

Feruloyl esterases, effects of glycosylation on activity, stability and immobilization



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BACKGROUND

Feruloyl esterases (FAE; EC 3.1.1.73) are a subclass of carboxylic ester hydrolases. They catalyze the hydrolysis of ester linkages in plant cell walls materials releasing ferulic acid and other hydroxycinnamic acids and therefore have been

PROFILES

The three forms of the enzyme were tested for pH and temperature profiles.



IMMOBILIZATION BACKGROUND

Immobilization is a powerful tool to allow enzyme reuse and therefore decrease their cost in the overall process.

Mesoporous silica particles (MPS) are porous supports made of silica which pore size can be tuned from 2 to 50 nm depending on the synthesis conditions [2]. A pore size matching the enzyme size can greatly reduce leaching after immobilization. The pore system also provides a sheltering and more packed environment, mimicking the inside of a cell.

classified in the CAZy database [1] in the carbohydrate esterase (CE) family. Currently, all FAEs in the CAZY database belong to the CE1 family.

We studied the Fae1a from *Miceliophtora thermophila* expressed in different host organisms and therefore having different glycosylation extent/patterns.

Our hypothesis was that glycosylation would influence the immobilization behavior of the studied FAE in mesoporous silica particles as well as its activity.

GLYCOSYLATION

Depending on the production host of the enzyme, the glycosylation extent and/or pattern can vary from none in *Escherichia coli* to hyper-glycosylation in *Pichia pastoris*. *M.thermophila* was expected to have an intermediate glycosylation level. Both glycosylated forms displayed similar behaviors with pH. The non-glycosylated enzyme was more tolerant to low pHs.



The orginal enzyme (B2Mt), having the intermediate glycosylation level, was the most tolerant to high temperatures. The non-glycosylated one (EcoMt) was the most robust at low temperatures.

Activity retained after 24h at 20°C

IMMOBILIZATION KINETIC

Immobilization on solid carriers can be achieved by different approaches, one of them is by physical adsorption. This technique relies mainly on the surface charges of the support and enzyme. Because they are negatively charged, mesoporous silica particles allow for rapid enzyme immobilization by physical adsorption. MPS of 10 nm pore size were used to immobilize

enzymes estimated to be 4-5 nm in diameter.





The different glycosylation extents were confirmed by PNGase F digestion and SDS-Page gel analysis.



рН	PipMt	B2Mt
5.0	64%	85%
6.0	79%	84%
7.0	66%	49%

Both glycosylated enzymes were relatively stable at 20°C for 24h.

At the determined preferred pH ant temperature, the kinetic constants of the enzymes were evaluated.

KINETIC CONSTANTS

Km, Vmax and kcat of the two glycosylated enzymes were evaluated.



For B2 Mt, at different pHs different behaviors were observed which can be linked to the enzyme pl, determined experimentally to be at 6.0 [4].



Based on the obtained profiles, PipMt seems to have a higher pl. This is in agreement with the fact that glycosylation are known to alkalify the pl. A different behavior is expected for the non glycosylated enzyme which pl should be closer to the theoretical one calculated by expasy to be at 4.4.

CONCLUSIONS

The different glycosylation extents were confirmed.
All three forms of Fae1a display the same pH and temperature optima with some differences in the profiles
Both glycosylated forms have the same kinetic parameters Km and kcat.
Different immobilization behaviors were observed which we hypothesized are linked to the enzyme's pl

	15		
Protein	Before (kDa)	After (kDa)	Δ (kDa)
PipMt	33	26	7
B2Mt	27	24	3

Glycosylation has been shown to alter activity and stability of enzymes [3]. It was therefore decided to investigate the behavior of the different forms of the same enzyme.

	1.5 1 0 0	0.1 0.2 0.3 Substr	0.4 0.5 ate (mM)	0.6 0.7	PipMt B2Mt	
PipMt				B2Mt		
Km (բ	ıM)	51.3 ± 2.1 52.9 ± 2.5				
Vmax (µl	M/min)	$3.2 \pm 3.2 \ 10^{-2}$		3.3 ± 3.9 10 ⁻²		
kcat (S⁻¹)	42.8 ± 0.4		31.8 ± 0.4		

Km was the same for both glycosylated enzymes. Small differences were observed on kcat. Future work:

- Stability of the enzymes
- The non-glycosylated form: EcoMt
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