant enzyme notably deficient in hydrolase activity. Using 2,6-DFP as acceptor substrate, E237Q was exclusively specific for catalyzing glucosyl transfer from αG1P to the phenolic hydroxy group whereas the wild-type enzyme showed primarily hydrolysis under otherwise identical conditions. E237Q could thus be a useful transglucosidase for reactions involving donors (e.g. αG1P or α-D-glucopyranosyl fluoride) and acceptors (hydroxy groups having low pK_a) whose reactivities do not critically depend on catalytic participation from a general acid–base on the enzyme. However, efficient glucosylation of phenols would require that the specific activity of E237Q be further enhanced.

Acknowledgements

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References

[1] K. Chiku, J. Uzawa, H. Seki, S. Amachi, T. Fujii, H. Shinoyama, Biosci. Biotechnol. Biochem. 72 (2008) 2285–2293.
[2] T. Nishimura, T. Kometani, H. Takii, Y. Terada, S. Okada, J. Ferment. Bioeng. 78 (1994) 31–36.
[3] R. Prodanovic, N. Milosavic, D. Sladic, J. Mol. Catal. B: Enzym. 35 (2005) 142–146.
[4] T. Sato, H. Nakagawa, J. Kurosu, K. Yoshida, T. Tsugane, S. Shimura, K. Kirimura, K. Kino, S. Usami, J. Biosci. Bioeng. 90 (2000) 625–630.
[5] S. Kitao, T. Ariga, T. Matsudo, H. Sekine, Biosci. Biotechnol. Biochem. 57 (1993) 2010–2015.
[6] S. Kitao, H. Sekine, Biosci. Biotechnol. Biochem. 58 (1994) 38–42.
[7] S. Kitao, H. Sekine, Biosci. Biotechnol. Biochem. 58 (1994) 419–420.
[8] S. Kitao, T. Matsudo, M. Saitoh, T. Horiuchi, H. Sekine, Biosci. Biotechnol. Biochem. 59 (1995) 2167–2169.
[9] S. Kitao, T. Matsudo, T. Sasaki, T. Koga, M. Kawamura, Biosci. Biotechnol. Biochem. 64 (2000) 134–141.
[10] O. Mirza, L.K. Skov, D. Sprogøe, L.A.M. van den Broek, G. Beldman, J.S. Kastrup, M. Gaihede, J. Biol. Chem. 281 (2006) 35576–35584.
[11] J.J. Mieyal, R.H. Abeles, in: P.D. Boyer (Ed.), The Enzymes, Academic Press, New York, 1972, pp. 512–532.
[12] A. Schwarz, B. Nidetzky, FEBS Lett. 580 (2006) 3905–3910.
[13] A. Schwarz, L. Brecker, B. Nidetzky, Biochem. J. 403 (2007) 441–449.
[14] C. Goedl, A. Schwarz, A. Minani, B. Nidetzky, J. Biotechnol. 129 (2007) 77–86.
[15] C. Goedl, T. Sawangwan, M. Mueller, A. Schwarz, B. Nidetzky, Angew. Chem., Int. Ed. 47 (2008) 10086–10089.
[16] D.H. Crout, G. Vic, Curr. Opin. Chem. Biol. 2 (1998) 98–111.
[17] F. van Rantwijk, M. Woudenberg-van Oosterom, R.A. Sheldon, J. Mol. Catal. B: Enzym. 6 (1999) 511–532.
[18] C. Eis, B. Nidetzky, Biochem. J. 341 (1999) 385–393.
[19] S. Saheki, A. Takeda, T. Shimazu, Anal. Biochem. 148 (1985) 277–281.
[20] A. Kunst, B. Draeger, J. Ziegenhorn, in: H.U. Bergmeyer (Ed.), Methods in Enzymatic Analysis, Academic Press, 1974, pp. 163–172.
[21] A. Kunst, B. Draeger, J. Ziegenhorn, in: H.U. Bergmeyer (Ed.), Methods in Enzymatic Analysis, Academic Press, 1974, pp. 178–185.
[22] M. Kim, T. Kwon, H.J. Lee, K.H. Kim, D.K. Chung, G.E. Ji, E. Byeon, J. Lee, Biotechnol. Lett. 25 (2003) 1211–1217.