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Structural analysis of srcSH3 intermediate on its folding pathway

Masaji Shinjo[1], Yasumasa Morimoto[2], Masaki Kojima[2], Jinsong Li[1], Xianju Jin[1], Yoshitaka Matsumura[1] and Hiroshi Kihara[1 *]
[1]Department of Physics, Kansai Medical University
[2]School of Life Science, Tokyo University of Pharmacy and Life Sciences

Introduction

SrcSH3 forms α-helix-rich intermediate in the early stage of its folding pathway[1]. In this study, we calculated molecular structure of the intermediate from the X-ray solution scattering profile by GASBOR program[2].

We also calculated molecular structure with atomic coordinate by SAXS_MD program[3]. The obtained structure is similar to the one obtained by GASBOR.

Result

Time-resolved refolding experiments were done by denaturant concentration-jump (stopped-flow) method with circular dichroism (CD) and X-ray solution scattering as probes. Unfolded protein solution in 5M GuHCl solution was mixed with 6 times volume of buffer. Thus, the protein solution was diluted 7 times (in 0.7 M GuHCl),which induces refolding.

Fig.1 shows a refolding curve by CD at 222 nm. Fig.2 shows refolding curve in terms of radius of gyration (*R*g). Src SH3 forms non-native α-helix-rich folding intermediate. *R*g of the intermediate is 18.5Å, much smaller than that of the unfolded state (27Å) and larger than that of the native state (14.6Å).[1]

We calculated molecular shape from scattering intensity by GASBOR[2] program. Calculation was done with 78 residues (srcSH3 64 residues and His-Tag 14 residues). Fig.3a shows the calculated molecular shape. The intermediate shows a bent shape around the center of the molecule. We also calculated the structure by SAXS_MD program[3] with 78 residues protein sequence. Obtained structure is similar to the one obtained by Gasbor although the expression of secondary structure is not enough. (Fig.3b)

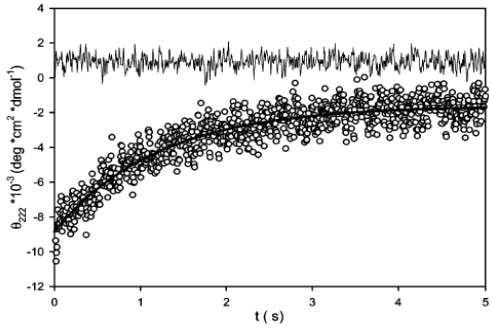

Fig.1 kinetic CD measurement of θ_{222} at 4°C[1]
The upper curve (initial) was obtained by mixing the unfolded protein solution with the unfolded buffer.

References

1) Li *et al*. (2007) Biochemistry 46, 5027-5082
2) Svergun *et al.* (2001) Biophys. J, 80, 2946-2953
3) Kojima *et al*. (2004) J. Appl. Cryst. 37, 103-109
*kihara@makino.kmu.ac.jp

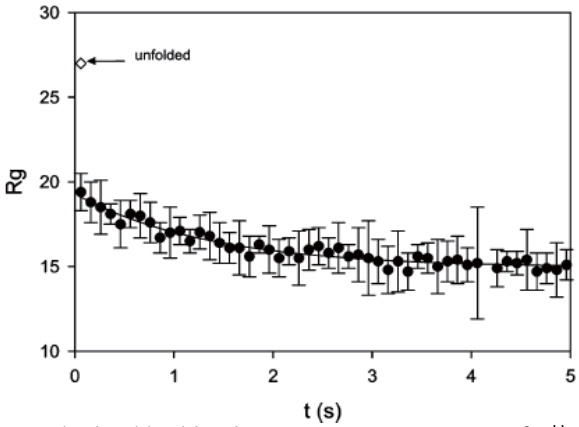

Fig.2 *R*g obtained by kinetic SAXS measurement at 4°C[1]
The "unfolded" Rg was separately obtained.

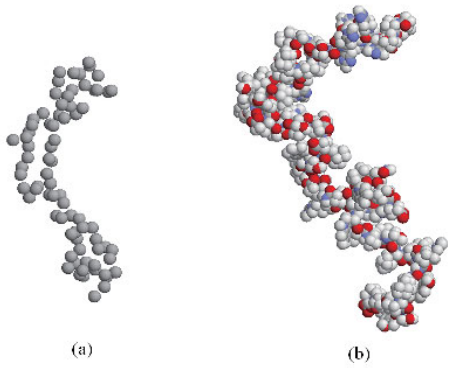

Fig.3 Reconstructed structures of the intermediate of srcSH3 (a) Cα atom model calculated by GASBOR[2]
(b) calculated by SAXS_MD[3]