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An Efficient Access to High Molecular Weight Co-Enzymes: Towards the Broadening of the Industrial Enzymatic Toolbox

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PURPOSE OF THE ABSTRACT

Metabolism is essential for life. It is the set of chemical reactions that occur within a living being, which allows organisms to grow and produce energy. Virtually, all these reactions are catalyzed by enzymes, governed by the principles of thermodynamics, and organized into metabolic pathways. Some enzymes require no additional chemical entities for catalytic activity other than their amino acid residues, but many require an additional component called a cofactor. The latter can be inorganic ions such as Fe2+, Mg2+, Mn2+, Zn2+ or organic molecules called coenzymes. Coenzyme A (CoA), Nicotinamide Adenine dinucleotide (NAD(H)), Nicotinamide Adenine dinucleotide phosphate (NADP(H)) are ones of these molecules.

These coenzymes are being involved in many metabolic pathways which makes them ubiquitous actors for in vivo and/or in vitro biochemical transformations and therefore key elements for the development of many biocatalysis processes. Unfortunately, the high cost of these molecules greatly hindered the development of such processes at an industrial scale. These high prices are closely linked to the difficulty to produce and purify them. Because they are involved in the energetic mechanism of every cellular organisms, in vivo production cannot reach high titration which makes this pathway not ideal. Moreover, the current purification methods involving ion exchange chromatography are tedious, solvent & salts consuming and required further desalting step downstream.

To overcome these obstacles, in vitro biocatalytic routes towards these coenzymes - that can be carried out on multigram per liter scale - must be developed as well as alternatives purification methods. A simple chemo-enzymatic route to Coenzyme A - that can be carried out up to 20 g/L ? has therefore been developed as well as an alternative purification method that overcome the limitations linked to the classical ions exchanges chromatography techniques and which significantly reduce the cost.1,2 This diafiltration method can be used for multiple high molecular weight adenosine-based coenzymes such as Coenzyme A, NAD(P)+, FADH2, etc.

FIGURES

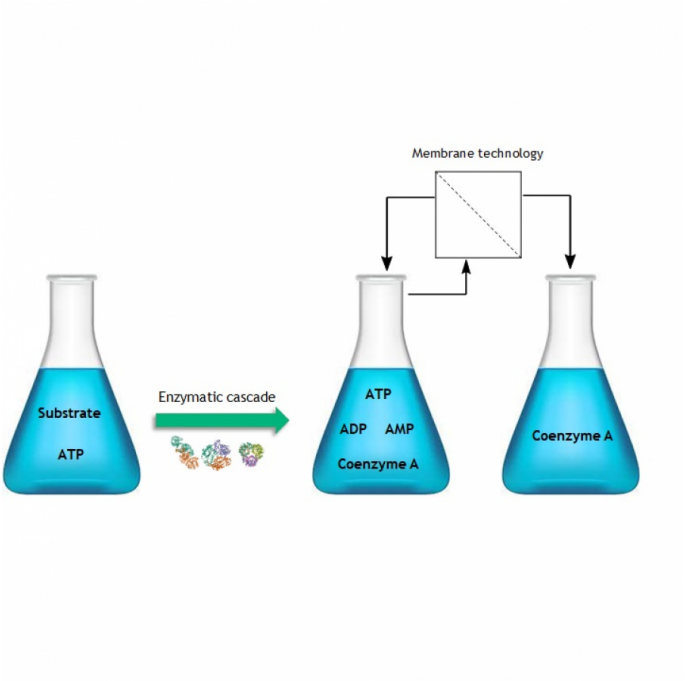

FIGURE 1

FIGURE 2

Production and purification of oxidized Coenzyme A

KEYWORDS

Coenzymes | Biocatalysis | Diafiltration

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