

Structural Stability of [2Fe-2S] Ferredoxin from the Extremely Halophilic Archaeon *Halobacterium salinarum* II

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Introduction

We have continued the investigations on [2Fe-2S] ferredoxin from *Halobacterium salinarum* and have found the protein to be structurally intact and functionally active at salt concentrations ≥ 1.5 M NaCl only [1]. At low salt concentration the protein partially unfolds in a time-dependent manner.

To dissect out the role of binding of salt ions on the extent of hydration of protein, we attempted to study the molecular dimensions & thereby the binding of salt ions / hydrated salt ions to the protein as a function of salt concentration by small angle X-ray scattering.

Methodology

The growth of the haloarchaeon and isolation and purification of ferredoxin was by a protocol reported earlier [1]. The purified ferredoxin solution (A_{420}/A_{280} of 0.349) was extensively dialyzed against 20 mM phosphate buffer pH 7.5 containing either 4.26 M NaCl (buffer A) or 1.0 M NaCl (buffer B) and concentrated.

SAXS measurements were performed on beamline 15A1 (Photon Factory, Tsukuba). The X-ray wavelength was 1.50 Å. The detector was a CCD camera. The data was corrected for image distortion, contrast & sensitivity, circularly averaged to one dimension and normalized to the incident X-ray intensity.

Results

Fig. 1 shows radius of gyration (R_g) at various salt concentrations. The R_g of native ferredoxin (in 4.2M NaCl) estimated to be 21Å, remains invariant in salt concentration as low as 1 M NaCl, whereas R_g becomes bigger at salt concentration lower than 1M. In Fig. 2, the Kratky plots of ferredoxin in 20mM NaCl is indicative of unfolded conformation whereas in 1M and 1.5M NaCl the protein retains its largely native, folded structure.

References

- [1] A.K. Bandyopadhyay *et al.*, Biophys. J. **79**, 501 (2000)
 [2] Deby *et al.*, PF activity report, 20, 254 (2003).
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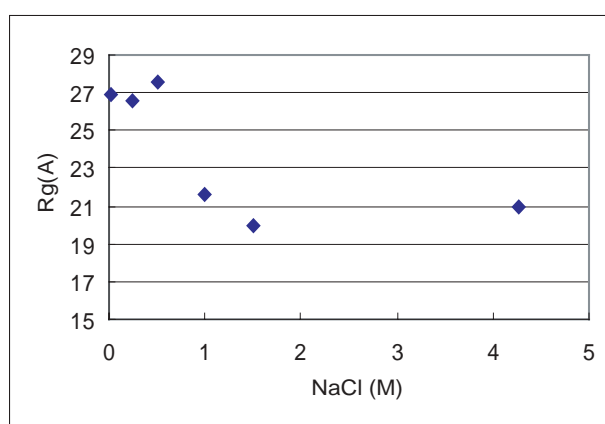


Fig. 1. R_g vs NaCl concentration

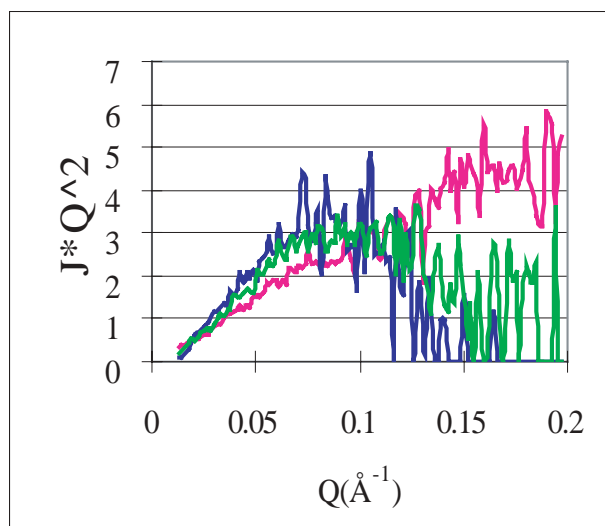


Fig.2. Kratky plot of ferredoxin at 20 mM (pink), 1M (blue) and 1.5 M (green) of NaCl.