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Biosynthesis of 3-Hydroxy Acids Through an ATP-Independent and Cell-Free Enzymatic Cascade Using Vinyl Esters as Smart Substrates

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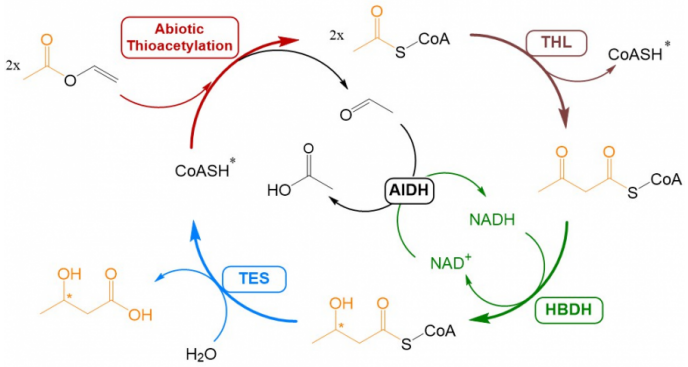
PURPOSE OF THE ABSTRACT

The production of compounds through in vitro biosynthetic cascades demands redox power and energy, usually in the form of NAD(P)H and ATP, respectively. The necessity of regenerate those cofactors, through chemicals, electricity or light, strongly increases the cost of the whole process, decreasing the atom economy or increasing the energy consumption. Nice examples of biosystems in which both cofactors are regenerated in situ could be found on the production of β-hydroxy acids or its polymers. These systems usually contain CoA-dependent Claisen condensation and a NAD(P)H-dependent asymmetric reduction steps. In vivo results, which this system was widely explored, reached up to 3 g/L[1,2]. On the contrary, in vitro systems barely reached 40 mM of poly(3-hydroxy butyrate)[3], alternatively artificial cell-free circular metabolisms, which includes those reactions, are emerging to fix CO2 into acyl-CoA, yielding C2-C3 compounds in the µM range[4,5]. In this work[6], we propose a new approach to produce β-hydroxy acids through an ATP-independent cascade by exploiting vinyl esters as dual acyl and electron donor. In that way, the substrate embedded the chemical energy to activate the acyl group and the redox power. The cell-free enzymatic cascade is wisely designed in 4 steps, an abiotic thiolysis, a non-decarboxylative Claisen condensation, an asymmetric reduction and a hydrolysis, including a NADH recycling step. The whole cascade is enzymatically catalyzed by 4 enzymes, achieving a titer of 3-hydroxy butyrate of 24 mM without ATP requirements, showing the potential of in vitro biocatalysis to transform simple molecules into multi-functional ones.

FIGURES



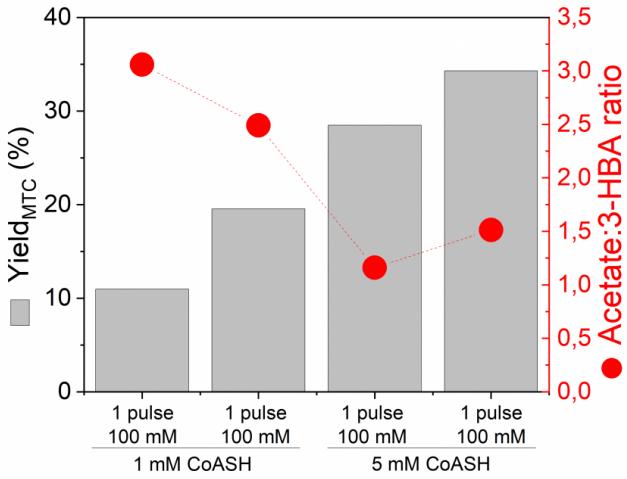


FIGURE 1

Reaction scheme of the Cell-Free Cascade for 3-Hydroxy Acids Biosynthesis

4-enzyme artificial biosynthetic pathway to synthesize 3-hydroxy butyrate through sequential abiotic thiolysis, Claisen condensation, reduction and hydrolysis.

FIGURE 2

Theoretical maximum conversion and acetate:3-HB molar ratio

Theoretical maximum conversion (YieldMTC) (grey bars) and acetate:3-HB molar ratio (red circles) using the cell-free enzyme systems starting at different CoASH concentration (1-5 mM) and adding the VA in different pulses.

KEYWORDS

Multi-enzyme systems | Cell-free biocatalysis | Coenzyme A | Thiolases

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