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IMPROVEMENT OF *SELENOMONAS RUMINANTIUM* β-XYLOSIDASE THERMAL STABILITY BY REPLACEMENT OF FREE BURIED CYSTEINES

E Dehnavi, M Aghaeepoor and A Akbarzadeh
Gene Transfer Pioneers (GTP) research group, Iran

Cysteine is an intriguing and enigmatic amino acid; while it is one of the least abundant amino acids in the structure of proteins, but it is frequently observed in functionally important sites of proteins such as catalytic, regulatory and cofactor binding sites. Moreover, its physio-chemical classification as a hydrophobic or polar residue is arguable. Whereas it has polar thiol group, free Cys in proteins is often buried and surrounded by a hydrophobic environment. It has been shown that both removal and insertion of Cys can lead to increase protein thermo-stability. Since *Selenomonas ruminantium* β-D-xylosidase (SXA) has four free cysteines, it used as a model. To characterize the role of cysteine residues in the structure, function and stability of SXA, we prepared and evaluated wild-type and four cysteines deficient SXA proteins. Buried cysteine residues, were replaced with valine using QuikChange site-directed mutagenesis. In comparison with the wild-type, the Km values remained relatively constant while the kcat decreased in mutants. The optimal pH and temperature were similar in the wild-type enzyme and its variants. The C101V and C286V displayed higher thermal stability than the wild-type at 55 and 60 °C. Secondary and tertiary conformational changes using circular dichroism and fluorescence spectroscopy revealed that changing a buried cysteine to a hydrophobic residue could lead to an increase in thermal stability with minimal perturbation of the overall wild-type protein structure. In addition to experimental methods, the stability of WT SXA and C101V and C286V mutants at 333 K was also studied by MD simulation. Our theoretical data had a good agreement with the experimental results.

Biography

E Dehnavi has graduated in Biochemistry in 2015 from Tarbiat Modares University, Tehran Iran. His doctoral dissertation was conducted in consultation with Prof Khajeh and examines the use of protein engineering methodology for improving kinetic properties of hemicellulosic enzymes. He has been working on the expression of *Selenomonas ruminantium* Xylosidase in yeast *Pichia pastoris*. Moreover, he worked to improve the thermal stability of some industrial enzyme by site-directed mutagenesis. Currently, he is a Team Leader in Gene Transfer Pioneers research group, the company is active in the field of biotechnology where optimization of protein expression of some industrial enzymes such as endoglucanase, xylosidase and phytase through protein engineering and producing more effective expression vectors is done. His current research is increasing enzymatic saccharification yields through cellulase and hemicellulase enzymes protein engineering. He has published 10 papers in reputed journals.

Ehsan.dehnavi@modares.ac.ir
bioehsan2000@gmail.com