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Ionic liquids as cosolvents for glycosylation by sucrose phosphorylase: balancing acceptor solubility and enzyme stability.

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Abstract

Over the past decade, disaccharide phosphorylases have received increasing attention as promising biocatalysts for glycoside synthesis. Unfortunately, these enzymes typically have a very low affinity for non-carbohydrate acceptors, which urges the addition of cosolvents to increase the dissolved concentration of these acceptors. However, commonly applied solvents such as methanol and dimethyl sulfoxide (DMSO) are not compatible with many intended applications of carbohydrate-derived products. In this work, the solubility of a wide range of relevant acceptors was assessed in the presence of ionic liquids (ILs) as alternative and 'green' solvents. The IL AMMOENG 101 was found to be the most effective cosolvent for compounds as diverse as medium- and long-chain alcohols, flavonoids, alkaloids, phenolics and terpenes. Moreover, this IL was shown to be less deleterious to the stability and activity of sucrose phosphorylase than the commonly used dimethyl sulfoxide. To demonstrate the usefulness of this solvent system, a process for the resveratrol glycosylation was established in a buffer containing 20 % AMMOENG 101, 1 M sucrose and saturated amounts of acceptor. A single regioisomer 3-*O*-α-D-glucopyranosyl-(*E*)-resveratrol was obtained as proven by NMR spectroscopy.

Keywords

Sucrose phosphorylase, Solubility, Stability, Ionic liquids, Resveratrol, Glycosylation

Introduction

Glycosylation of small molecules can substantially improve their physicochemical and biological properties, thereby extending their application potential in various industries[1-4]. Prominent examples include the increased solubility of therapeutic flavonoids[5], the prolonged stability of ascorbic acid in cosmetic formulations[6], and the modified perception of flavours and fragrances[7]. Despite the continuous development of new procedures, chemical synthesis of glycosides still suffers from low yields because of the need for (de)protection and activation steps[8, 9]. Biocatalytic approaches are, therefore, an attractive alternative that enables one-step reactions with high regio- and stereoselectivity[10]. Enzymatic glycosylation reactions typically generate 5-fold less waste and have a 15-fold higher space-time yield, which is a tremendous improvement in the eco-efficiency[2].

Currently, few enzymes are available to perform glycosylation reactions cost-efficiently at the industrial scale[11]. On the one hand, glycosyl transferases (GT) require nucleotide-activated sugars that are relatively expensive[12] and glycoside hydrolases (GH) suffer from low yields when used in the synthetic direction[13]. Consequently, disaccharide phosphorylases have received increasing attention in recent years as promising biocatalysts for glycoside synthesis[14-16]. These enzymes catalyze the reversible phosphorolysis of glycosidic bonds, and thus only require a glycosyl phosphate as donor substrate for synthetic reactions.

Unfortunately, disaccharide phosphorylases have typically very low affinity (K_m > 1 M) for non-carbohydrate acceptors[17, 18]. The catalytic efficiency of sucrose phosphorylase (SP) towards such compounds is usually not significantly higher than the contaminating hydrolytic activity[17]. Nevertheless, an efficient process for the production of 2-*O*-(α-D-glucopyranosyl)-*sn*-glycerol has been developed with this enzyme by careful optimization of the substrate

concentrations[19, 20]. For hydrophobic acceptors like flavonoids and polyphenols, however, concentrations compensating for the high K_m values are not readily achieved in aqueous solutions. Enzymatic glycosylation of these compounds is, therefore, commonly performed in the presence of organic cosolvents, such as DMSO or methanol. However, these solvents are not compatible with numerous applications of carbohydrate-derived products[21], and their presence can lead to the partial inactivation of the biocatalyst[17, 22, 23].

Ionic liquids (ILs) have emerged recently as a completely new class of solvents for biocatalytic applications[24, 25]. These green solvents consist of salts with a low melting point (<100 °C) that possess no vapor pressure and are inflammable[26]. Due to their ability to dissolve both polar and hydrophobic substrates, ILs have been successfully applied for the synthesis of glycosidic compounds[27-30]. For example, the yield for the galactosylation of *N*-acetylglucosamine with β-galactosidase from *Bacillus circulans* could be doubled upon addition of 25 % [MMIM][MeSO4][26]. However, no attention has yet been paid to the use of ILs as cosolvents for disaccharide phosphorylases.

In this work, the stability and activity of the SP from *Bifidobacterium adolescentis* was evaluated in the presence of various ILs, and the solubility of relevant acceptors in these systems was determined.

1. Results and discussion

1.1 Solubility of acceptors in IL cosolvent systems

The enzymatic glycosylation of hydrophobic compounds is complicated by their low solubility in aqueous systems[22, 31]. Therefore, the effect of various ILs on solubility was evaluated here, and compared with that of conventional cosolvents at a concentration of 20 % (Table 1). Optimal values (pH 6.5 and 60 °C) for the sucrose phosphorylase from *B. adolescentis*[32] were used. Furthermore, the influence of sucrose (1M) was also assessed, since this substrate serves as glycosyl donor for SP and will thus be present during the actual reactions. The initial screening was performed with resveratrol and quercetin as target compounds with powerful anti-oxidant and anti-inflammatory properties but a very low solubility in water[33-35].

Table 1 Solubility at pH 6.5 and 60 °C, in the presence of 20 % cosolvent and 1 M sucrose.

	Solubility resveratrol (mM)		**Solubility quercetin (mM)**	
Solvent	**- sucrose**	**+ sucrose**	**- sucrose**	**+ sucrose**
Water	0.2 ± 0.1	2.4 ± 0.3	< 0.1	< 0.1
MeOH	2.2 ± 0.2	5.8 ± 0.6	0.2 ± 0.1	0.4 ± 0.1
DMSO	37.5 ± 2.9	46.3 ± 3.8	9.4 ± 0.9	12.1 ± 1.1
Tween 20	44.5 ± 3.3	56.1 ± 4.2	10.5 ± 1.1	11.8 ± 2.0
Triton X-100	11.5 ± 1.3	18.6 ± 0.9	1.6 ± 0.2	2.2 ± 0.4
[MMIM][$MeSO_4$]	13.8 ± 1.3	15.4 ± 0.8	0.5 ± 0.1	0.5 ± 0.2
[BMIM][BF_4]	10.1 ± 1.2	12.6 ± 1.1	0.4 ± 0.1	0.5 ± 0.2
[BMIM][dca]	11.8 ± 1.6	16.4 ± 1.8	0.5 ± 0.1	0.6 ± 0.1
[BMIM][I]	39.5 ± 3.2	47.3 ± 3.9	0.8 ± 0.2	1.2 ± 0.3
[EMIM][$EtSO_4$]	14.6 ± 1.2	18.1 ± 1.4	0.6 ± 0.1	0.9 ± 0.1

[EMIM][dca]	130.4 ± 9.8	145.1 ± 11.6	1.5 ± 0.2	1.8 ± 0.4
AMMOENG 101	246.6 ± 15.2	286.5 ± 12.9	21.2 ± 1.1	24.9 ± 2.0

As expected only traces of resveratrol or quercetin could be dissolved in the cosolvent-free system. The addition of 20 % ILs resulted in a remarkable increase in solubility, without affecting the ability to dissolve 1 M sucrose. All ILs easily outperformed methanol as cosolvent. Furthermore, both [EMIM][dca] and AMMOENG 101 proved to have better dissolving properties than DMSO, which was recently used for the enzymatic synthesis of resveratrol glucosides[31]. The nature of the anion as well as the cation appears to be important for the immidazolium-based ILs, which is in agreement with previous observations[21, 36]. Interestingly, the presence of 1 M sucrose was found to further increase the solubility, although the effect was rather limited in most cases.

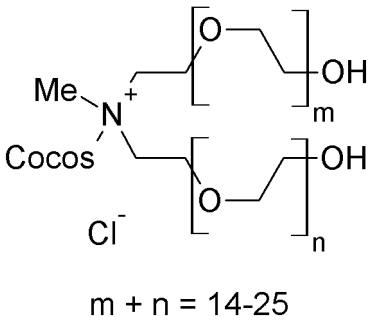

Figure 1 Chemical structure of the ethoxylated quaternary ammnonium salt AMMOENG 101.

The highest solubility was obtained when using AMMOENG 101 (Figure 1), an ethoxylated quaternary ammnonium salt manufactured by Solvent Innovation. This IL, also commercialized by Evonik Industries as TEGO K5, has already been used to capture carbon dioxide from fuel gas streams[37], and to solubilize water-insoluble ketones[38]. The solubilizing properties of AMMOENG 101 were then examined in more detail, by varying the amount of

cosolvent added (Figure 2). As could be expected, increasing the concentration of IL resulted in an increased solubility of the acceptors. However, a maximum was observed at 60 % AMMOENG 101, after which the solubility decreased. Resveratrol could be dissolved up to an impressive 2.0 M, whereas the highest solubility of quercetin was found to be 0.2 M. When the temperature was lowered from 60 °C to 37 °C, the peak shifted to 40 % IL, corresponding to a maximal resveratrol and quercetin solubility of 1.2 M and 0.1 M, respectively (data not shown).

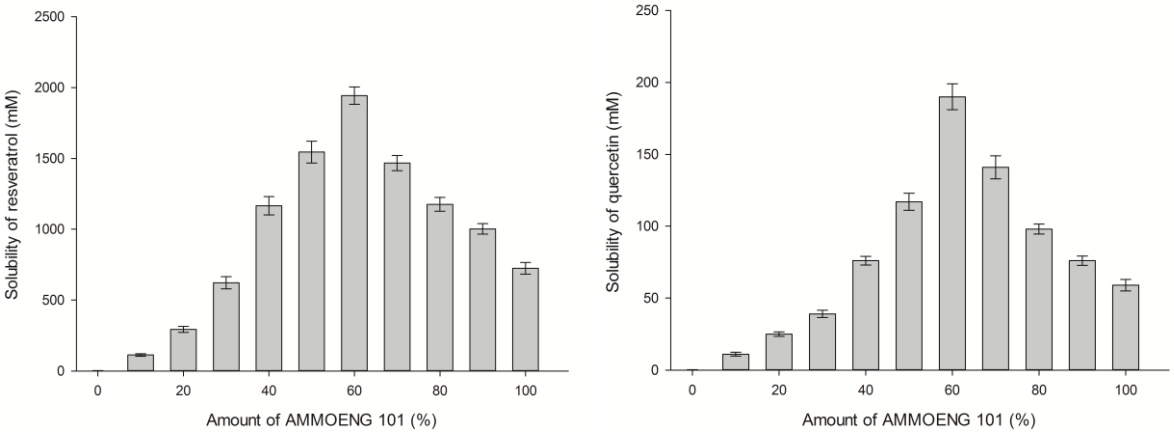

Figure 2 Solubility at pH 6.5 and 60 °C in the presence of various concentrations of AMMOENG 101.

Finally, the solubility of other glycosylation acceptors was determined in both DMSO and AMMOENG 101 cosolvent systems (Table 2). Based on their structure, these acceptors can be divided into five classes, *i.e.* flavonoids, medium- and long-chain alcohols, terpenes, alkaloids and phenolics. The addition of cosolvent enhanced the solubility of all of them but the IL was remarkably more efficient than DMSO. For example, the solubility of sparingly soluble substances such as curcumin and dodecanol could be increased to 46 mM and 510 mM, respectively, by the addition of 40 % AMMOENG 101. The same condition resulted in a tenfold increased solubility of 3-hydroxy-2-nitropyridine and cinnamyl alcohol. Solubility of

hexanol and geraniol was increased by two ordes of magnitude, resulting in a concentration of 3.1 M for the former. In conclusion, the IL was found to boost the solubility of various chemicals, from rather hydrophilic to very hydrophobic, regardless of their type.

Table 2 Solubility of various compounds at 60 °C in a 50 mM MES buffer at pH 6.5 containing 20 and 40 % DMSO and AMMOENG 101 respectively.

Compound	Solubility (mM)				
	Buffer	DMSO		AMMOENG 101	
		20 %	40 %	20 %	40 %
Resveratrol	0.2 ± 0.1	37.5 ± 2.9	97.5 ± 6.9	246 ± 15	1166 ± 95
Quercetin	< 0.1	9.4 ± 0.9	22.1 ± 1.3	21.2 ± 1.1	76.5 ± 5.2
Curcumin	< 0.1	8.2 ± 0.5	19.1 ± 1.1	13.5 ± 0.8	45.9 ± 1.8
Dodecanol	1.3 ± 0.2	3.6 ± 0.2	18.1 ± 1.7	268 ± 18	508 ± 39
Hexanol	6.3 ± 0.8	12.3 ± 0.9	31.1 ± 2.2	1664 ± 106	3108 ± 159
Geraniol	4.3 ± 0.3	9.3 ± 0.3	19.1 ± 1.4	403 ± 26	1861 ± 94
Linalool	5.2 ± 0.6	7.3 ± 0.4	16.1 ± 1.2	294 ± 18	1130 ± 82
3-Hydroxypyridine	255 ± 20	457 ± 26	923 ± 68	654 ± 51	1395 ± 109
3-Hydroxy-2-nitropyridine	261 ± 18	602 ± 54	1356 ± 96	968 ± 63	2652 ± 153
Saligenin	352 ± 16	409 ± 11	920 ± 48	721 ± 52	2560 ± 192
Salicylic acid	7.3 ± 0.7	61.8 ± 3.6	162 ± 12	271 ± 21	854 ± 66
Vanillyl alcohol	70.4 ± 5.9	452 ± 11	796 ± 66	817.3 ± 75.6	1018 ± 72
Cinnamyl alcohol	17.4 ± 1.3	40.6 ± 2.9	137 ± 9	937 ± 46	2751 ± 196
4-Nitrophenol	167 ± 12	187 ± 16	442 ± 31	642 ± 52	1219 ± 86

1.2 Stability and activity in IL cosolvent systems

Although the addition of ILs allows to dissolve a wide variety of compounds, their presence could negatively affect the stability of the biocatalyst[22, 27]. Therefore, the half-life (t_{50}) of the SP from *B. adolescentis* was determined in the presence of different cosolvents (Table 3). In agreement with previous studies of other enzymes, [MMIM][$MeSO_4$], AMMOENG 101 and DMSO were found to be less deleterious for the stability of SP than for example [BMIM][I], [BMIM][BF_4] and methanol[27, 28, 38, 39]. Moreover, the presence of sucrose appears to be a critical parameter for the enzyme's stability. Indeed, the addition of 1 M sucrose resulted in a 25-fold increased half-life when 20 % DMSO or AMMOENG 101 is present. Similar patterns were obtained with the well-known protectant trehalose,[40] which is not a substrate of SP (data not shown). The results indicate that stabilization does not originate from the binding of substrate in the active site but rather from non-specific interactions at the protein surface[41, 42].

Table 3 Stability of SP at pH 6.5 and 60 °C, in the presence of 20 % cosolvent and 1 M sucrose.

	t_{50} (min)	
Solvent	**- sucrose**	**+ sucrose**
Water	3210 ± 126	4261 ± 192
[EMIM][$EtSO_4$]	16.0 ± 2.1	54.7 ± 3.2
[EMIM][dca]	5.1 ± 0.3	32.5 ± 2.6
[BMIM][dca]	4.8 ± 0.2	29.5 ± 1.9
[BMIM][I]	<1	2.1 ± 0.1
[BMIM][BF_4]	3.2 ± 0.1	33.8 ± 2.7
[MMIM]][$MeSO_4$]	32.6 ± 3.2	85.3 ± 6.6
AMMOENG 101	128 ± 6	3162 ± 198

DMSO	106 ± 9	2622 ±144
MeOH	3.2 ± 0.5	26.8 ± 2.4

166

167 Next, the stability of SP was determined at varying concentrations of both AMMOENG 101
168 and DMSO (Figure 3), the former being superior allowing 24 h half-life at the concentration
169 of 60 %, whereas such a t_{50} was already reached at a DMSO concentration of only 30 %.
170 Activity of SP in both cosolvents was assessed as well. The enzyme displayed a relative
171 activity of 98 and 87 % in 20 and 40 % IL, respectively, compared to 86 and 34 % in
172 identical concentrations of DMSO. According to these results, the IL AMMOENG 101 is a
173 much better cosolvent than the commonly used DMSO.

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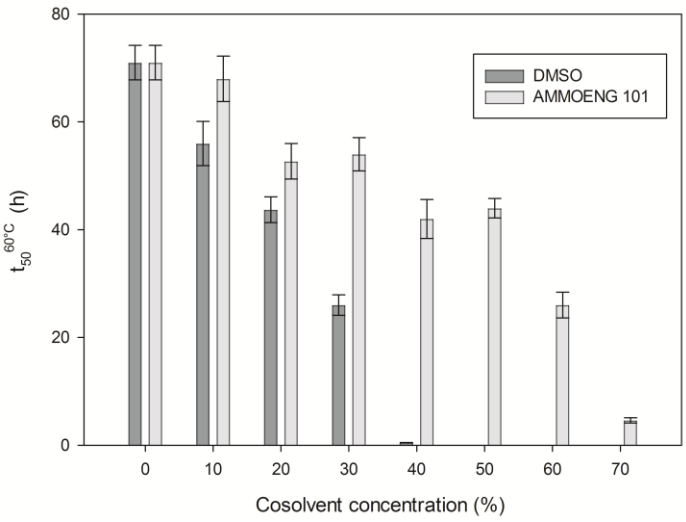

175

176 **Figure 3 Half-life of SP at pH 6.5 and 60 °C, in the presence of 1 M sucrose and varying cosolvent concentrations.**

177

178 Although the SP from *B. adolescentis* already is quite stable, a stabilized variant ('LNFI')
179 containing 6 mutations has recently been created[23]. Furthermore, immobilized preparations of
180 the enzyme have also been prepared, either by the covalent coupling to Sepabeads[32] or by the
181 production of a cross-linked enzyme aggregate (CLEA)[43, 44]. All of these are known to be

more thermostable that the native enzyme and they are shown here to be also more resistant to the presence of 20 % AMMOENG 101 (Figure 4). Indeed, the enzyme's t_{50} could be increased by about 15 and 40 h when using the LNFI variant or Sepabeads immobilisate, respectively. The CLEA was the most stable, and exhibited an impressive half-life of 100 h. These results support previous findings that thermal and solvent stability are closely related[27, 45, 46].

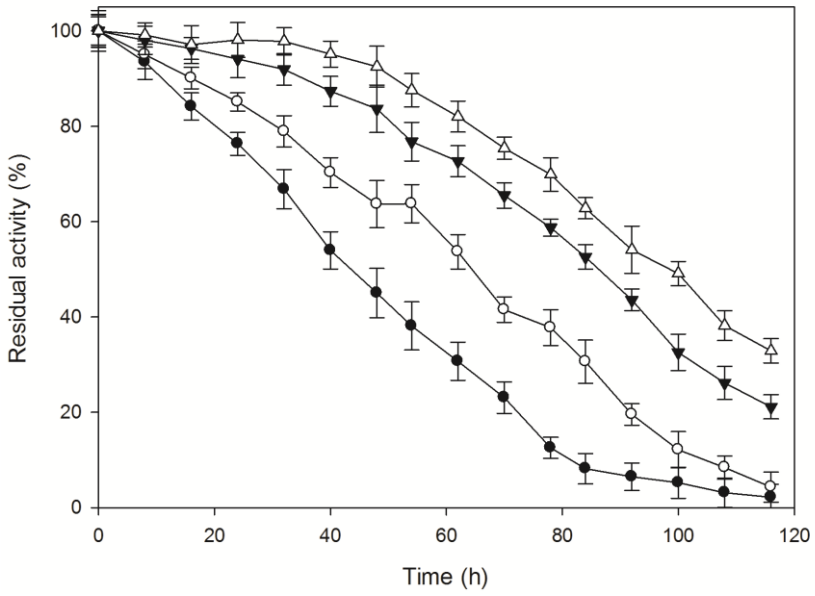

Figure 4 Stability of various SP formulations at 60 °C and pH 6.5, in the presence of 20 % AMMOENG 101 and 1 M sucrose. Wild-type enzyme (●), LNFI variant (○), Sepabeads immobilizate (▼) and CLEA immobilizate (Δ).

1.3 Production of glycosides in ILs

The applicability of AMMOENG 101as cosolvent for the production of glycosides with SP was assessed using the glycosylation of resveratrol as case study. After 24 h reaction of sucrose with resveratrol saturated in 20 % AMMOENG 101, a single new peak could be observed at 8.5 min, next to the signal of resveratrol at 10.9 min (Figure 5). The product was purified by silica gel chromatography, and identified as 3-*O*-α-D-glucopyranosyl-(*E*)-

resveratrol by NMR spectroscopy. Glycosylation of resveratrol has already been achieved with *Bacillus cereus* and *Streptococcus mutans* as the whole-cell biocatalysts[47, 48]. More recently, the synthesis of a mixture of α-glucosyl derivatives using cyclodextrin glucanotransferase was described[31]. However, this is the first report on the use of a purified enzyme for the glucosylation of resveratrol yielding a single product.

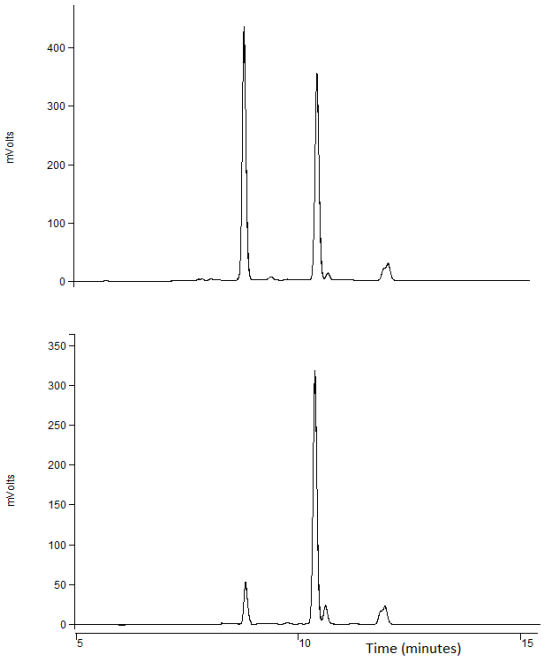

Figure 5 Production of α-glycosyl resveratrol with SP at pH 6.5 and 60 °C, using 1 M sucrose and resveratrol saturated in 20 % AMMOENG 101 (above) or DMSO (beneath) after 24 h incubation.

In contrast, about 10 times less product was formed when DMSO was used as cosolvent under optimal reaction conditions. The difference was even more pronounced with quercetin as acceptor substrate. Indeed, numerous attempts using 20 and 40 % of DMSO, DMF, methanol, ethanol and acetone as organic cosolvents failed to yield product. Only minor amounts of quercetin could be dissolved in 20 % of these solvents, whereas the addition of 40 % resulted in rapid inactivation of the enzyme. However, glycosylated products could clearly be detected when quercetin was saturated in 20 % AMMOENG 101 (data not shown). In addition, the glycosylation of all compounds from Table 1 was evaluated using similar

reaction conditions. Glucosides of hexanol, geraniol, 3-hydroxypyridine, saligenin, vanillyl alcohol and cinnamyl alcohol were observed by means of TLC analysis. These results identified the IL AMMOENG 101 as a powerful tool for glycosylation reactions with disaccharide phosphorylases, efficiently balancing acceptor solubility and enzyme stability.

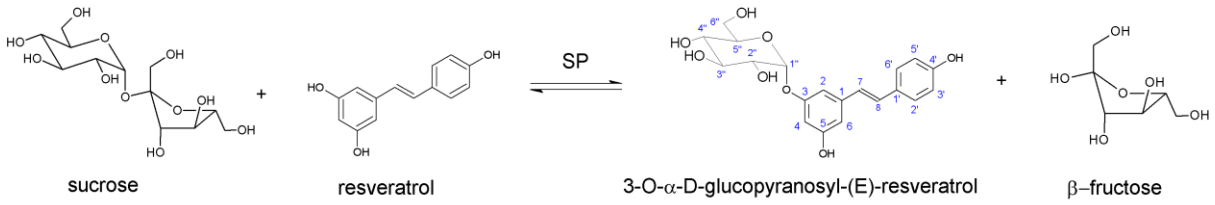

Figure 6 Enzymatic glucosylation of reveratrol catalyzed by thermostable SP in an ionic liquid cosolvent system.

2. Conclusions

Following the work on glycoside synthesis with SP in conventional solvents[49], this is the first report on the use of ionic liquids with disaccharide phosphorylases. The stability and activity of SP, as well as the solubility of a wide range of interesting acceptors was assessed. The results show that the solubility of flavonoids, medium- and long-chain alcohols, terpenes, alkaloids and phenolics can be improved significantly by addition of AMMOENG 101. Moreover, this IL was found to be less deleterious to the stability and activity of SP compared to organic solvents like DMSO or methanol. The presence of 1M sucrose as a substrate significantly improves the enzyme stability in the presence of cosolvents. As proof of concept, the production of 3-*O*-α-D-glucopyranosyl-(*E*)-resveratrol was demonstrated to be much more efficient in AMMOENG 101 compared to DMSO as cosolvent. Consequently, the use of ILs could find widespread application for glycoside synthesis with disaccharide phosphorylases.

3. Experimental

3.1 Materials and Enzymes

TEGO K5 and amino-epoxy (EC-HFA) Sepabeads were kindly provided by Evonik Industries AG and Resindion S.R.L (Mitsubishi Chemical Corporation), respectively. The other ILs were purchased from IoLiTec Ionic Liquids Technologies GmbH, and had a purity of at least 99 %. All other chemicals were analytical grade and purchased from Sigma-Aldrich. Recombinant SP was produced and partially purified by heat treatment as described recently[19]. The immobilized SP variants were produced according to protocols published earlier[32, 43].

3.2 Instruments

NMR spectra were measured on a Bruker Avance III (400 MHz) spectrometer, HPLC measurements were performed on a Varian Prostar, and UV absorbance at 340 nm was measured in a Bio-Rad microplate reader 680XR.

3.3 HPLC analysis

HPLC analysis was performed on a reversed phase column (Alltech Prevail C-18, 250 mm × 4.6 mm), with milliQ water (solvent A) and methanol (solvent B), both containing 0.05 % formic acid, as the mobile phase. The flow rate and temperature were set at 1.0 mL min^{-1} and 30 °C, respectively. The gradient elution was as follows: 100 % of solvent A (0 - 2 min), 0 to 90 % solvent B (2 - 20 min), 90 % solvent B (20 – 25 min), 90 to 0 % solvent B (25 – 30 min) and 100 % solvent A (30 – 38 min). Adequate detection was obtained with an Alltech 2000ES evaporative light scattering detector (ELSD) for dodecanol, hexanol, geraniol, linalool, 3-hydroxypyridine, 3-hydroxy-2-nitropyridine, saligenin and salicylic acid. The tube temperature, gas flow and gain were set at 25 °C, 1.5 L min^{-1} and 4, respectively. The other compounds were quantified using a Varian Prostar 320 UV/Vis detector at 306, 373, 335, 254, 317 and 246 nm for resveratrol, quercetin, curcumin, cinnamyl alcohol, 4-nitrophenol

and vanillyl alcohol, respectively. The obtained peaks were calibrated using standard curves of the specific compounds, prepared in DMSO or methanol. All HPLC analyses were performed in triplicate.

3.4 Solubility measurements

The dissolution of all compounds in the different cosolvent systems was performed in a water bath at 37 or 60 °C with ± 0.1 °C accuracy. Varying amounts of cosolvent and sucrose were added to 50 mM MES buffer at pH 6.5, and transferred to a 10 mL falcon. Next, the desired compounds were added until clear precipitation was observed. The samples were vortexed multiple times and allowed to equilibrate for 48 h. Undissolved solids were removed by filtration trough a 0.45 µm microporous membrane at the same temperature. Finally, the obtained solutions were diluted in DMSO or methanol, and subjected to HPLC analysis.

3.5 Activity assays

Phosphorolysis of sucrose was measured with an enzymatic assay, in which the production of α-D-glucose 1-phosphate (αG1P) is coupled to the reduction of NAD^+ in the presence of phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G6P-DH)[50]. The assay solution consisted of 2 mM EDTA, 10 mM $MgSO_4$, 2 mM β-NAD, 10 µM glucose-1,6-bisphosphate, 1.2 U PGM, 1.2 U G6P-DH and 100 mM sucrose in 100 mM phosphate buffer at pH 7 and 37 °C. The reactions were followed continuously or discontinuously by inactivation of samples (10 min at 95 °C) at regular intervals, for the free and immobilized SP respectively. The absorbance of the samples was measured at 340 nm in a microplate reader. One unit of SP activity corresponds to the release of 1 µmol fructose from 100 mM sucrose in 100 mM phosphate buffer at pH 7 and 37 °C. All assays were performed in triplicate and had a CV of less than 10 %.

3.6 *Stability assays*

The thermostability of SP was determined by incubating 25 U mL^{-1} in 50 mM MES buffer pH 6.5 in a water bath at 60 °C. If required, sucrose and cosolvents were added. At regular intervals, samples were taken and diluted 100 times in 50 mM MOPS buffer at pH 7. The samples were stored at 4 °C, and the activity at 37 °C was determined using the continuous αG1P assay. The t_{50}-values were calculated from the equations obtained by fitting the linear part of the stability curves. The stability of the immobilized biocatalysts was evaluated by incubating 1 and 15 mg of CLEAs and Sepabeads, respectively, in 300 µL 50 mM MES buffer at pH 6.5 containing 20 % IL AMMOENG 101 and 1 M sucrose. At regular intervals, samples were taken and diluted 100 times in 50 mM MOPS buffer at pH 7. The samples were stored at 4 °C, and the activity at 37 °C was determined using the discontinuous αG1P assay.

3.7 *Production and purification of glycosides*

The glycosylation of flavonoids was carried out at 10 mL scale in a 50 mM MES buffer at pH 6.5 containing 1 M sucrose and 20 % cosolvent. The substrate solutions were saturated with resveratrol or quercetin, and 50 U mL^{-1} of the SP variant 'LNFI' was added. After 24 h, the reactions were stopped by inactivation (10 min at 95 °C) and centrifuged (12000 g, 4 °C, 15 min) to remove all particulates. Next, the supernatant was diluted, and subjected to HPLC analysis. Alternatively, the aqueous buffer was evaporated in vacuo and the residue was purified by column chromatography on silica gel (EtOAC/MeOH/H_2O = 30:5:4; v/v/v). TLC analysis (EtOAC/MeOH/H_2O = 30:5:4; v/v/v) was conducted on Merck Silica gel 60 F_{254} precoated plates. Detection was achieved by spraying with 10 % (v/v) H_2SO_4 and heating. The chemical structure of the purified resveratrol glycoside was inferred from the combination of 1D NMR (^{1}H NMR and ^{13}C NMR) and 2D NMR (gCOSY, gHSQC and gHMBC) spectroscopic analysis; *J* values are given in Hz.

δ_H(400 MHz; CD_3OD) 7.39 (2 H, d, *J* = 8.6, 2'-H and 6'-H), 7.04 (1 H, d, *J* = 16.3, 8-H), 6.87 (1 H, d, *J* = 16.3, 7-H), 6.86 (1 H,s, 2-H),6.80 (2 H, d, *J* = 8.6, 3'-H and 5'-H), 6.62 (1 H, s, 6-H), 6.54 (1 H, s, 4-H), 5.50 (1 H, d, *J* = 3.5, 1"-H), 3.87 (1 H, dd, *J* = 9.7 and 8.9, 3"-H), 3.76 (2 H, m, 6"-Ha, 6"-Hb), 3.72 (1 H, ddd, *J* = 9.7, 4.2 and 2.5, 5"-H), 3.60 (1 H, dd, *J* = 9.7 and 3.6, 2"-H) and 3.48 (1 H, dd, *J* = 9.7 and 8.9, 4"-H).

δ_C(100 MHz; CD_3OD) 160.23 (C-3), 159.87 (c-5), 158.73 (C-4'), 141.75 (C-1), 130.63 (C-1'), 130.25 (C-8), 129.18 (C-2' and C-6'), 127.01 (C-7), 116.83 (C-3' and C-5'), 108.70 (C-6), 107.85 (C-2), 104.88 (C-4), 99.61 (C-1"), 75.28 (C-3"), 74.63 (C-5"), 73.64 (C-2"), 71.81 (C-4"), 62.63 (C-6").

According to the COSY experiment, the proton spectrum displays a –CH=CH- group, AA'BB' and AMX spin systems of para-disubstituted and 1,3,5-trisubstituted aromatic rings, and one saccharide unit. The set of extracted vicinal constants (d, *J* = 3.5 Hz) identified α-glucose, while the magnitude of $J_{7\text{-H},\ 8\text{-H}}$ (16.3 Hz) proved the *trans*-conformation of resveratrol. The position of the glycosylation was confirmed by the HMBC contact of the C-3 with 1"-H.

Abbreviation	**Full name**
IL	ionic liquid
AMMOENG 101 / TEGO K5	quaternary ammonium compounds, coco alkylbis (hydroxyethyl)methyl, ethoxylated, chlorides, methyl chloride
MES	2-(*N*-morpholino)ethanesulfonic acid
DMSO	dimethyl sulfoxide
[MMIM]][$MeSO_4$]	1-methyl-3-methylimidazolium methylsulfate
[BMIM][BF_4]	1-butyl-3-methylimidazolium tetrafluoroborate

[BMIM][dca]	1-butyl-3-methylimidazolium dicyanamide
[BMIM][I]	1-butyl-3-methylimidazolium iodide
[EMIM][$EtSO_4$]	1-ethyl-3-methylimidazolium ethylsulfate
[EMIM][dca]	1-ethyl-3-methylimidazolium dicyanamide
αG1P	α-D-glucose 1-phosphate
PGM	phosphoglucomutase
CLEA	cross-linked enzyme aggregate
GT	glycosyl transferases
GH	glycoside hydrolases
SP	sucrose phosphorylase

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