Structural analysis of srcSH3 intermediate on its folding pathway

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Introduction

SrcSH3 forms α-helix-rich intermediate in the early stage of its folding pathway¹). In this study, we calculated molecular structure of the intermediate from the X-ray solution scattering profile by GASBOR program²).

We also calculated molecular structure with atomic coordinate by SAXS_MD program³). 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 (Rg). Src SH3 forms non-native α-helix-rich folding intermediate. Rg of the intermediate is 18.5 Å, much smaller than that of the unfolded state (27 Å) and larger than that of the native state (14.6 Å)¹).

We calculated molecular shape from scattering intensity by GASBOR²) 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 program³) 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 θ222 at 4°C¹)

The upper curve (initial) was obtained by mixing the unfolded protein solution with the unfolded buffer.

References

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2) Svergun et al. (2001) Biophys. J, 80, 2946-2953

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