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Does spatial proximity matter?

Distance between molecules of the xylanase *NpXyn11A* impacts product profile from methylglucuronoxylan

Cédric Montanier¹, Antoine Bouchoux¹, Anne-Marie Di Guilmi², Mathieu Fanuel³

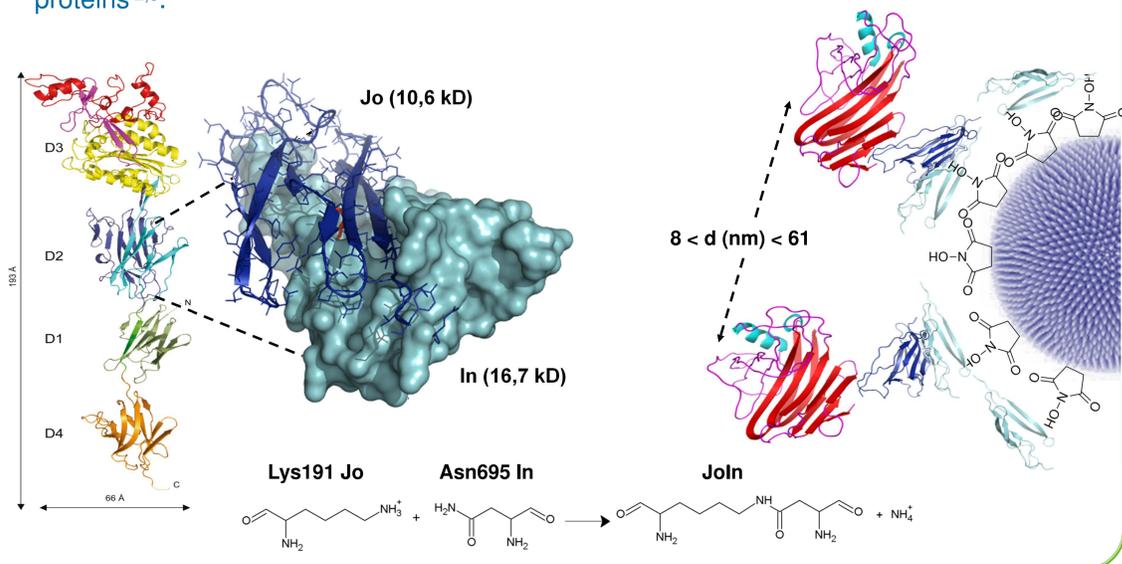
1- LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France. 2- Institut de Biologie Structurale (IBS), Univ. Grenoble Alpes, CEA, CNRS, 38044 Grenoble, France.

Context and Objective

In Nature, the plant-based organic carbon contained within plant cell walls is mainly recycled by the action of cellulolytic microorganisms, such as bacteria and fungi, which produce complex arrays of cell wall-degrading enzymes. Many of the enzymes that degrade plant cell wall polysaccharides are modular proteins, which contain single or multiple copies of both catalytic domain(s) and CBM(s). Cellulolytic microorganisms developed a couple of strategies to break down the complex plant cell wall; secrete free enzymes into their surroundings or produce large multi-component cell-bound structures, which harbour several enzymes displaying complementary activities. In both case, substrate is attacked by different enzymes acting together at different regions, and much more than sum of individual different enzymatic activities, synergism drives the efficiency of such system. However, spatial proximity between enzymes and its impact over synergism is rarely investigated. Here we report an original way to easily and precisely control distance between molecules of the xylanase *Xyn11A* from *Neocallimastix patriciarum*¹ through a covalent immobilization on a solid support and we analysed the impact of such immobilization according to the size of the products released using HPAEC-PAD and MALDI-ToF analysis.

Results

1 Precise control of the distance between immobilized enzymes using Jo and In recombinant proteins^{2,3}.



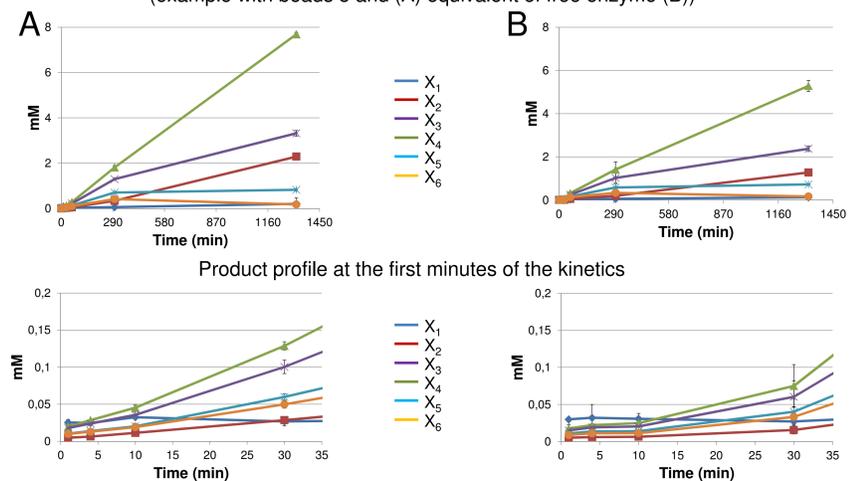
3 The size of the substrate reflects close proximity between molecule of *NpXyn11A*.

Beads	Specific activity (IU.mg ⁻¹)			Specific activity (IU.mg ⁻¹)		
	3 μl of beads	free enzyme	4.47 μg of immobilized enzyme	1 μl of beads	free enzyme	
1	2.12	2.29	2.14	391.2 \pm 48.4	919.0 \pm 48.4	
2	2.88	2.48	2.14	705.4 \pm 41.0	971.2 \pm 36.4	
3	2.52	2.45	2.10	784.4 \pm 55.8	934.0 \pm 24.5	
4	2.56	2.38	2.12	932.9 \pm 7.6	922.1 \pm 70.2	
5	2.19	2.14	2.10	1004.2 \pm 79.4	1092.48 \pm 90.5	

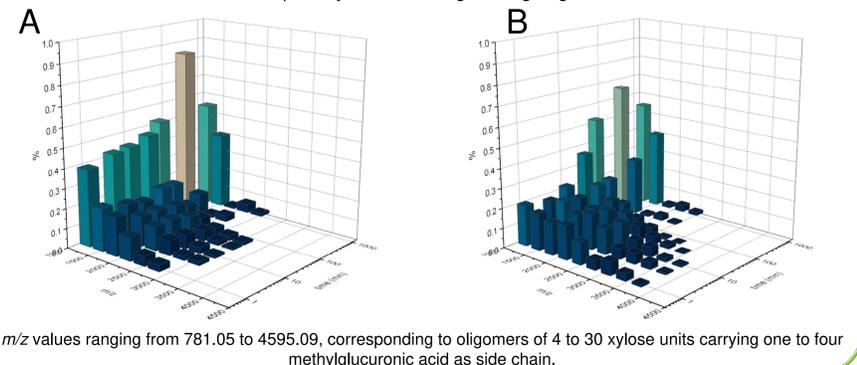
5 mM 4-nitrophenyl- β -xylotriside 2 % Beechwood methylglucuronoxylan

4 Comparing hydrolysis products of methylglucuronoxylan released from beads and equivalent amount of free enzymes.

HPAEC-PAD was used to quantify the release of small oligosaccharides over the time (example with beads 5 and (A) equivalent of free enzyme (B))



MALDI-TOF was used to quantify the releasing of long oligosaccharides over the time.



2 Distance between immobilized molecules and kinetics.

Distance between molecules of *InNpXyn11A* from *Neocallimastix patriciarum* is controlled and measured:

- commercial beads
- decreasing amount of enzymes
- Langmuire surface adsorption (BET)

Effect of the immobilization is investigated using small and long substrate and compared to the equivalent amount of free enzymes

Beads	Amount of immobilized enzyme per μl of beads (10% v/v)			D (nm)
	InNpXyn11A (μg)	Density (nm^2)		
0	2.42	75.01		8.7
1	1.49	123.45		11.1
2	0.38	472.03		21.7
3	0.22	809.98		28.5
4	0.11	1523.08		39.0
5	0.05	3695.26		60.8

	InNpXyn11A	Beads 0	Beads 2	Beads 4	Beads 5
V_{max} (mM.min ⁻¹)	0.5 \pm 0.1	0.7 \pm 0.1	0.7 \pm 0.1	0.5	0.7
K_m (mg.ml ⁻¹)	1.8 \pm 0.7	3.6 \pm 0.5	3.66 \pm 0.49	2.63	2.1
k_{cat} (min ⁻¹)	46.1 $\times 10^3 \pm 8.8 \times 10^3$	8.9 $\times 10^3 \pm 0.9 \times 10^3$	22.4 $\times 10^3 \pm 2.65 \times 10^3$	43.5 $\times 10^3$	55.1 $\times 10^3$
k_{cat}/K_m (min ⁻¹ .mg ⁻¹ .ml)	25.6 $\times 10^3 \pm 6.5 \times 10^3$	2.5 $\times 10^3 \pm 0.07 \times 10^3$	6.15 $\times 10^3 \pm 9.5 \times 10^1$	16.5 $\times 10^4$	26.2 $\times 10^3$

12 mM citric acid 50 mM Tris/HCl pH7 5.1 mg/mL, BSA at 37°C. 2% Beechwood xylan.

Conclusions

Here we report an original way to easily and precisely control distance between molecules of the xylanase *Xyn11A* from *Neocallimastix patriciarum* through Joln covalent immobilization on a solid support. Immobilization did not modify the enzymatic activity on chromogenic substrate and Beechwood xylan. Distance is the only parameter. This approach allowed us to control distance between enzymes from 8 to 60 nm and compare the kinetic to the equivalent amount of free enzymes. Product profile for both immobilized and free enzymes were assessed by HPAEC-PAD and MALDI-TOF. A distance of 20 nm seems to increase dramatically the production of small oligosaccharides probably because of an homogeneous production of medium sized oligomers.

1 - Vardakou et al., 2008 2 - Izoré et al., 2010 3- Bonnet et al., 2017

