A STOCHASTIC APPROACH OF BUILDING UP THE ENZYME hGSTA1-1 BY IDENTICAL REPEAT MOTIFS CAN BE PROMISING FOR THE DEVELOPMENT OF BIOMATERIALS

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ABSTRACT

The design of supramolecular enzyme architectures on molecular level (so called "bottom-up" fabrication) is considered a new trend in synthetic biotechnology research. Enzyme nanostructures are considered as effective materials for the development of assembled structures, with customized characteristics that can be fabricated [1,2]. In the same vein, protein engineering is a prominent method for the design and creation of new enzymes with innovative catalytic and structural features [3]. GSTs are multi-functional enzymes of high-importance for a number of biotechnological applications. They are considered as good model systems for engineering studies, due to their modular catalytic and binding features [4]. This work was inspired by "lego'' chemistry, aiming to the design and development of novel protein folds that can be self-assembled into complex-structures, by exploiting the modularity capabilities of human GSTA1-1 enzyme (hGSTA1-1) through stochastic approach, which follows the principles for a higher order and geometrically irregular assembly [5]. Surprisingly and in contrast with the complex topology of the globular enzyme hGSTA1-1, a motif with regularized interactions was found. Therefore, GST can be evolved not only by site-saturation mutagenesis and directed evolution, but also by the addition of whole repeats.

Based on the analysis of the crystal structure of hGSTA1-1 (PDB identification code: 1K3Y) [6], the residues Lys140 and Ser141 were selected as potential hot-spots for step-by-step site-saturation mutagenesis. Four mutated enzymes were designed and constructed: hGSTA1-1H (K140H), hGSTA1-12H (K140H, S141H), hGSTA1-1S (with the motif KVLH before the mutation K140H) and hGSTA1-1GS (with more repetitive motifs KVLH). These GST genes were cloned in 5EXP-CT TOPO vector and expressed in E. coli. The recombinant enzymes were purified by affinity chromatography and their biochemical properties were studied.

The results showed that the mutant and "lego" enzymes appear to exhibit improved catalytic efficiency without losing their structural stability. The discovery and characterization of these mutants depicts the potential of globular enzymes (e.g. GSTs) to be explored for the generation of complex architectures through protein engineering, by inserting specific repeat motifs. Therefore, it is a new way to enlarge the library of GST scaffolds of biomaterials for advanced applications.

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REFERENCES

[1] Luo Q, Hou C, Bai Y, Wang R, Liu J. (2016). *Chem. Rev. 116*, 13571-13632.

[2] Gradišar H, Jerala R. (2014). *J. Nanobiotechnol. 12*, 4.

[3] Damborsky J, Brezovsky J. (2014). *Curr. Opin. Chem. Biol. 19*, 8-16.

[4] Axarli I, Muleta AW, Chronopoulou EG, Papageorgiou AC, Labrou NE. (2017). *Biochim. Biophys. Acta: General Subjects. 1861*, 3416-3428.

[5] Lai Y-T, King NP, Yeates TO. (2012). *Trends Cell Biol*. 22, 653-661.

[6] Le Trong I, Stenkamp RE, Ibarra C, Atkins WM, Adman ET. (2002). *Proteins., 48*, 618-627.