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SAXS study of Trigger Factor and its mutants

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Introduction

Trigger Factor (TF) is a 3-domain protein, taking a shape like crouching dragon at its native state[1]. The present study aims to investigate the solution structure of TF and the effect of C-terminal truncation to elucidate which part is important to the folding and which part is related to function. To this end, the wild type and 6 mutant proteins were constructed, namely C419, C389, C380, C360, NM and MC in which the C-terminal 13, 43, 52, 72 residues or the whole C domain or the whole N domain of TF were deleted, respectively.

Result

. We performed X-ray scattering experiments of the concentration dependence of TF and its mutants at its native state. Radius of gyration (Rg) were estimated from Guinier plot. They were much bigger than that calculated from the crystallographic data (36Å). In case of TF, MC, C360, C380, C389 and C419, Kratky plots show a peak, indicating them as compact, whereas the Kratky plot does not show a peak in case of NM. We then estimated Rg from the peak position of Kratky plot by using equation $R_g = \sqrt{3/h_p}$, where h_p is the peak position of scattering vector h, as far as the protein is folded[2] Obtained Rg are plotted in Fig. 2 as a function of protein concentration. Rg of TF thus estimated is 34.1Å, in good agreement with the one calculated from the crystallographic data. Rg of MC, C360, C380, C389 and C419 (at 0 mg extrapolated) were 29.4, 34.1, 32.3, 31.1, 29.4, respectively.

C360 shows Rg as large as TF, which suggests that the mutant C360 is less packed than TF and other mutants.

Reference

1) L. Ferbitz *et al*, (2004) Nature. 431, 590-596.

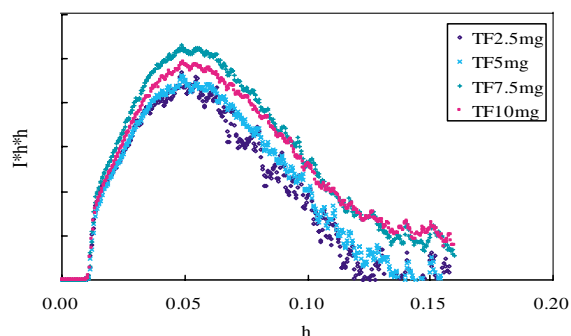2) G. Semisotnov *et al*. (2003) PF activity report.20, 256
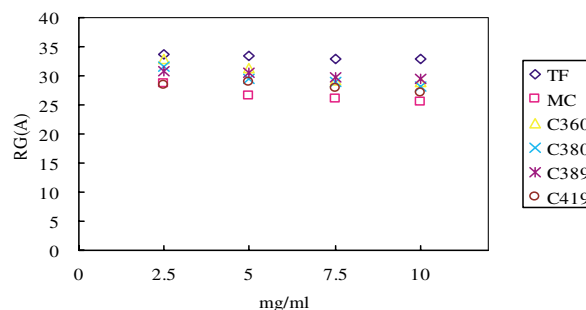
Fig1.   Kratky plot of TF at its native state

Fig 2.   Concentration dependence of Rg

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