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Regio- and stereoselective glucosylation of diols by sucrose phosphorylase using sucrose or glucose 1-phosphate as glucosyl donor

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ABSTRACT

Previously it has been shown that glycerol can be regioselectively glucosylated by sucrose phosphorylase from *Leuconostoc mesenteroides* to form 2-*O*-α-D-glucopyranosyl-glycerol (Goedl et al., Angew. Chem. Int. Ed. 47 (2008) 10086–10089). A series of compounds related to glycerol were investigated by us to determine the scope of the α-glucosylation reaction of sucrose phosphorylase. Both sucrose and glucose 1-phosphate (G1P) were applied as glucosyl donor. Mono-alcohols were not accepted as substrates but several 1,2-diols were readily glucosylated, proving that the vicinal diol unit is crucial for activity. The smallest substrate that was accepted for glucosylation appeared to be ethylene glycol, which was converted to the monoglucoside for 69%. Using high acceptor and donor concentrations (up to 2.5 M), sucrose or G1P hydrolysis (with H_2O being the 'acceptor') can be minimised. In the study cited above, a preference for glucosylation of glycerol on the 2-position has been observed. For 1,2-propanediol however, the regiochemistry appeared to be dependent on the configuration of the substrate. The (*R*)-enantiomer was preferentialy glucosylated on its 1-position (ratio 2.5:1), whereas the 2-glucoside is the major product for (*S*)-1,2-propanediol (1:4.1). d.e.$_p$s of 71–83% were observed with a preference for the (*S*)-enantiomer of the glucosides of 1,2-propanediol and 1,2-butanediol and the (*R*)-enantiomer of the glucoside of 3-methoxy-1,2-propanediol. This is the first example of stereoselective glucosylation of a non-natural substrate by sucrose phosphorylase. 3-Amino-1,2-propanediol, 3-chloro-1,2-propanediol, 1-thioglycerol and glyceraldehyde were not accepted as substrates. Generally, the glucoside yield is higher when sucrose is used as a donor rather than G1P, due to the fact that the released phosphate is a stronger inhibitor of the enzyme (in case of G1P) than the released fructose (in case of sucrose). Essentially the same results are obtained with sucrose phosphorylase from *Bifidobacterium adolescentis*.

1. Introduction

Glucosylation of alcohols and phenols is an important reaction in nature and industry. Glucosides, oligo- and polysaccharides are used by nature e.g. for energy storage, for strengthening cells and tissues, and for molecular recognition. In pharmaceutical industry, glucosylation is used to increase the solubility of drugs. Last but not least, regio- and stereoselectively formed glucosides are used as intermediates for other (bio)chemicals. Enzymatic glucosylation naturally occurs mostly by tailor-made glucosyltransferases, with a different glucosyltransferase for each glucosyl acceptor [1]. These transferases usually use UDP-glucose as glucosyl donor and therefore the equilibria of the reactions catalysed are far on the glucoside side. However, the expensive nature of this donor is one of the major bottlenecks in industrial application of these enzymes.

A class of enzymes that has been intensely studied in their ability to catalyse glucosylation with glucose as the donor are glucosidases [1–4]. The equilibrium of the reaction catalysed by these enzymes is however on the side of hydrolysis of the glucoside, leading to often low yields with these enzymes. The equilibrium can be influenced by the use of glucose derivatives with good leaving groups, like 4-nitrophenol [4], but these substrates are rather expensive. Alternative approaches involve media with minimal water content [5–9] or continuous removal of the product [10] and they sometimes give reasonable yields, but still they have inherent limitations.

More recently, phosphorylases have been extensively studied with respect to glucosylation, one of these being sucrose phosphorylase. The enzyme from *Bifidobacterium adolescentis* has been shown to catalyse the regioselective glucosylation of several sugars such as L-arabinose [11]. The natural reaction catalysed by this enzyme is the phosphorolysis of sucrose, with the concomitant formation of α-glucose 1-phosphate (G1P) and fructose:

$$\text{sucrose} + P_i \rightarrow \text{G1P} + \text{D-fructose} \quad (1)$$

The reaction proceeds through a β-glucosyl intermediate, which is formed upon reaction of the enzyme with sucrose, as shown by

Abbreviations: G1P, Glucose α-1-phosphate; SUC, Sucrose; Fru, Fructose; Glc, Glucose; P_i, Phosphate; d.e.$_p$, Diastereomeric excess of the product.

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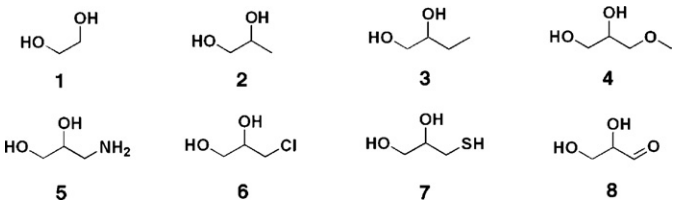

Fig. 1. Potential glucosyl acceptors similar to glycerol used in this study.

X-ray crystallography studies [12]. If phosphate is absent, other glucosyl acceptors can be glucosylated:

$$\text{sucrose} + \text{acceptor} \rightarrow \alpha\text{-glucoside} + \text{D-fructose} \quad (2)$$

This reaction occurs with net retention of the α-glucosidic bond. The equilibrium of this reaction is between the extreme situations of the glucosyl transferases and glucosidases; the exact equilibrium depends on the nature of the glucosyl acceptor. Both sucrose and G1P can be used to form the glucosyl–enzyme intermediate. In the latter case phosphate is released rather than fructose:

$$\text{G1P} + \text{acceptor} \rightarrow \alpha\text{-glucoside} + P_i \quad (3)$$

Recently, Nidetzky and co-workers [13] have shown that glycerol can be regioselectively converted with high yield using sucrose phosphorylase from *Leuconostoc mesenteroides*. 2-*O*-(α-D-glucopyranosyl)-*sn*-glycerol, a natural osmolyte, is formed and is currently marketed under the trade name Glycoin® [14]. To determine the scope and selectivity of sucrose phosphorylase, a series of compounds related to glycerol were investigated by us (Fig. 1), using the enzymes from *L. mesenteroides* and *B. adolescentis*. The formation of glucosylated ethylene glycol (**1**), 1,2-propanediol (**2**), 1,2-butanediol (**3**) and 3-methoxy-1,2-propanediol (**4**) is described, using sucrose or G1P as the glucosyl donor. The glucosylation products of 1,2-propanediol, 3-methoxy-1,2-propanediol and 1,2-butanediol were isolated and analysed. The glucosylation reactions of 1,2-propanediol, 1,2-butanediol and 3-methoxy-1,2-propanediol show interesting regio- and stereoselectivity.

2. Experimental

2.1. Materials

Sucrose phosphorylase from *L. mesenteroides*, sucrose, glucose 1-phosphate, glycerol, ethylene glycol, (*R*,*S*)-1,2-propanediol, (*R*)-1,2-propanediol, (*S*)-1,2-propanediol, (*R*,*S*)-1,2-butanediol, (*R*,*S*)-3-methoxy-1,2-propanediol, 3-chloro-1,2-propanediol, 1-thioglycerol, (*R*,*S*)-3-amino-1,2-propanediol, (*R*)-3-amino-1,2-propanediol, (*S*)-3-amino-1,2-propanediol, glyceraldehyde, magnesium chloride, MES buffer, disodium hydrogenphosphate, hydrogen chloride were obtained from Sigma–Aldrich (Zwijndrecht, The Netherlands). Sucrose phosphorylase from *B. adolescentis* was produced and isolated as described before [11]. Methanol (Biosolve, Valkenswaard, The Netherlands) and ethylacetate (Fischer Scientific, Loughborough, UK) were HPLC grade. NANOpure water (18.3 MΩ cm) was used during all experiments (Barnstead, Thermo Scientific). Pre-packed flash purification silica cartridges (SNAP 10 g KP-SIL) were obtained from Biotage.

2.2. Enzymatic glucosylation

Typical enzymatic reactions (250 μL) were done using 100 mM G1P or 100 mM sucrose. A stock solution of G1P was brought to pH 6.6 using HCl without adding additional buffer, whereas in the case of sucrose (100 mM) MES buffer pH 6.6 (50 mM) was added. Incubations at $T = 30\,^{\circ}C$ further contained glucosyl donor (see below), 10 mM $MgCl_2$ and 25 U/mL sucrose phosphorylase. The highest practical glucosyl acceptor concentration was used, namely: 2.5 M ethylene glycol, 2.5 M (*R*,*S*)-1,2-propanediol, 2.5 M (*R*)-1,2-propanediol, 2.5 M (*S*)-1,2-propanediol, 2.5 M 1,2-butanediol, 2.5 M 3-methoxy-1,2-propanediol, 0.25 M 3-chloro-1,2-propanediol, 2.5 M 1-thioglycerol, 0.25 M glyceraldehyde, 1.7 M 3-amino-1,2-propanediol. The 3-amino-1,2-propanediol stock solution was brought to pH 7 with 37% HCl prior to use. Conversions were followed in time by HPLC. The pH of the mixtures was checked after completion. In the case of G1P a maximum increase to pH 7.0 was observed. Two standard controls for all reactions were performed: (i) same conditions without enzyme; (ii) same conditions without glucosyl acceptor. For product isolation with a charcoal column, 1.0 M sucrose and 1.0 M 1,2-butanediol were used (1 mL scale); this reaction was followed using HPLC.

2.3. HPLC analysis

An Alltech OA-1000 column plus a wide pore C4 Security Guard (Phenomenex, Utrecht, The Netherlands) were used with 25 mM H_2SO_4 as eluent at a flow rate of 0.4 mL min^{-1}, coupled to a refractive index detector (Gilson M 131, Gilson France, Villiers le Bel, France). Samples were diluted 10-fold in water after which 20 μL was injected via a fixed volume loop. Calibration curves for phosphate, glucose, fructose, sucrose and glucosyl acceptors were linear in the concentration range measured; the same holds for G1P after correction for the overlap with the negative void peak. Concentrations of these compounds were calculated using these curves; for the glucosides the concentrations were calculated assuming that glucose production + glucoside production = glucoside-1-phosphate consumption (=phosphate production).

2.4. TLC analysis

Prior to product isolation reactions were checked by TLC analysis to determine optimal eluent conditions, using various ratio's of H_2O, methanol and ethyl acetate. Spots were colored with a molybdate-cerium based reagent (42 g L^{-1} $Mo_7O_{24}(NH_4)_6{\cdot}4H_2O$, 3.6 g L^{-1} $Ce(NH_4)(SO_4)_4{\cdot}2H_2O$, 6.2% (v/v) H_2SO_4).

2.5. Product isolation

2.5.1. Silica column

Glucoside products from (*R*,*S*)-1,2-propanediol, (*R*)-1,2-propanediol, (*S*)-1,2-propanediol, (*R*,*S*)-1,2-butanediol and (*R*,*S*)-3-methoxy-1,2-propanediol obtained with G1P as glucosyl donor were isolated using pre-packed silica cartridges using the Isolera One flash chromatography system from Biotage. This procedure led to the removal of most of the excess glucosyl acceptor, some remaining G1P, the formed phosphate and most of the formed glucose; glucoside regio- or stereoisomers could not be separated in this way. A mixture of ethyl acetate:methanol:water = 7:2:1 was used for elution. Fractions were analysed by HPLC and pooled fractions were freeze dried and taken up in D_2O for NMR analysis.

2.5.2. Charcoal column

Using equimolar concentrations of 1,2-butanediol and sucrose (1.0 M) resulted in 87% sucrose consumption after 96 h reaction time. The product mixture consisted of 85% glucosides (corresponding to 750 mM) and 15% glucose (120 mM). The glucoside product mix was isolated with a charcoal column based on the procedure that Goedl et al. [13,14] used for the isolation of the glucoside of glycerol. This method removed sucrose, formed fructose and glucose and remaining 1,2-butanediol, but could not separate product