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Enzymatic synthesis, purification and immobilization of coenzymes of interest: the example of NADP(H).

AUTHORS

Celestin BOURGERY / URD ABI - AGROPARISTECH, CEBB, 3 RUE DES ROUGES TERRES, POMACLE
David MENDOZA / BIORESOURCE PROCESSING RESEARCH INSTITUTE OF AUSTRALIA (BIOPRIA), DEPARTMENT OF CHEMICAL ENGINEERING, MONASH UNIVERSITY, CLAYTON
Gil GARNIER / BIORESOURCE PROCESSING RESEARCH INSTITUTE OF AUSTRALIA (BIOPRIA), DEPARTMENT OF CHEMICAL ENGINEERING, MONASH UNIVERSITY, CLAYTON
Louis MOUTERDE / URD ABI - AGROPARISTECH, CEBB, 3 RUE DES ROUGES TERRES, POMACLE
Florent ALLAIS / URD ABI - AGROPARISTECH, CEBB, 3 RUE DES ROUGES TERRES, POMACLE

PURPOSE OF THE ABSTRACT

Biocatalysis is significantly developing these last decades due to its great potential to strengthen the chemical and pharmaceutical industries through the use of enzymes, which can (1) lead to sustainable and energy-efficient production methods, and (2) enable the production of high-value fine chemicals and pharmaceutical compounds that would otherwise be inaccessible. Although some enzymes do not require any additional chemicals for their catalytic activity other than their amino acid residues, many require a complementary component called cofactor. Therefore, to study or take benefit of all naturally occurring enzymes, it is necessary to have an affordable access to these cofactors. Although the use of inorganic ions seems straightforward, it is not so obvious when it comes to coenzymes. Indeed, their price can be prohibitive: 1600 ?/g, 30 000 ?/g or 370 ?/g for Coenzyme A (CoA), its disulfide (CoAS2) and Nicotinamide Adenine Dinucleotide Phosphate (NADP(H)), respectively. Oxidoreductases, which represent one of the largest classes of enzymes (25% of all known enzymes), are of real interest. Indeed, thanks to their enantioselectivity and intrinsic specificity, they are able to promote bio oxido/reduction reactions that are vital for the global pharmaceutical and chemical market. However, these enzymatic oxido/reduction depend on a coenzyme: the nicotinamide adenine dinucleotide NAD(H) or its phosphorylated form NADP(H). Due to theirs high costs, the use of the NAD(P)+ /NAD(P)H-dependent enzymes is not viable on an industrial scale without an efficient and adapted regeneration system. Inspired by previous works on another coenzyme of interest, the coenzyme A1, a new pathway towards NADP(H) involving an in vitro enzymatic cascade has been designed to greatly decrease the price of this coenzyme and allow its use at a stochiometric level. Moreover, membrane-based purification to access high purity NADP+ was developed2. Finally, in collaboration with Monash University, we developed an efficient click-chemistry-based synthetic strategy3 to graft Adenosine, Adenosine mono- and triphosphate onto cellulose nanocrystals, that offers a novel sustainable approach for cofactor immobilization. The latter opens the way for the development of fast-growing fields such as flow chemistry applied to biocatalysis.

FIGURES

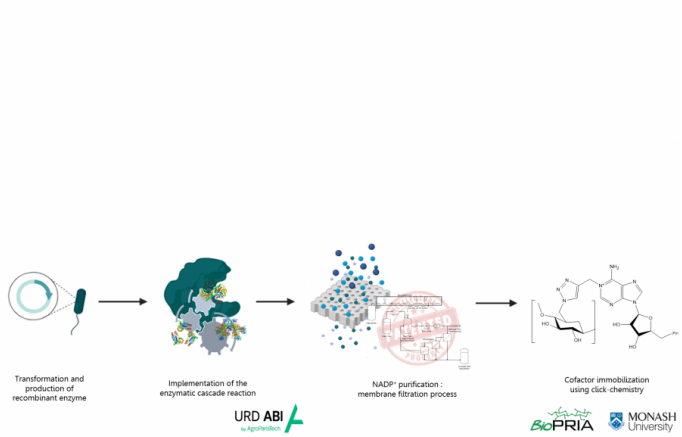

FIGURE 1

Enzymatic synthesis, purification and immobilization of coenzymes of interest: the example of NADP(H).

FIGURE 2

KEYWORDS

Biocatalysis | NADP(H) | Membrane-based purification | Cofactor immobilization

BIBLIOGRAPHY