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*₁*₂*₁*₂*₁. The unit cell parameters were *a* = 40.9 Å, *b* = 43.3 Å, *c* = 54.4 Å, *α* = *β* = *γ* = 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 Ca atoms: 0.89 Å), thioredoxin from the extreme thermophile *Thermus thermophilus* (PDB id: 2CVK, RMSD for Ca atoms: 1.00 Å) and the reconstructed precambrian thioredoxins (PDB id: 2YNX, 4BA7 and 3ZIV, RMSD for Ca 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.](image)

Table 1. Structural comparison of HsTRX and homologous thioredoxins

<table>
<thead>
<tr>
<th>Thioredoxin</th>
<th>HsTRX</th>
<th>2E0Q</th>
<th>2YNX</th>
<th>2CVK</th>
<th>4BA7</th>
<th>3ZIV</th>
<th>2GZY</th>
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<tbody>
<tr>
<td>RMSD for Ca atoms (Å)</td>
<td>0.89</td>
<td>0.96</td>
<td>1.00</td>
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<td>39</td>
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<tr>
<td>[D+E]/[K+R]</td>
<td>20 / 7 (2.86)</td>
<td>15 / 15 (1.00)</td>
<td>16 / 13 (1.23)</td>
<td>21 / 14 (1.50)</td>
<td>18 / 15 (1.20)</td>
<td>18 / 10 (1.80)</td>
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References

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