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# Conformational analysis of trigger factor and its mutant I. Kratky plot and Rg

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## Introduction

We have studied trigger factor (TF), and its mutants. TF is a 3-domain protein, namely composed of N-terminal domain, middle part domain and C-terminal domain. To investigate conformational analysis, We prepared wild type TF and its truncated mutants, called C419, C360, NM and MC. They mean: 13 and 72 residues from C-terminal are truncated, whole C-domain is truncated, and whole N-domain is truncated, respectively. The number of amino acids for TF, C419, C360, NM and MC are 432, 419, 360, 251 and 287, respectively.

## Result

SAXS experiments were performed at BL-15A with CCD detector. Kratky plots for all samples in the native condition are shown in Fig.1. Wild type TF has a clear peak and mutants, C419, NM and MC have broad peaks, while C360 shows no peaks. Apparently mutants take less stable forms, and C360 does not fold though it might take partially compact form. We also performed urea titration for TF, C419, C360 and MC to investigate transitions from the folding state to the unfolding state. All mutants are less stable in comparison with TF, judging from peaks of Kratky plots. The transition mid-points of TF, C419, C360 and MC were 2.05 M, 1.2 M, 0.37 M and 1.04 M, respectively. So the order of stability is: TF> C419> MC> C360. This result implies C-domain has important role to stabilize structure.

At the native condition, TF existed as a monomer, MC shows some aggregation, and C419, C360 and NM show strong aggregation in Guinier plot.

We calculated P(r) functions by GNOM program [1-4]. TF showed good coincidence with crystal data. For mutants, to obtain monomer component from scattering data, we used two-exponential equation,

$$I(h) = I_1 \exp(-\frac{R_{g1}^2 h^2}{3}) + I_2 \exp(-\frac{R_{g2}^2 h^2}{3})$$

where I is intensity, $R_g$ is radius of gyration and h is scattering vector. The smaller Rg gives the Rg of the monomer, while the larger one gives the average Rg of oligomers, if they are well separated (unpublished data). By subtracting oligomers part, we could calculate P(r) functions as monomer component. C360 showed two peaks in this function. Others showed single peaks.

Table.1 shows radius of gyration in the native state. Rg values from Guinier analysis were calculated by two-exponential equation, and values from Kratky plot were calculated by $R_g = \sqrt{3/h_p}$, where $h_p$ is the peak position of scattering vector $h$ [5]. In this experiment, as Kratky plots of mutants have broad peaks, $h_p$ values contain large errors. But they are not so much different with Guinier analysis.

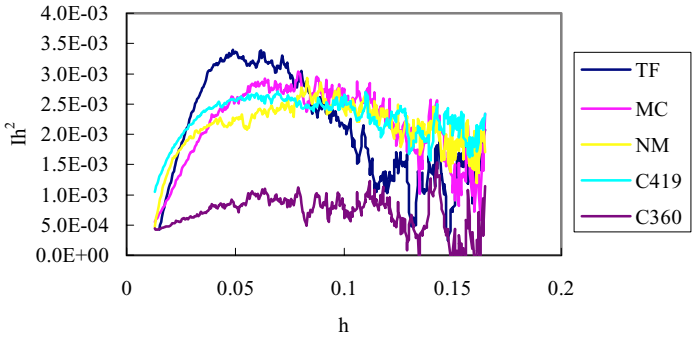


Fig.1. Kratky plot. All samples are in native condition

Table.1. Radius of gyration

| Protein | Kratky | Guinier |
|---|---|---|
| TF | 31.8 Å | 32.7 Å |
| MC | 22.2 Å | 26.8 Å |
| NM | 19.9 Å | 22.3 Å |
| C419 | 27.1 Å | 31.2 Å |
| C360 | 26.6 Å | 29.7 Å |

## References

[1] Svergun D.I. & Semenyuk A.V. (1987). *Doklady AN SSSR*, **297**,1373-1377 (in Russian)
[2] Svergun D.I., Semenyuk A.V. & Feigin L.A. (1988). *Acta Cryst.*, **A44**, 244-250
[3] Svergun D.I. (1991). *J.Appl.Cryst.*, **24**, 485-492
[4] Svergun D.I. (1992). *J. Appl. Cryst.*, 495-503
[5] G. Semisotnov *et al*. (2003) PF activity report.20,256

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