Exploring the glucosylation potential of glucansucrases

Devlamynck, Tim Nick

Chapter 3

Improving the low operational stability of Gtf180-ΔN from *Lactobacillus reuteri* 180 by means of its immobilization

Tim Devlamynck[1,2], Wim Soetaert[1], Lubbert Dijkhuizen[2]
Ghent University[1], University of Groningen[2]

Abstract

Glucansucrases are increasingly targeted as biocatalyst for the glycosylation of non-carbohydrate acceptor substrates. A major obstacle in their industrial use remains their relatively low operational stability at high temperatures and in systems containing cosolvents and high acceptor substrate concentrations. As a consequence, glycosylation of poorly soluble compounds results in low yields and productivities as only low concentrations of these acceptor substrates can be supplied in the reaction mixture. This chapter focused on overcoming the low operational stability of Gtf180-ΔN from *Lactobacillus reuteri* 180 by cross-linking the enzyme to mesoporous silica particles, yielding an immobilized enzyme with enhanced activity at temperatures above 50 °C and in systems containing 20% DMSO, reaction conditions detrimental for the free enzyme. As a result, the glycosylation of gallic acid (GA), caffeic acid (CA) and catechin (CT) could be performed in reaction mixtures containing much higher concentrations of acceptor substrate. Their conversion was improved accordingly: from 63% to 82%, from 59% to 80%, and from 4% to 11%, for GA, CA and CT, respectively.

1. Introduction

Glucansucrases are glycoside hydrolase enzymes (GH70), catalyzing sucrose hydrolysis (minor activity) and the conversion of sucrose into α-glucan polysaccharides (major activity), linking the α-D-glucopyranosyl units by (α1→2), (α1→3), (α1→4) or (α1→6) bonds, depending on the enzyme specificity[73,74]. In addition, they catalyze so called acceptor reactions, thereby glycosylating various (poly)phenolic and aliphatic compounds, using sucrose as donor substrate[111-114]. As such, glucansucrases offer a cheap alternative for "Leloir" glycosyltransferases[18], which require rare and expensive nucleotide-activated sugars as donor substrate[13]. Due to their broad acceptor substrate specificity and their use of inexpensive sucrose as donor substrate, glucansucrases have attracted considerable interest from academia and industry for their application as glycosylation biocatalyst. Glycosylation may result in an increased solubility of hydrophobic compounds[33], an improved stability of labile molecules against light and oxidation[7], or a modified taste profile[8].

Although several alternative acceptor substrates are indeed glycosylated by glucansucrases, very often incomplete conversions, with low to moderate yields, are obtained. Alternative acceptor substrates are per definition not the natural acceptor substrates of glucansucrases and, hence, they generally have rather high K_m values (Chapter 2)[110]. To outcompete α-glucan synthesis and hydrolysis as possible glucansucrase reactions, high concentrations of acceptor substrate are consequently required. Shifting the relative balance between the three glucansucrase reactions towards the acceptor reaction by applying high acceptor substrate concentrations was demonstrated as early as 1993 by Su and Robyt[132]. Furthermore, high space-time yields, which greatly reduce production costs, can only be achieved if the acceptor substrate concentrations are correspondingly high. It was demonstrated in Chapter 2 that suppressing α-glucan synthesis by mutational engineering enhanced the glycosylation of several phenolic and aliphatic compounds[110]. The biggest improvements were observed for small molecules such as catechol and butanol. The glycosylation of larger molecules,

such as flavonoids, was still problematic, mostly due to the low water solubility of these compounds, preventing their addition in higher concentrations. Chapter 2 also revealed that glucansucrases were inhibited by high concentrations of non-carbohydrate acceptor substrates: the glycosylation of catechol by Gtf180-ΔN from *Lactobacillus reuteri* 180 was inhibited at concentrations of catechol higher than 400 mM[110]. Similarly, catechol displayed inhibitory effects on glucansucrase GtfD from *Streptococcus mutans* GS-5 at a concentration of 200 mM[125]. Finally, also the inhibition of amylosucrase (GH13) from *Neisseria polysaccharea* by several flavonoids has been reported[124].

As mentioned earlier, the limited water solubilities of many (poly)phenolic and aliphatic acceptor substrates complicate their glycosylation by glucansucrases. Increasing the solubility of acceptor substrates can be achieved by the addition of organic cosolvents such as DMSO, ethanol, acetone, etc. The main drawback of this strategy is that enzyme activity –and stability typically decrease with increasing solvent concentrations[172]. Determination of the initial activity of the dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F in the presence of organic solvents revealed a 50% loss in activity in 20% DMSO, 15% ethanol, 15% acetone, 10% DMF and 7% acetonitrile[134]. Diglyme or bis(2-methoxyethyl) ether (MEE) displayed a lower inhibitory effect on glucansucrases: the dextransucrase from *L. mesenteroides* NRRL B-512F and the alternansucrase from *L. mesenteroides* NRRL B-23192 retained more than 50% activity at an MEE concentration of 30%[120]. The previous examples indicate that the combined use of high concentrations of certain acceptor substrates and high solvent concentrations may be even more detrimental for glucansucrase activity. Hence, a compromise between acceptor substrate concentration, solvent concentration and enzyme activity needed to be found.

An alternative approach comprises the application of a biphasic glycosylation system. In this way, the inhibitory effect of cosolvents can be circumvented while the solubility of the acceptor substrates is still enhanced. In a biphasic glycosylation system, the aqueous phase contains the biocatalyst and the donor substrate sucrose, whereas the organic phase contains the hydrophobic acceptor

substrates. Upon stirring, the acceptor substrates are transferred to the aqueous phase, where they are enzymatically glycosylated. Enzyme stability is correlated with solvent polarity: water miscible cosolvents distort the essential water layer that stabilizes the enzyme, whereas hydrophobic solvents will leave this layer of water molecules intact, resulting in a higher enzyme stability. Solvent polarity is expressed by the logarithm of the octanol/water partition coefficient (log P). Solvents with a log P < 2 will deactivate enzymes more distinctly than hydrophobic solvents with a log P > 4[173]. Furthermore, the use of a biphasic glycosylation system minimizes enzyme inhibition by acceptor substrates due to their low actual concentration in the aqueous phase; hydrophobic compounds are only slowly released from the organic phase, which acts as a substrate reservoir[174,175]. The use of a biphasic glycosylation system was already demonstrated for the glycosylation of several phenolic and aliphatic compounds with sucrose phosphorylase from *Bifidobacterium adolescentis*, resulting in enhanced glycosylation yields[23].

Immobilization is a well-known strategy to increase the operational activity and stability of an enzyme. It may alleviate the decrease of enzyme activity and stability provoked by high solvent and acceptor substrate concentrations[140]. An additional advantage is the possibility of recycling the immobilized biocatalyst, which can drastically lower the economic cost of the enzymatic process[141]. Glucansucrases have been described as troublesome to covalently immobilize, mainly due to inactivation of the enzyme during immobilization, e.g. by the participation of a lysine residue in the active site. Typical immobilization yields (ratio of activity of immobilized enzyme to the activity of enzyme prior immobilization) range from 3% to 22%[143-146]. In contrast, encapsulation of glucansucrases in alginate has been more successfully applied: several studies report immobilization yields up to 90%[147,148]. However, the resulting alginate beads are not durable, since the accumulation of α-glucan polysaccharides inside the beads ultimately results in their rupture.

To our knowledge, the inhibitory effects of acceptor substrates and cosolvents on glucansucrases have never been countered by applying enzyme immobilization

nor by using a biphasic system. This chapter investigates both strategies to overcome the low operational stability of Gtf180-ΔN from *L. reuteri* 180: the application of a biphasic system with ethyl acetate as second phase, and the covalent immobilization of this enzyme on mesoporous silica particles.

2. Materials and methods

2.1. Production and purification of recombinant Gtf180-ΔN

Recombinant, N-terminally truncated Gtf180-ΔN from *Lactobacillus reuteri* 180 was produced and purified as described previously[80].

2.2. Protein determination

Protein concentration was determined using the BCATM Protein Assay kit from Pierce. The protocol as described in the kit was used. In short, 200 µL of freshly prepared assay solution was added to 25 µL of protein sample. After incubation at 37 °C for 30 min, the absorbance was measured at 562 nm. As standard series, bovine serum albumin (BSA) was used in a range from 0-2 mg/mL.

2.3. Activity assay for free and immobilized Gtf180-ΔN

Enzyme activity assays were performed at 37°C with 100 mM sucrose in 25 mM sodium acetate (pH 4.7) and 1 mM $CaCl_2$ unless indicated otherwise. Samples of 100 µL were taken every min over a period of 8 min and immediately inactivated with 20 µL 1 M NaOH for 30 min. The released glucose and fructose were quantified enzymatically by monitoring the reduction of NADP with the hexokinase and glucose-6-phosphate dehydrogenase/phosphoglucose isomerase assay (Roche) as described previously[166,167], allowing the determination of the total- (fructose release) and hydrolytic (glucose release) activities, and calculation of the transglycosylation activity.

One unit (U) of total activity corresponds to the release of 1 µmole fructose from 100 mM sucrose in 25 mM sodium acetate (pH 4.7) and 1 mM $CaCl_2$ at 37 °C.

2.4. TLC analysis

TLC analysis of transglycosylation products was performed on silica gel 60 F_{254} plates (Merck). The eluent consisted of *n*-butanol-acetic acid-water (2:1:1 by volume). Detection was achieved by UV absorption (254 nm) and/or staining with 10 % (v/v) H_2SO_4 containing 2 g/L orcinol.

2.5. HPLC analysis

An Agilent MetaCarb 67H column (300 mm × 6.5 mm) was used under isocratic conditions with 2.5 mM H_2SO_4 as the mobile phase. The flow rate and temperature were set at 0.8 mL/min and 35 °C, respectively. Detection was achieved with an RID detector. Calibration of the obtained peaks was accomplished using the corresponding standard curves.

2.6. Solubility measurements

The solubility of gallic acid (GA) was determined in 250 µL of ultrapure water, incubated in a thermoblock at 20 °C. GA was added until clear precipitation was noticeable, after which the samples were vortexed multiple times and allowed to equilibrate for 24 h. The supernatants were diluted in ethanol and subsequently subjected to HPLC analysis. Calibration was accomplished using the appropriate standard curve. The analysis was performed in duplicate.

2.7. Immobilization of Gtf180-ΔN on silanized silica particles

Before silanization (the functionalization of the silica surface with alkoxysilane molecules), the silica particles (10 g) were boiled in distilled water for 30 min. The wetted particles were then dissolved in 750 mL of a 10% solution of (3-aminopropyl)triethoxysilane (APTES) at pH 4 and subsequently heated in a water

bath at 75 °C for 4 h. The particles were washed three times with water and dried overnight at 80 °C. The silanized particles were stored at room temperature until further use.

Immobilization consisted of two steps: (1) adsorption of the enzyme on the support and (2) cross-linking of the adsorbed enzyme onto the support. In a 1.5 mL-Eppendorf tube, a suspension of 2 mg of silanized particles and 1 mL of buffered enzyme solution (containing 0.2 mg enzyme/mL) was shaken at room temperature for 1 h to ensure that adsorption equilibrium was reached. Subsequently, 10 µL of cross-linker (glutaraldehyde) was added and the suspension was shaken at room temperature for another hour. Afterwards, the supernatant was decanted using a benchtop centrifuge (10,000 g, 2 min) and the particles were washed with distilled water until no enzyme activity was detected (see 2.3.) in the wash solution.

3. Results and discussion

3.1. Solvent engineering: Applying Gtf180-ΔN in a biphasic –or cosolvent system

To improve the solubility of acceptor substrates, cosolvents such as DMSO and acetone are typically added to the glycosylation reaction mixture. The disadvantage of this strategy is that enzyme activity and stability decrease substantially. Alternatively, a biphasic glycosylation system can be implemented; hydrophobic solvents provoke less inhibition than hydrophilic ones. In 2014, De Winter et al. reported the application of a biphasic glycosylation system with sucrose phosphorylase, using ethyl acetate as second phase[23]. The effect of using this system on Gtf180-ΔN catalyzed glycosylation reactions was consequently investigated. The glycosylation of ethyl gallate (EG), added to food products as antioxidant (E313) and representing a poorly soluble acceptor substrate with inhibitory effects on Gtf180-ΔN, was demonstrated previously[137] and chosen as model reaction. The enzyme was each time incubated with 1000

mM sucrose, while the concentration of EG and ethyl acetate (0 or 37.5% v/v) was varied (Figure 1).

Glycosylating 100 mM EG in the aqueous system resulted in a high conversion degree (Figure 1A), whereas the glycosylation of 100 mM EG in 37.5% ethyl acetate displayed a much lower conversion (Figure 1B). This effect can be explained by diffusion limitations between the ethyl acetate phase and the aqueous phase. The actual EG concentration near the enzyme is consequently substantially lower than 100 mM, resulting in relatively more α-glucan synthesis from sucrose at the expense of the acceptor reaction. Increasing the EG concentration to 400 mM yielded a higher product concentration, however, the EG conversion degree remained lower than in the aqueous system (Figure 1C). Conclusively, the application of a second phase to dissolve poorly soluble acceptor substrates should be avoided for glucansucrase mediated glycosylation reactions, due to the resulting decrease of acceptor substrate conversions.

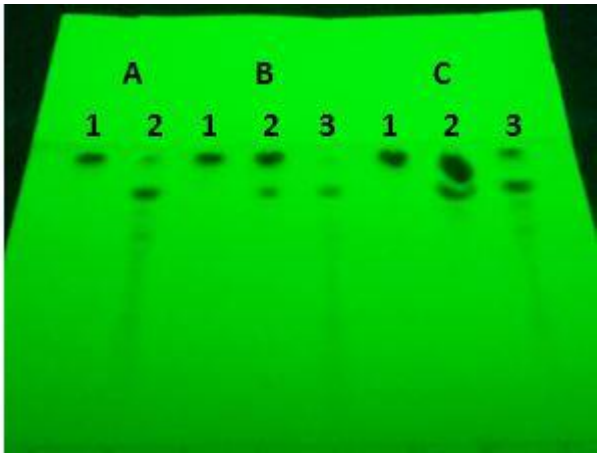

Figure 1. TLC analysis of ethyl gallate (EG) glycosylation by free Gtf180-ΔN (1000 mM sucrose, 4 U/mL enzyme), visualized by UV (254 nm). **A** 100 mM EG, 0% ethyl acetate; **B** 100 mM EG, 37.5% ethyl acetate; **C** 400 mM EG, 37.5% ethyl acetate. **A1**, **B1**, **C1** Incubation mixture without enzyme; **A2** 60 min incubation; **B2**, **C2** 60 min incubation (ethyl acetate phase); **B3**, **C3** 60 min incubation (aqueous phase).

Alternatively, the effect of using DMSO as cosolvent on Gtf180-ΔN catalyzed glycosylation reactions was investigated. The glycosylation of gallic acid (GA), a potential medicine for the treatment of Alzheimer's[176] and Parkinson's disease[177], served as case study. The solubility of GA in water at 37 °C was determined to be roughly 80 mM, which could be increased by using DMSO as cosolvent. Reactions with the free enzyme in 0%, 10% and 20% DMSO however illustrated the detrimental effect of cosolvent and acceptor substrate on Gtf180-ΔN activity (Figure 2).

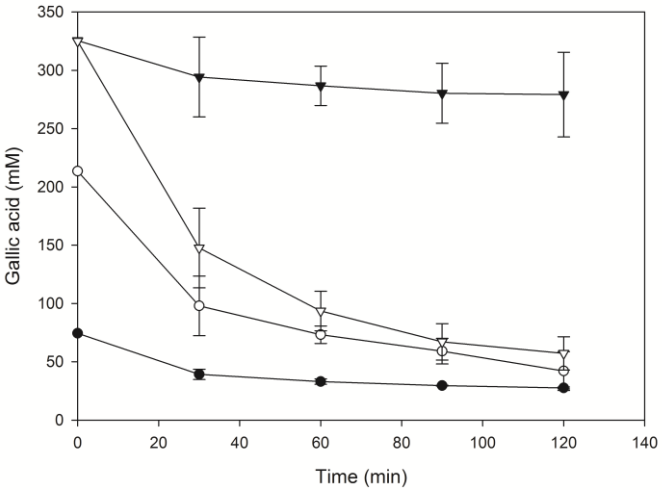

Figure 2. Depletion of gallic acid (GA) due to its glycosylation by free Gtf180-ΔN (1000 mM sucrose, 4 U/mL enzyme): ● 80 mM GA, 0% DMSO; ○ 210 mM GA, 10% DMSO; ▼ 325 mM GA, 20% DMSO; and by immobilized Gtf180-ΔN (1000 mM sucrose, 4 U/mL enzyme): ▽ 325 mM GA, 20% DMSO.

Glycosylation of GA by Gtf180-ΔN without the addition of DMSO (80 mM GA) resulted in a conversion of 63%. Interestingly, the GA conversion degree in 10% DMSO (210 mM GA) was significantly higher (80%) than in 0% DMSO (63%), indicating that the relative balance between α-glucan synthesis, sucrose hydrolysis and GA glycosylation was shifted towards the latter, due to the improved availability of the acceptor substrate GA. Glycosylation in 20% DMSO (325 mM GA) displayed a GA conversion degree of only 14%, which can be attributed to inactivation of the enzyme. Performing the glycosylation in 20%

DMSO might consequently further increase the GA conversion degree, provided that the enzyme is sufficiently stabilized.

3.2. Immobilization of Gtf180-ΔN on mesoporous silica particles

Previous paragraph was another illustration of the undesired inhibitory effects of acceptor substrates and cosolvents on Gtf180-ΔN, resulting in suboptimal glycosylation yields and conversion degrees. Immobilization of Gtf180-ΔN may alleviate this inhibition, more specifically by improving enzyme activity at high acceptor substrate –and cosolvent concentrations. Of all immobilization techniques, covalent immobilization provides the strongest interaction between enzyme and support, stabilizing the enzyme most thoroughly. Immobilization of Gtf180-ΔN on mesoporous silica particles was therefore first optimized, after which the characteristics of the immobilized enzyme were determined.

3.2.1. Optimization of Gtf180-ΔN immobilization

Several factors influence the covalent immobilization of enzymes on mesoporous silica, the most important being adsorption pH, concentration of cross-linker (mM), and enzyme loading (mg protein/g support)[178]. Immobilizing a single layer of proteins (2-3 mg protein/m^2 support) may result in improved immobilization yields due to the reduction of substrate diffusion limitation and undesired conformational changes, both associated with multilayers[179]. The specific surface area of the applied silica particles was roughly 50 m^2/g. A single layer of protein was consequently formed when 100-150 mg Gtf180-ΔN/g or 4000-6000 U Gtf180-ΔN/g was adsorbed.

The pH was found to be crucial for adsorption –and cross-linking of the biocatalyst (Figure 3). An enzyme loading of 100 mg/g resulted in a nearly complete (>95%) adsorption at a pH range of 5.5-6. At a pH of 4.5 not even half of the enzyme was adsorbed, whereas 75% adsorption was achieved at a pH of 7. These results could only partly explain the sharp immobilization yield optimum: at a pH lower than 5.5, the enzyme was not efficiently cross-linked (as could be

visually observed by the absence of a red color, Figure S1), resulting in very low immobilization yields. Between a pH of 5.5 and 7, the immobilization yield decreased with increasing pH (Figure 3), suggesting that the enzyme was 'locked' in a more active conformation at slightly acidic conditions, as was demonstrated by Kondo et al. for α-amylase[180].

The optimal concentration of cross-linker (glutaraldehyde, GLU) generally is strongly dependent on the enzyme and carrier under consideration. For example, the immobilization of laccase using a similar protocol only required the use of 1 µmol GLU per mg silica (~ 10 mM) to maximize the immobilization yield[181], whereas Demarche et al. used 8 µmol GLU per mg silica for laccase immobilization[171]. Increasing the concentration of GLU from 10 mM to 100 mM (~ 2.5 to 25 µmol GLU per mg silica) had no effect on the immobilization yield with Gtf180-ΔN nor on its thermo-activity (data not shown). Therefore, 10 mM GLU was selected for further experimenting.

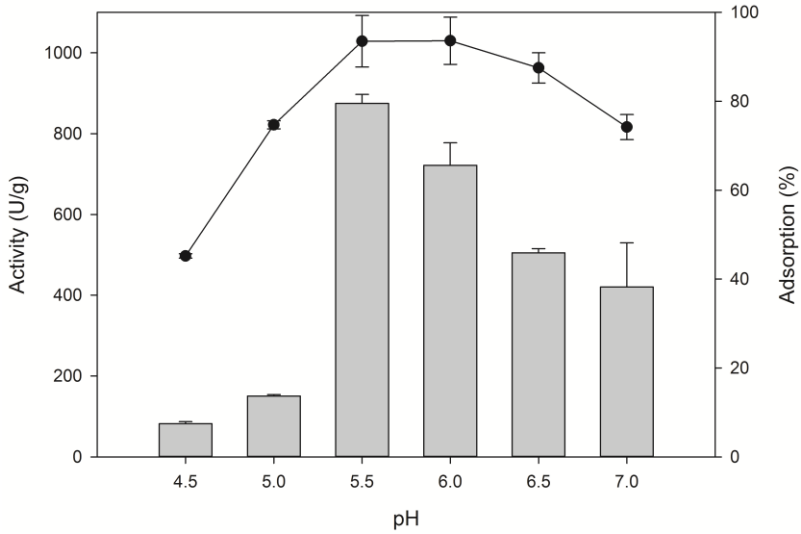

Figure 3. Effect of pH on adsorption and cross-linking of Gtf180-ΔN on mesoporous silica (4000 U/g, 10 mM GLU). **Line graph:** enzyme adsorbed (%). **Bar graph:** activity (U/g) after cross-linking.

3.2.2. Characterization of immobilized Gtf180-ΔN

Immobilized enzymes display very different characteristics compared to their soluble counterparts. Their stability and activity at high temperatures and high solvent concentrations is typically enhanced due to the 'rigidification' of the enzyme[182], which may be advantageous for the glycosylation of poorly soluble acceptor substrates. The properties of glucansucrase Gtf180-ΔN immobilized on mesoporous silica were therefore compared with those of the free enzyme.

3.2.2.1. Effect of immobilization on thermo -and solvent activity

The optimal temperature for activity of the immobilized Gtf180-ΔN enzyme was found to be 55 °C, compared to 50 °C for the free enzyme. The immobilized enzyme even retained activity up to 65 °C, whereas the free enzyme lost all activity at 55 °C (Figure 4A). Improved thermo-activities are usually an indication of enhanced activities in cosolvent systems. Determination of the activity of immobilized and free enzyme in media with different concentrations of DMSO confirmed this hypothesis. The immobilized enzyme was remarkably more active in the presence of DMSO, retaining 87.3% of its solvent-free activity in 37.5% DMSO. In contrast, the free enzyme only retained 24.4% of its solvent-free activity in 25% DMSO and lost all activity at 37.5% DMSO (Figure 4B).

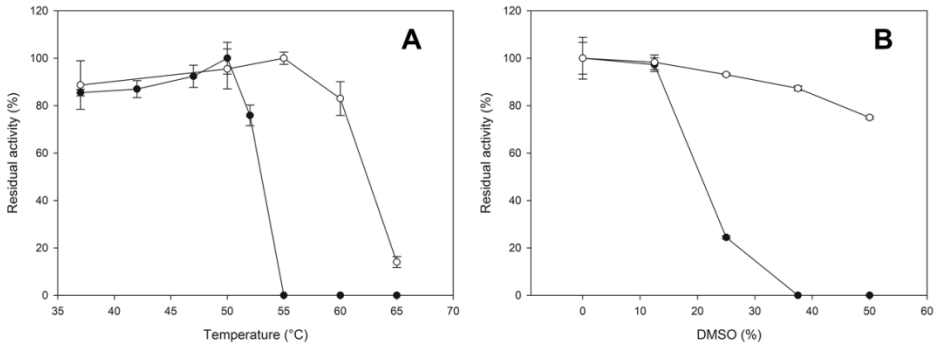

Figure 4. A. Thermo-activity of free (●) and immobilized (○) Gtf180-ΔN (100 mM sucrose, pH 4.7). **B.** Activity in solutions containing DMSO of free (●) and immobilized (○) Gtf180-ΔN (100 mM sucrose, pH 4.7, 50 °C).

3.2.2.2. Effect of immobilization on activity at high acceptor concentrations

Glucansucrases are inhibited by high concentrations of non-carbohydrate acceptor substrates, as was demonstrated for Gtf180-ΔN: the model substrate catechol could not be glycosylated at concentrations above 400 mM due to severe inhibition[110]. The immobilized enzyme was incubated with 400, 600 or 800 mM catechol and 1000 mM sucrose to determine the effect of immobilization on the glycosylation of non-carbohydrate acceptor substrates (Figure 5).

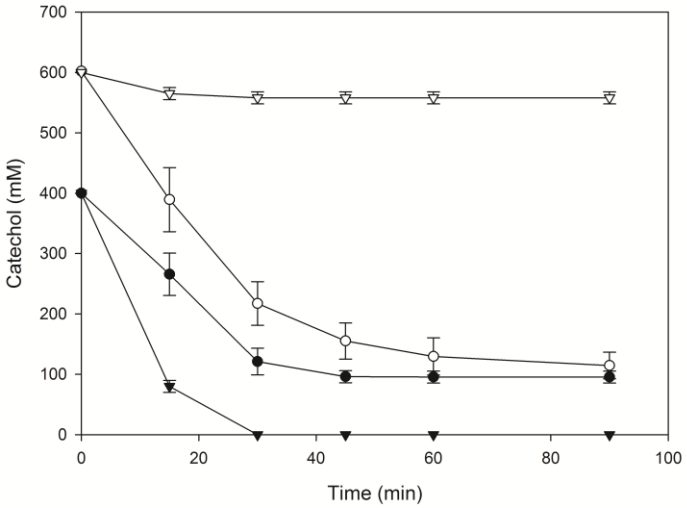

Figure 5. Depletion of catechol (CAT) due to its glycosylation by free and immobilized Gtf180-ΔN (1000 mM sucrose, 4 U/mL enzyme). ● Immobilized Gtf180-ΔN, 400 mM CAT; ○ Immobilized Gtf180-ΔN, 600 mM CAT; ▼ Free Gtf180-ΔN, 400 mM CAT; ▽ Free Gtf180-ΔN, 600 mM CAT.

Incubation of the immobilized enzyme with 400 mM catechol resulted in a 78% conversion of the latter into glycosides compared to 100% for the free enzyme. In contrast, sucrose was completely converted in both cases, indicating that the immobilized enzyme underwent undesired changes in the active site, resulting in a decreased affinity for the acceptor substrate catechol. Furthermore, 600 mM catechol was converted by the immobilized enzyme into glycosides with a conversion degree of 81%, slightly better than when incubated with 400 mM and significantly better than for the free enzyme (7%) (Figure 5). This confirms the

results of the thermo –and solvent activity experiments and demonstrates the improved operational stability of the immobilized enzyme. A concentration of 800 mM catechol resulted in deactivation of not only the free but also the immobilized enzyme (data not shown). In conclusion, the immobilized enzyme remained active at higher acceptor substrate concentrations, higher solvent concentrations and higher temperatures, compared to the free enzyme. However, its acceptor substrate affinity was altered, possibly due to participation of one or more amino acid residues in the active site during cross-linking. In fact, this is known to be one of the main disadvantages related to the use of GLU as cross-linker. Due to its small size, GLU can penetrate easily into the active site of enzymes and subsequently react with amino acid residues.

In order to prevent GLU from causing undesired changes in the active site, protecting agents, typically enzyme substrates, are used[183]. Moreover, the activity of the immobilized enzyme towards the protecting agent/enzyme substrate can be improved by this 'molecular imprinting' of the enzyme's active site[184]. It is important that the substrate is not converted during immobilization to obtain the optimal protecting effect; catechol and maltose were consequently tested in different concentrations (100-400 mM) as protecting agent during Gtf180-ΔN immobilization. Unfortunately, the addition of catechol prevented cross-linking of the enzyme, whereas the addition of maltose had no effect on catechol glycosylation.

3.3. Glycosylation with immobilized Gtf180-ΔN

It was previously shown that GA glycosylation proceeds with improved conversion degrees in systems containing high GA concentrations, until DMSO and GA reach a concentration that is inhibitory to Gtf180-ΔN. In order to further improve GA glycosylation, immobilized Gtf180-ΔN was incubated in 20% DMSO, containing 325 mM GA (Figure 2).

Immobilization of Gtf180-ΔN alleviated the inhibiting effect of DMSO and GA, resulting in a GA conversion degree of 82%, which is slightly higher than the

conversion obtained after incubation of the free enzyme in 10% DMSO. In addition, the glycosylation in 20% DMSO of caffeic acid (CA), catechin (CT), and quercetin (QU) with immobilized Gtf180-ΔN was evaluated and compared with the glycosylation potential of the free enzyme in solvent-free reaction medium (Figure 6).

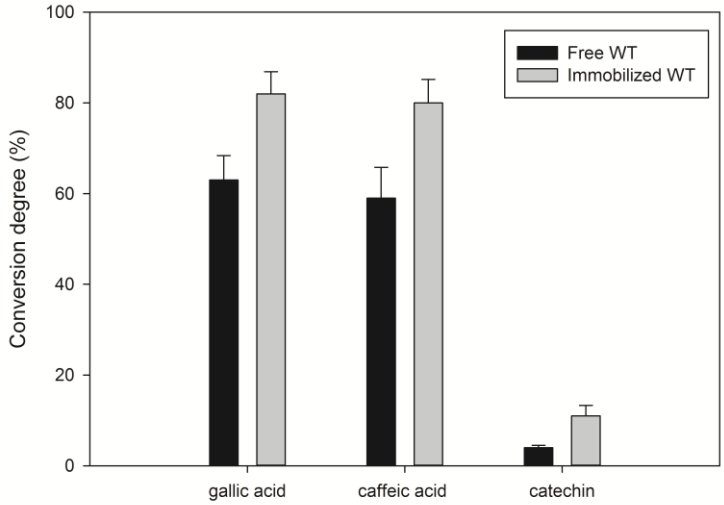

Figure 6. Conversion degrees for the glycosylation of gallic acid (GA), caffeic acid (CA) and catechin (CT) by immobilized Gtf180-ΔN in 20% DMSO and free Gtf180-ΔN in 0% DMSO (1000 mM sucrose, 4 U/mL enzyme).

CA and CT were glycosylated in 20% DMSO by the immobilized enzyme, and in 0% DMSO by the free enzyme. Similarly to GA glycosylation, higher conversions were obtained by the glycosylation systems containing 20% DMSO. QU was not glycosylated by the immobilized enzyme nor by the free enzyme, indicating that the applied strategy was ineffective in this case. The apparent very low affinity of Gtf180-ΔN for QU is clearly not sufficiently compensated by increasing its concentration in the reaction mixture. Also the suppression of α-glucan synthesis by mutational engineering (Chapter 2) did not result in improved QU conversion degrees, only causing more sucrose hydrolysis (as determined by screening of the mutant library described in Chapter 2[99,110]). This indicates that specific

mutational engineering of Gtf180-ΔN's active site is required to enhance the glycosylation of QU and other related flavonoids.

4. Conclusions

The relatively low operational stability of Gtf180-ΔN at high temperatures and in systems containing cosolvents and high acceptor substrate concentrations ultimately results in suboptimal acceptor substrate conversion degrees. By means of its immobilization on mesoporous silica particles, the activity of Gtf180-ΔN under such conditions was substantially improved. The immobilized enzyme displayed enhanced activity at temperatures above 50 °C and in systems containing 20% DMSO, allowing the glycosylation of GA, CA and CT in systems containing much higher acceptor substrate concentrations. As a result, their conversion into glycosides was improved substantially: from 63% to 82%, from 59% to 80%, and from 4% to 11%, for GA, CA and CT, respectively. The glycosylation of QU, a compound which was very poorly glycosylated by the free enzyme, was also not successful using immobilized Gtf180-ΔN in 20% DMSO. Suppressing α-glucan synthesis by enzyme engineering (Chapter 2) nor solvent engineering (Chapter 3) were effective strategies to enhance QU's glycosylation, as this most probably requires specific mutational engineering of Gtf180-ΔN in order to increase its very low affinity for this molecule. Improving the glycosylation of related flavonoids, such as luteolin, or stilbenoids, such as resveratrol, will most probably demand the same strategy.

5. Supplementary information

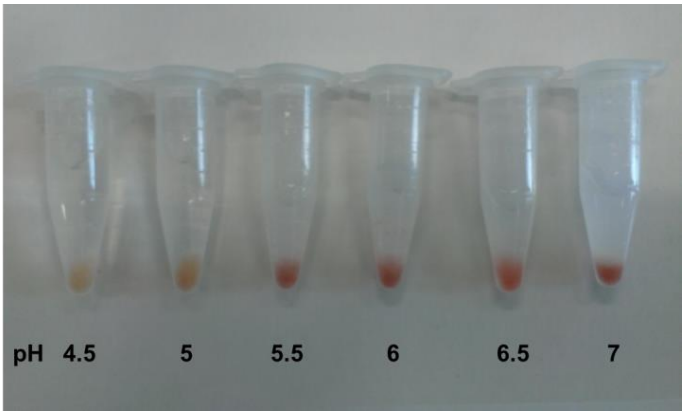

Figure S1. Successful cross-linking is indicated by the presence of a red color, caused by Rayleigh scattering.