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Effects of sorbitol and glycerol on the structure, dynamics, and stability of *Mycobacterium tuberculosis* pyrazinamidase

Mehrnoosh Khajehzadeh [a], *Faramarz Mehrnejad* [b], *Mohammad Pazhang* [a], *Farahnoosh Doustdar* [c,*]

[a] *Department of Biology, Faculty of Science, Azarbaijan Shahid Madani University, Tabriz, Iran*
[b] *Department of Life Science Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran, Iran*
[c] *Department of Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

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ABSTRACT

Objective/background: *Mycobacterium tuberculosis* pyrazinamidase (PZase) is known an enzyme that is involved in degradation of pyrazinamide to ammonia and pyrazinoic acid. Pyrazinamide is an important first-line drug used in the short-course treatment of tuberculosis. Previous investigations have indicated that the pyrazinamide (PZA)-resistant *M. tuberculosis* strains are caused by point mutations in the PZase enzyme which is the activator of the prodrug PZA. Although the general fold of PZase was determined, the structural and functional properties of the enzyme in solution were not understood very well. In this study, the PZase enzyme was overexpressed and purified. In addition, two polyols, namely sorbitol and glycerol, were chosen to study their effects on the structure, dynamics, and stability of the enzyme. To gain a deeper insight, molecular dynamics simulation and spectroscopic methods, such as fluorescence spectroscopy and circular dichroism (CD), were used.
Methods: The genes were cloned in *Escherichia coli* BL21 (DE3), harboring the recombinant pET-28a (+) plasmid, overexpressed and purified by Ni-NTA Sepharose. The far UV–visible CD spectra were measured by a Jasco-810 spectropolarimeter. The intrinsic fluorescence spectra were measured on a Cary Varian Eclipse spectrofluorometer. For molecular dynamics (MD) simulations, we have applied GROMACS4.6.5.
Results: The results showed that glycerol and sorbitol increased the enzyme activity up to 130% and 110%, respectively, at 37 °C. The stability of PZase was decreased and the half-life was 20 min. Glycerol and sorbitol increased the PZase half-life to 99 min and 23 min, respectively. The far UV CD measurements of PZase indicated that the CD spectra in glycerol and sorbitol give rise to an increase in the content of α-helix and β-sheets elements. The average enzyme root mean square deviation (RMSD) in sorbitol solution was about 0.416 nm, a value that is higher than the enzyme RMSD in the pure water (0.316). In dictionary of protein secondary structure (DSSP) results, we observed that the secondary structures of the protein are partially increased as compared to the native state in water. The experimental and simulation data clearly indicated that the polyols increased the PZase stabilization in the order: glycerol > sorbitol.

* Corresponding author at: Department of Microbiology, Faculty of medicine, Shahid Beheshti University of Medical sciences, Tehran, Iran.
E-mail address: f_doustdar@yahoo.com (F. Doustdar).
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Conclusion: It can be concluded that the native conformation of the enzyme was stabilized in the sorbitol and glycerol and tend to exclude from the PZase surface, forcing the enzyme to keep it in the compactly folded conformation. The glycerol molecules stabilized PZase by decreasing the loops flexibility and then compacting the enzyme structure. It appears that more stability of PZase in glycerol solution correlates with its amphiphilic orientation, which decreases the unfavorable interactions of hydrophobic regions.

Conflict of interest

None declared.

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