A combination of disulfide bridge creation mutations in turn and β-sheet regions of organophosphorus hydrolase enzyme for increasing of the thermal stability.

Mostafa Akbariqomi[1], Ali Mohmmad Latifi[1], Morteza Mirzaei[1], Gholamreza Farnoosh[1*]

[1]Applied Biotechnology Research Centre, Baqiyatallah University of Medical Sciences, Tehran, Iran

Corresponding author email: rzfarnoosh@yahoo.com

Introduction

Thermostability enhancement of enzymes used commercially or industrially would due to increased enzymatic competence and cost-effectiveness. Many stabilizing agents have been proposed to be the base of thermal stability, like disulfide bonds creation, proline replacements, ionic pair networks creation and surface loop truncation.The organophosphorus hydrolase (OPH) has been used to degrade chemical warfare agents, as one of the most frequently used biological decontamination methods. For utilize Organophosphorus hydrolase (OPH) enzyme in various applications, stability at high temperature is an important character. This study evaluates synergist effect of two disulfide bridge mutations in **γ**-turn, **β**-turn and **β**-sheet regions of organophosphorus hydrolase enzyme for increasing of the thermal stability.

Material and method

Here, two combinations of the stabilizing point mutations were used to improvement the thermal stability of the Organophosphorus hydrolase (OPH). As is described in our previous study, mutations A175C/T205C, G45C/A49C and T99C/ G124C were designed and introduced by Disulfide by Design software prediction. Activity of OPH-wt and OPH-M4(A175C/T205C-T99C/ G124C) and OPH-M5(G45C/A49C-T99C/ G124C) combination mutants were evaluated by monitoring the production of the p-nitrophenol (PNP), from paraoxon as the specific substrate. The thermostability of the wild type and mutant's enzymes were investigated by half-life and ∆Gi analysis.

Results

Results confirmed an increase half-life and ∆Gi of OPH-M4 in compared to OPH-wt while OPH-M5 was reduced. Although the activity of both mutants was slightly reduced compared to OPH-wt.

Discussion

Based on these results, a strong document is presented for thermostability improvement of OPH enzyme by rigidity of turn and **β**-sheet regions. Consequently, the results not only assist us for rigid of the flexible regions in OPH by disulfide bridge creation, but also promote our knowledge in enzymes engineering for defense applications.

Key words: Organophosphorus hydrolase, Disulfide Bridge, Thermostability, chemical warfare agents