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Enhancement of the overexpression and stability of strictosidine synthase from Rauvolfia serpentina by protein engineering

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PURPOSE OF THE ABSTRACT

The Pictet–Spengler reaction offers a valuable route to 1,2,3,4-tetrahydro-β-carboline (THBC) and isoquinoline molecules. These scaffolds are found in important pharmaceuticals such as Tadalafil, a phosphodiesterase 5 (PDE5) inhibitor, administered to treat male erectile dysfunction.[1] Moreover, due to their pharmacological activities they are investigated for their potential use as antivirals, fungicides, antimalarials, antileishmanial, antithrombotic, analgesic and anticancer drugs.[2] However, one of the main challenges during the synthesis TBHCs derivatives is the introduction of chirality of C1. In this sense, a promising alternative to the traditional organic synthesis strategies is the application of biocalatalysis. Strictosidine synthases (STRs) catalyze the Pictet–Spengler condensation of tryptamine and the aldehyde secologanin to give (S) strictosidine as a key intermediate in indole alkaloid biosynthesis (Scheme 1).[3] Thus, STRs could be valuable asymmetric biocatalysts for applications in synthesis. In this sense, STRs also accept short-chain aliphatic aldehydes to give enantioenriched alkaloid products with up to 99% ee.[4] However, a drawback for the implementation of STRs in the synthesis of pharmaceutical compounds is their low heterologous overexpression, solubility and stability.

Hence, to overcome this issue the work presented here describes the computer-aided design of novel enzyme variants based on the STR from Rauvolfia serpentina (RsSTR) with improved protein solubility and stability. Amino acid changes to increase the stability and/or the solubility of RsSTR were identified. For this purpose, different bioinformatic tools were used, thus, positions relevant for the stability were detected using the Protein Repair One-Stop Shop (PROSS)[5] server and via calculation of ‘stability hot spots' using the HotSpot Wizard (FireProt),[6] while mutations reducing the aggregation tendency were predicted with the webserver SolubiS.[7] Overexpression levels of the different variants were studied in terms of RsSTR percentage present in the total soluble protein fraction, milligram of RsSTR per gram of E. coli cells and mg of purified RsSTR per gram of E. coli cells. Protein stability was tested by quantifying the melting temperature of the variants and the wt. Additionally, to determine the possible effects that the new introduced amino acid changes could have on the enzymatic activity, specific activity of the produced SrSTR variants was measured and compared with the activity displayed by the wt enzyme.

FIGURES

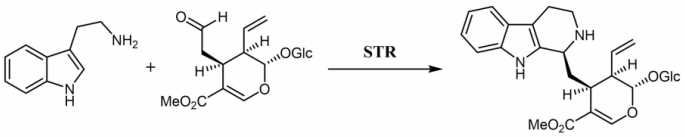

FIGURE 1

Biocatalytic Pictet-Splenger condensation

Strictosidine synthase synthesis of (S)-strictosidine by Pictet-Splenger condensation of tryptamine and secologanine

FIGURE 2

KEYWORDS

Strictosidine synthase | Pictet-Splenger reaction | Stability and solubility enhancement | Protein engineering

BIBLIOGRAPHY

[1] a).- A. Daugan, P. Grondin, C. Ruault, A. C. Le Monnier de Gouville, H. Coste, J. M. Linget, J. Kirilovsky, F. Hyafil and R. Labaudini□ret, J. Med. Chem. 2003, 46, 4533-4542; b).- A. Daugan, P. Grondin, C. Ruault, A. C. Le Monnier De Gouville, H. Coste, J. Kirilovsky, F. Hyafil and R. Labaudini□re, J. Med. Chem. 2003, 46, 4525-4532.

[2] J. Wang, F. Gong, T. Liang, Z. Xie, Y. Yang, C. Cao, J. Gao, T. Lu and X. Chen, Eur. J. Med. Chem. 2021, 225, 113815.

[3] R. Roddan, J. M. Ward, N. H. Keep and H. C. Hailes, Curr. Opin. Chem. Biol. 2020, 55, 69-76.

[4] D. Pressnitz, E.-M. Fischereder, J. Pletz, C. Kofler, L. Hammerer, K. Hiebler, H. Lechner, N. Richter, E. Eger and W. Kroutil, Angewandte Chemie, International Edition 2018, 57, 10683-10687.

[5] a).- A. Goldenzweig, M. Goldsmith, Shannon E. Hill, O. Gertman, P. Laurino, Y. Ashani, O. Dym, T. Unger, S. Albeck, J. Prilusky, Raquel L. Lieberman, A. Aharoni, I. Silman, Joel L. Sussman, Dan S. Tawfik and Sarel J. Fleishman, Mol. Cell 2016, 63, 337-346; b).- J. J. Weinstein, A. Goldenzweig, S. Hoch and S. J. Fleishman, Bioinformatics 2020,

[6] D. Bednar, K. Beerens, E. Sebestova, J. Bendl, S. Khare, R. Chaloupkova, Z. Prokop, J. Brezovsky, D. Baker and J. Damborsky, PLoS Comput. Biol. 2015, 11, e1004556.

[7] J. Van Durme, G. De Baets, R. Van Der Kant, M. Ramakers, A. Ganesan, H. Wilkinson, R. Gallardo, F. Rousseau and J. Schymkowitz, Protein Eng. Des. Sel. 2016, 29, 285-289.