Enhancing the thermal stability and activity of the artificial self-sufficient P450SPα-SOX by switching the domains linker

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Fusion proteins are powerful tools to facilitate the combination of two or more enzyme functionalities in a cascade reaction, to increase the protein stability and to enhance the catalytic performance of a multi-enzyme system. Our laboratory recently developed an artificial multi-enzyme fusion protein to increase the peroxidase activity of the fatty acids α-hydroxylase $P450_{SP\alpha}$ (CYP152B1) by fusing it to a H_2O_2-donor sarcosine oxidase (SOX) through a flexible poly-glycine linker. In order to investigate the effect of the linker on the biophysical and catalytic properties of the multi-enzyme system we modified the amino acid sequence of the linker between $P450_{SP\alpha}$ and SOX, obtaining a new fusion protein characterized by a high structural rigidity. The $P450_{SP\alpha}$-rigid-SOX displayed a higher energy barrier to thermal denaturation compared to the flexible construct, as shown by an increase in the T_{onset} of 10°C and an increase of 227 cal/mol of the unfolding enthalpy measured by differential scanning calorimetry (DSC). We investigated the effect of thermal inactivation on the heme-thiolate ligand by UV-VIS spectroscopy for both flexible and rigid $P450_{SP\alpha}$-SOX constructs. The data indicates that the rigid linker has a positive effect on the stabilization of a P420 semi-folded state. Furthermore residual CO-binding experiments also demonstrated a 5.7 °C increase of the T_{50} for the $P450_{SP\alpha}$-rigid-SOX. Most importantly, $P450_{SP\alpha}$-rigid-SOX showed a increased total turnover for the oxidation of p-nitrophenol to p-nitrocatechol, herein reported for the first time as $P450_{SP\alpha}$ substrate. Finally, $P450_{SP\alpha}$-rigid-SOX was used for the turnover of styrene and also in this case it outperformed $P450_{SP\alpha}$-flexible-SOX. Overall, we demonstrated that the rigid linker improved the fusion enzyme thermal stability and its catalytic performance.