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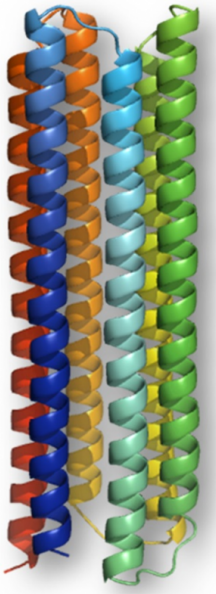
De novo design of a multifunctional thermostable protein catalyst with a novel fold

AUTHORS
Wael ELAILY / INSTITUTE OF BIOCHEMISTRY, TU GRAZ, PETERSGASSE 12/2, GRAZ

PURPOSE OF THE ABSTRACT
The design of a stable de novo protein scaffold that can be functionalized to catalyze a wide range of reactions is a promising but challenging task. This study aims to use the Rosetta software to parametrically design a novel thermostable helix bundle with the ability to accommodate an active site. Our initial design (6H5L) forms a helical barrel structure with a hydrophobic channel and is comprised of six straight antiparallel helices, which are connected by loops (Figure 1). As a first reaction model, we designed several variants to catalyze the retro-aldol reaction, which has extensive application in biocatalysis and could be extended to perform various non-native carboligation reactions. All variants are readily produced in E. coli and characterized using biochemical and biophysical methods including circular dichroism spectroscopy (CD), UV-Vis and fluorescence spectroscopy, SAXS analysis, and X-ray crystallography studies. The CD spectra of the designs confirm their alpha-helical fold and, moreover, a thermal stability up to 95°C with only minimal loss of signal at high temperatures and complete refolding after cooling back to 20°C. To improve protein production, we redesigned the protein's surface using ProteinMPNN, which increased protein production 10-fold. Alphafold2 showed a high predicted lDDT score of over 90 for the originally designed sequence and most variants. To test retro-aldolase activity, we performed an inhibition reaction assay with a naphthalene diketone derivative which reacts with a nucleophilic lysine residue and forms an imine intermediate that can be monitored photometrically. The retro-aldol reaction of our designs with 4-hydroxy-4-(6-methoxy-2-naphthyl)-2-butanone (methodol) as a substrate was followed by measuring the increase of fluorescence upon product formation. 6H5L and all its variants showed noticeable retro-aldolase activity. To increase the activity, we used rational and computational design approaches. The best variant showed a 10-fold activity increase compared to the initial design (Figure 2). SAXS measurements of all variants gave an overall good fit between the measured and the calculated scattering profiles with low chi^2 values. We were able to determine the crystal structures of the apo design (2.2 Å) and a variant with the covalently bound ligand (naphthalene diketone derivative, 3.0 Å). To demonstrate the multifunctional use of our designs, we assayed them for other reactions and found some variants which showed notable Michael-addition activity and good PLP binding affinity. We are currently working on improving all catalytic activities.

FIGURES



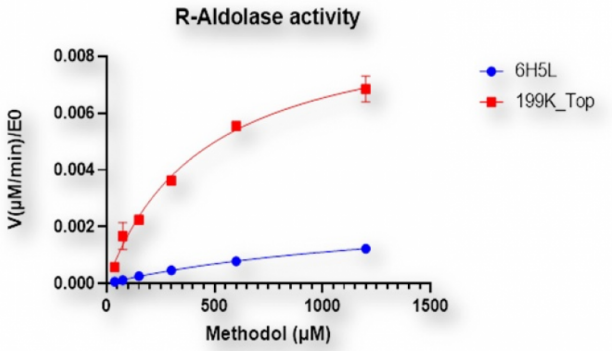


FIGURE 1

6H5L design

Structure representation of 6H5L in side view.

FIGURE 2

Retro-aldolase activity

Michaelis Menten plots of 6H5L and 199K_Top variant with Methodol as a substrate. E0, total enzyme concentration; V, initial reaction velocity.

KEYWORDS

De novo enzyme design | Thermostable protein | Multifunctional enzyme | Rosetta software

BIBLIOGRAPHY