BIOTRANS
LA ROCHELLE 2023

N°1450 / PC
TOPIC(s) : Artifical intelligence / computational methods / Biocatalytic cascade reactions

The LightCas process: A customised solution for a biocatalytic and light regulated three step cascade producing tetrahydroisoquinolines (THIQ)

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PURPOSE OF THE ABSTRACT

Biocatalysis is a potential key technology for the development of carbon neutral and circular economy in the future [1]. One important task for biocatalytic applications is the enzyme catalysed synthesis of stereoisometrically pure active pharmaceutical ingredients (APIs) from renewable sources. An example is the asymmetric synthesis of tetrahydroisoquinolines (THIQ) in a three step enzymatic cascade (shown in Figure 1a) [2]. However, cross reactivities in the first and the third reaction step (shown in Figure 1b) cause a significantly more complicated reaction process and require a separation of the individual reaction step in either time or space. To realise the time separation, the individual enzymes can be fused with photosensitizers, which, upon light exposure produce reactive oxygen species (ROS), inactivating the to the photosensitiser attached enzyme [3]. Nonetheless, even if the light-induced inactivation of the enzymes enables a separation of the reaction steps in time (Figure 1a) and thereby prevents cross reactivities (Figure 1b), the process is still labour intensive for experimenters, as the concentrations of the substrates, products and intermediates of the individual reaction steps have to be measured continuously and the light inactivation as well as the induction of the respective reaction steps need to be performed manually. To solve these challenges, a system is required, which automatically monitors the reaction, performs the light inactivation, and induces the individual reaction steps.

The realisation of the just mentioned challenges is the goal of the LightCas process. Using Arduino microcontrollers, we have created a hardware infrastructure, able to irradiate the reaction mixture with light as well as the feeding of the respective enzyme and cosubstrate required for the individual reaction steps. With the automated biocatalysis and benchtop NMR application (ABBA), we have developed a customised software, which automatically monitors the reactions by a benchtop NMR and controls the hardware infrastructure by communicating with the Arduino microcontrollers. With the combination of the Hardware infrastructure and the ABBA software, we have established an automated process (the LightCas process), which handles the complex reaction control, occurring due to the cross reactivities in the synthesis of THIQ (Figure 1a, b). As an additional functionality, a communication with the BioCatHub platform enables the automated storage and publication of the collected data, based on the Findable, Accessible, Interoperable and Reusable guiding principles due to an implemented interface to the BioCatHub platform.

FIGURES

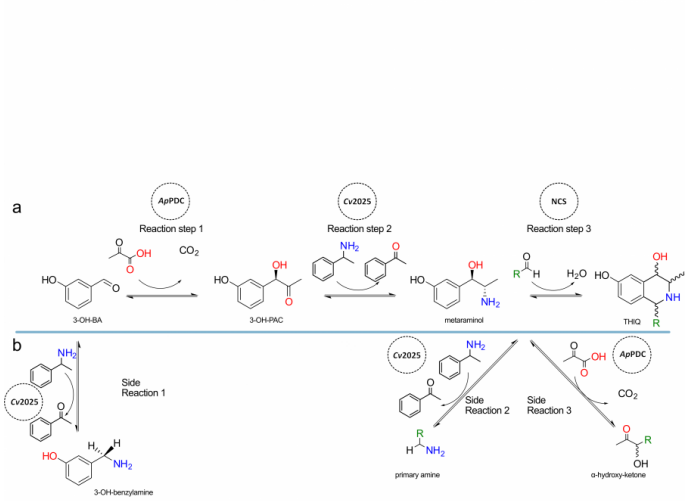

FIGURE 1

Figure 1

Reaction schema displaying the asymmetric synthesis of THIQ. ApPDC: Acetobacter pasteurianus pyruvate decarboxylase, Cv2025: Chromobacterium violaceum transaminase, NCS: Norcolaurine synthase from Thalictrum flavum.

FIGURE 2

KEYWORDS

Biocatalysis | Automation | Synthetic enzyme cascades | NMR

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