BIOTRANS
LA ROCHELLE 2023

N°1366 / PC
TOPIC(s) : Enzyme production, immobilization / Artifical intelligence / computational methods

Production and characterization of bacterial aminoacylases by an experimental/numerical approach for their implementation in a green N-acylation process

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PURPOSE OF THE ABSTRACT

The N-acylation reaction leads to the addition of an acyl group to the amine function of a molecule which can be an amino acid or a peptide. These acylated derivatives have a high application potential because of their technofunctional properties, among others emulsifying and foaming, or their biological activities. On an industrial scale, they are produced by the Schotten-Baumann reaction which is highly efficient but non-selective and polluting. These acylated derivatives can also be produced by an enzymatic way, in particular using aminoacylases.
Aminoacylase activities were discovered in the culture supernatant of Streptomyces ambofaciens [1]. Four genes of S. ambofaciens were identified, presenting a very high percentage of homology with the gene sequences of aminoacylases identified and characterized in Streptomyces mobaraensis by Koreishi's team [2]. The construction of deletion mutants of S. ambofaciens confirmed the activity of the enzymes encoded by the four genes [3]. The characterization of these enzymes began using culture extracts [4] and revealed the particular interest of SamAA that catalyzes the acylation of the α amine function of lysine selectively, which is rare in the world of enzymes.
First attempts to produce this enzyme by heterologous expression showed a significant production under the form of inclusion bodies. To workaround this, a strategy based on the modification/combination of various experimental conditions was adopted for the production of SamAA. Recombinant SamAA was produced by the Escherichia coli BL21 Gold (DE3) strain with an optimized gene, for 24 hours on an LB medium supplemented or not with cobalt. Induction was done with the addition of IPTG (0.1 mM) at 0.6 or 0.4 of Do(600 nm), respectively in the absence or in the presence of cobalt in the culture medium. SDS-PAGE analyzes did not reveal any visible band of over-expression at the expected size of SamAA in the soluble fractions obtained after lysis with a French press. The presence of the recombinant protein is going to be confirmed by Western blot analysis. Unpurified concentrated soluble fractions were used for a synthesis of N-α-lauryol-L-lysine at 45°C and pH8 in 48h, which showed an apparent specific activity of 710 mg/L of product/ 100mg of protein/ L of crude extract with cobalt in the culture medium while the activity is zero without cobalt in the culture medium. Purification tests on IMAC column are in progress.
Along with this experimental study, a numerical approach was implemented to gain knowledge of the 3D structure and the catalytic mechanism of SamAA that remain unknow until now. Templates that are defined as proteins having high percentages of identity and similarity with the target sequence and whose structures are known were identified using Blastp and Swiss Model's « Search for templates » tools. The structures of PDB entries 1Q7L, 5VO3,

4PPZ, 4RUH and 7LGP were identified as SamAA templates with identity percentages between 17 and 33%. Basing on these templates, 3D homology models were built using Modeller from Discovery Studio suite, Swiss Model [5] and AlphaFold2 [6] and compared. The best 3D structure of SamAA according to energy calculations and presence of conserved residues was shown to be that obtained by ColabFold (Figure 1). Based on the literature survey relative to the templates [7], metal ions were added and structural characteristics were determined : SamAA would be a homodimeric protein with two zinc atoms per subunit. The His88, Asp120, Glu155, Glu182 et His416 residues would be responsible for the binding of divalent ions. The potential catalytic residues are Asp90 and Glu154: the latter would play the acid/base residue essential to the catalytic mechanism. Energy minimization is applied to the retained models. Docking simulations are in progress aiming to determine the key residues and interactions that are responsible for the selectivity of SamAA towards the lysine α amino group.

FIGURES

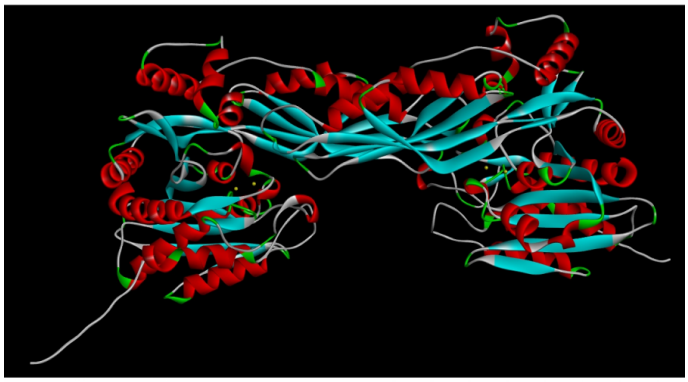

FIGURE 1

3D structure of SamAA

Structure of SamAA obtained by ColabFold after adding metal ions

FIGURE 2

KEYWORDS

N-acylation enzymatic process | aminoacylase | production of recombinant enzymes | molecular modelling

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