

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

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Introduction

Proteins from extreme haloarchaeal organisms are adapted to function in multimolar salt concentration [1]. We have been studying [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 [2]. 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 [2]. 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

The Rg of native ferredoxin (in 4.2M NaCl) could not be obtained due to technical reasons. We obtained Rg values at the salt concentration of 1 M with the average value of 19.9 Å (Fig. 1). This value is much higher than that estimated from its NMR structure (16.4 Å) [3]. Kratky plots at 1 M NaCl is shown in Fig. 2 with the calculated one from NMR data. Figure shows conformation at 1 M shows less peak height, indicating the conformation at 1 M is not at native state, but a state with larger Rg value. It might suggest that the conformation at 1 M NaCl is a kind of molten globule state.

References

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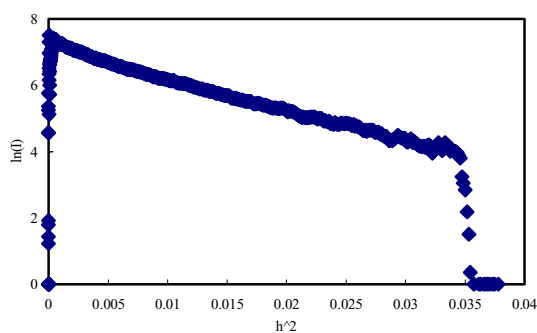


Fig. 1 Guinier plot of halophilic Ferredoxin

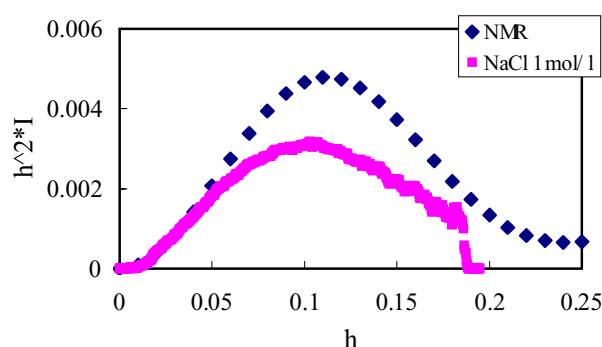


Fig. 2. Kratky plot of halophilic Ferredoxin