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SAXS study on the halophilic ferredoxin. Salt concentration dependence

Haripalsingh SONAWAT[1], Jinsong LI[2], Masaji SHINJO[2], Yoshitaka MATSUMURA[2], Satoshi NAKAMURA[3], Toshiharu HASE[4] and Hiroshi KIHARA[2]

[1]Dept. of Chemical Sciences, Tata Institute of Fundamental Research, Mumbai India
[2]Dept. of Physics, Kansai Medical University, 18-89 Uyama-Higashi, Hirakata 573-1136, Japan
[3]Dept. of Bioengineering, Tokyo Institute of Technol., 4259 Nagatsuda, Yokohama 226-8501, Japan
[4]Institute of Protein Research, Osaka University, 3-2 Yamadaoka, Suita 565-0871, Japan

Introduction

Ferredoxin is a group iron-sulfur protein that was found in almost all living cultures. Although the ferredoxins have wide range diversity, from primary sequence to the composition of amino acid, they share similar structure and biological function. Halophilic archaea are microorganisms that require 3-5 mol of salt for a proper life style that are lethal for common organisms. Ferredoxin from Halobactrium salinrum is composed by 128 amino acids with a Fe2-S2 center and it works as the electron carrier in the decarboxylation of a-ketoacids [1]. The sequence and structure of this halophilic ferredoxin is very similar with plant type ferredoxin. However, the halophilic ferredoxin has an additional domain at the N-terminus that includes large amount of negative charges. This suggests the negative charge play an important role to stabilize the halophilic ferredoxin in the high salt concentration.

Here, we report the conformational study on halophilic ferredoxin in the presence of various concentration of sodium chloride by small angle x-ray scattering.

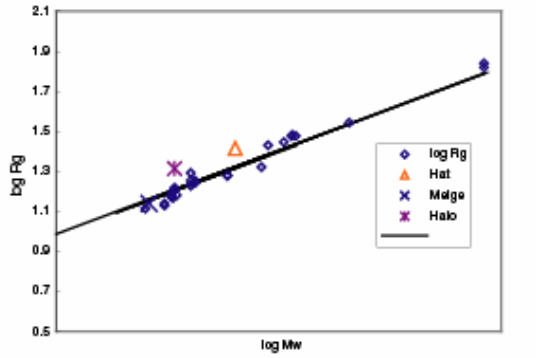
Result

The halophilic ferredoxin is keeping compact when [NaCl] higher than 1M, judging from radius of gyration (Rg) and Kratky plot. It is interesting that the Rg of the protein, 20 Å, is larger than we predicted from its molecule weight and that calculated based on the protein data bank. At low salt concentration, the protein was expanded to 27 Å. This value is similar with the Rg of the chemically denatured state. It indicates halophilic ferredoxin is totally expanded at low salt concentration. In addition, we measured other two kinds of ferredoxins in the extremely high salt concentration and at the physiological condition. Maize ferredoxin is distributed differentially in mesophyll and bundle-sheath cells from maize [2]. The Kratky analysis indicated the Maize ferredoxin is kept compact both in the extremely high salt and in the physiological condition. Gunier-plot fitting analysis gave the Rg of maize ferredoxin 14.2 Å at the native state.

Another halophilic ferredoxin from Haloarcula japonica strain TR1 is a 32 kD protein [3]. Guinier-plot fitting analysis gave the Rg of the protein in 4.26M NaCl as 28 Å, while that in 0M NaCl buffer was 56 Å.

Data demonstrates; (1) mesophilic ferredoxin from maize is compact with Rg of 14.1 Å both in 0M and in 4.26M NaCl. (2) Both halophilic ferredoxin from Halobacterium salinarum and Haloarcula japonica are compact in 4.26M NaCl, while they are unfolded in 0M NaCl buffer. The molecule size is proportional to molecule weight as far as they are globular except halophilic ferredoxins in 4.26M NaCl, as shown in Fig 1.

Fig 1 relationship between the molecule size and molecule weight



Using this linear relation, the molecular weight of the halophilic ferredoxin and Haloarcula japonica, predicting from its molecule size, is very close to the double of their molecular weight, about 26kD, as shown in table 1. This implies the proteins mainly take dimer in solution.

Table 1 Calculated molecular weight from Rg

Sample	Mw[a)]	Rg[b)]	Rg [c)]	Mw[d)]
HAT	32KDa	20.9	26	60.5KDa
Maize	10.5Kda	14.5	13.8	9KDa
Halo	14.6KDa	16.1	20.6	30.1KDa

[a)]the molecular weight was calculated from sequence
[b)] the Rg was obtained from the molecular weight
[c)]the Rg was obtained from the experimental result
[d)]The molecular weight was calculated from Rg

References

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*kihara@makino.kmu.ac.jp