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Thermostabilizing strategies performed by two computational methods in a DyP-type peroxidase from Pseudomonas putida

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PURPOSE OF THE ABSTRACT
Dye-decolorizing peroxidases (DyPs) are enzymes that can oxidize many substrates like anthraquinone and azo dyes, phenolic and nonphenolic lignin units, metals, among others, while reducing hydrogen peroxide to water. This makes DyPs interesting candidates for biotechnological purposes.

The computational methods PROSS [1] and FireProt [2] were used to design thermostable DyP variants from Pseudomonas putida (PpDyP) with since this enzyme lacks robust stability properties required for industrial processes and an efficient mechanism to oxidize many substrates with biotechnological interest.

The variant PpDyP PR, generated by PROSS, included 29 mutations and exhibited a melting temperature of 88°C, a 20°C increase compared to the wild type (WT). On the other hand, the variant PpDyP FP, generated by FireProt, included 21 mutations and exhibited a melting temperature of 75°C, a 10°C increase from the WT. Additionally, both variants show approximately 5-fold higher catalytic efficiency (kcat/Km) for hydrogen peroxide and ABTS (unpublished data).

The structure of these thermostable variants was determined by X-ray crystallography. Both variants generated thin orange needle-shaped crystals and diffraction data was collected at ALBA Synchrotron Light Facility (Barcelona, Spain) in the BL13-XALOC beamline. Data was processed with a resolution of 2.10 Å for PpDyP PR and 2.35 Å for PpDyP FP. PpDyP PR crystals belong to P 21 21 21 space group and contain two protein molecules per asymmetric unit, whereas PpDyP FP crystals belong to P 32 2 1 space group and show four protein molecules per asymmetric unit.
The structures were determined by molecular replacement using PpDyP WT structure (PDB 7QYQ [3]) as search model. The structure of PpDyP PR shows an R-free and R-work values of 23.6% and 19.0%, respectively. Refinement of PpDyP FP is ongoing.

Enzymes must have a degree of thermostability to overcome the thermal stress common from industrial processes. To better understand the role of the mutations on the improved thermostability, suggested by PR and FP, we used the webserver Protein Interactions Calculator (PIC; http://pic.mbu.iisc.ernet.in/) to identify interactions in proteins such as hydrophobic interactions, H-bonds and salt bridges, known to contribute for the higher stability in enzymes. Moreover, some other thermostabilizing strategies employed by hyperthermophilic enzymes were reported in the literature and may include structural elements such as shorter loops, maximized protein packing, lower solvent accessible surface, decreased number of internal cavities, among others.

Comparative analysis of PpDyP WT and PR variant structures showed, in the PR variant, an increase of the internal

packing due to decreased cavities number and volume. The analysis performed at PIC revealed an increase of 14 new hydrophobic interactions in PR relative to the WT, where some involve mutations (6) present in this variant. Moreover, we found that PR has 34 new H-bonds, and 15 mutations are involved in the appearance of these interactions in the variant. Regarding the salt bridges, we did not find any significant differences between the two enzymes. The comparative analysis involving PpDyP FP will also be performed.

The structural analysis of these variants will help to understand the variety of stabilization mechanisms that enzymes acquire to become more thermostable which is essential for their application in biotechnology industry.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

DyP-type peroxidase | X-ray crystallography | Protein interactions | Thermostability

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