LPMO immobilization on Carbon nanotubes: a first investigation toward higher stability and catalytic activity

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Thanks to their excellent functional properties, enzymes are able to catalyze several complex chemical processes under mild conditions and are potentially excellent catalysts for a more sustainable chemical industry. However, limitations, such as low stability and a tendency to be inhibited by substrates, products or solvents, limit their application for nonbiological purposes. One way of tailoring enzymes for industrial applications is immobilization onto a suitable carrier, following simple and cost-effective protocols, in order to increase enzyme stability. Lytic polysaccharide monooxygenases (LPMOs) are mononuclear copper-containing enzymes found in the majority of cellulolytic fungi and actinomycete bacteria that are able to oxidize C–H bonds of the glycoside linkages in polysaccharides[1–3]. However, LPMOs are prone to oxidative damage, particularly in the absence of an adequate substrate[2]. The aim of this contribution is to develop a possible bio-catalyst for C-H activation reactions, based on bioconjugation of LPMOs to Carbon Nanotubes (CNTs), which is done to stabilize the biological partner without jeopardizing its catalytic ability. CNTs have been selected since they are conductive, thus allowing the electron transfer processes that are needed to optimize the LPMO activity. After the optimization of the immobilization protocol with different model proteins, two different LPMOs were selected, *Sc*AA10C, from *Streptomyces coelicolor* A3 and LsAA9A from *Lentinus similis*. Oxidized CNTs were firstly activated by using N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) and then mixed under stirring with the enzyme solution. After the reaction, the suspension was dialysed and the bioconjugates were analyzed by means of UV-Vis spectroscopy, fluorescence spectroscopy and circular dichroism. Lastly, the catalytic activity of the LPMO bioconjugates was assessed by determining their oxidase[4] and peroxidase[5] activities, which are known side activities of LPMOs involving small molecule substrates.

References

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