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X-ray crystallographic analysis of the highly acidic thioredoxin from an extreme halophile *Halobacterium* sp. NRC-1

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1 Introduction

Halophilic proteins have unique structural characteristics: high content of acidic residues creating negatively charged surface, high reversibility of tertiary structure and activity even in high salt concentration. As part of our structure-function studies for halophilic proteins [1-3], we determined a tertiary structure of the thioredoxin derived from the extreme halophile *Halobacterium* sp. NRC-1 (HsTRX) having the highest acidic amino acid content ([D+E]/[K+R]=9.0) in comparison with proteins in PDB.

2 Experiment

Diffraction datasets were taken at BL-5A, 17A and NW12A beamlines. All datasets were collected at 100 K and crystals were cryoprotected with NVH oil (Hampton Research, CA). The HsTRX crystal diffracted up to 1.6 Å resolution, and belonged to space group $P2_12_12_1$. The unit cell parameters were a = 40.9 Å, b = 43.3 Å, c = 54.4 Å, $\alpha = \beta = \gamma = 90°$. Diffraction data were integrated and scaled using the *HKL2000* suite of programs. Overall R_{merge}, completeness, I/σ and redundancy values were 4.5%, 99.9%, 12.9 and 6.9, respectively. Initial phase information for HsTRX was obtained by the molecular replacement (MR) method using the program *Phenix AutoBuild* in which the structure of thioredoxin from *Bacillus subtilis* (PDB ID: 2GZY) was used as a search model. The modelling and refinement were carried out using programs *Phenix .refine* and *Coot*.

3 Results and Discussion

The crystal structure of HsTRX was determined to 1.6 Å resolution with an R-factor of 16.9% (R_{free} 20.6%) (Figure 1). One asymmetric unit includes one HsTRX molecule comprising 113 residues and 102 waters. This is the first structure determination of halophilic thioredoxin.

Interestingly, the tertiary structure of HsTRX was similar to those of thioredoxin from the extreme thermophile *Sulfolobus tokodaii* (PDB id: 2E0Q, RMSD for Cα atoms: 0.89 Å), thioredoxin from the extreme thermophile *Thermus thermophiles* (PDB id: 2CVK, RMSD for Cα atoms: 1.00 Å) and the reconstructed precambrian thioredoxins (PDB id: 2YNX, 4BA7 and 3ZIV, RMSD for Cα atoms < 1.13 Å) (Table 1).

These results might give us hints for a clarification of the relationship between the environmental adaptation mechanism and the molecular evolution of proteins.

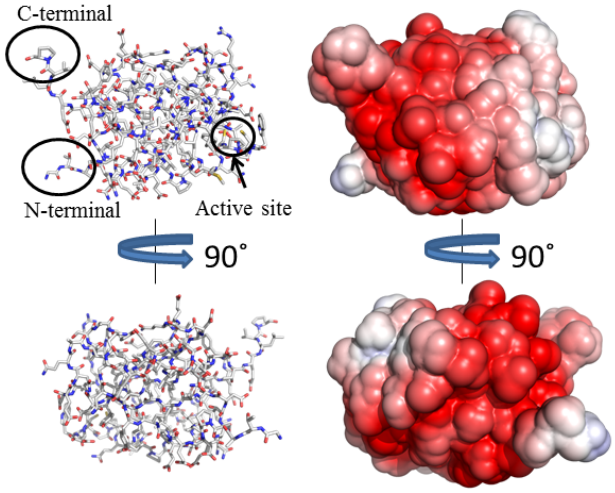

Figure 1. Tertiary structure (left) and molecular surface (right) of HsTRX. In the right figure, the negatively charged surface is colored in red.

References

[1] Arai S. et al., *Protein Sci.* **21**, 498 (2012).
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Table 1. Structural comparison of HsTRX and homologous thioredoxins

Thioredoxin	HsTRX	2E0Q	2YNX	2CVK	4BA7	3ZIV	2GZY
RMSD for Cα atoms (Å)	0	0.89	0.96	1.00	1.09	1.13	1.17
Sequence identity (%)	100	31	38	38	39	39	43
[D+E] / [K+R]	27 / 3 (9.00)	20 / 7 (2.86)	15 / 15 (1.00)	16 / 13 (1.23)	21 / 14 (1.50)	18 / 15 (1.20)	18 / 10 (1.80)