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SITE-DIRECTED MUTATION STUDY ON HYPERTHERMOSTABILITY OF RUBREDOXIN FROM PYROCOCCUS FURIOSUS USING MOLECULAR DYNAMICS SIMULATIONS IN WATER

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The hyperthermostable protein, rubredoxin from *Pyrococcus furiosus* is 53-residue protein with a three-stranded anti-parallel β-sheet and several loops. To investigate the effect of changes of electrostatic and hydrophobic interactions on the structure and dynamic property of *P. furiosus* rubredoxin, molecular dynamics simulations in water were performed on three mesophilic rubredoxins, *P. furiosus* rubresoxin, and 5 mutants of *P. furiosus* rubredoxin. Glu 14 of *P. furiosus* rubredoxin has a backbone hydrogen bond with N-terminal and multiple electrostatic interactions with Ala 1, Trp 3, and Phe 29. The multiple electrostatic interactions make the residues around the N-terminal stable and the hydrogen bond between Glu 14 and Ala 1 remains at higher temperature. The flexibility of a loop from Asp 15 to Gly 26 is reduced by making the loop closer to the main part of rubredoxin by virtue of the multiple electrostatic interactions of Glu 14. In the middle of the β-sheet, three hydrophobic residues, Val 4, Ile 11, and Leu 51 makes the cluster binding three strands of the β-sheet. This cluster aggregates tightly to stabilize the β-sheet and furthermore whole protein.