

SAXS study of oligomeric state of metal-dependent novel peptidase - endolysin from bacteriophage T5

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1 Introduction

Endolysins are a group of proteins encoded by bacteriophages and destroying the peptidoglycan of bacterial cell wall in the final stage of lytic cycle of phage development. One of the interesting object is L-alanoyl-D-glutamate peptidase encoded by bacteriophage T5 (EndoT5). This protein is the main component of the lysis system of bacterial cell wall and it is necessary for phage burst from the cell. The endolysin of bacteriophage T5 was firstly cloned by authors in *E.coli* cells and was purified to homogeneous state [1]. The enzyme is Ca²⁺-dependent L-alanoyl-D-glutamate peptidase and belongs to the Zn-containing peptidase family of M15 family of MD. This is the first example of L-alanoyl-D-glutamate peptidase founded in virulent phage infecting the Gram-negative bacteria. The great number of diseases caused by Gram-negative bacteria (e.g., enterobiasis) permits to consider the endolysin as a potential bacteriolytic agent with narrow spectrum of action which is perspective in cases when the application of antibiotics is undesirable or ineffective. Here we present SAXS patterns of EndoT5 in the presence of EDTA and its complexes with Zn²⁺ and Ca²⁺.

2 Experiment

EndoT5 was prepared as described in [1]. The buffer 25 mM Tris-HCl pH 8.0, 150mM NaCl was used. To eliminate metal ions we used 1mM EDTA. To obtain complexes with metal ions we added to protein solutions 0.5 mM ZnSO₄, 10 mM CaCl₂, respectively. The protein concentration was 4.1 mg/ml. Synchrotron X-ray measurements were done on a small-angle camera BL-6A (Photon Factory, Tsukuba) using PILATUS 100K detector. The range of scattering vectors Q=0.01-0.25 Å⁻¹.

3 Results

SAXS pattern of EndoT5 with EDTA is presented in Fig.1. One can see that initial part of scattering curve plotted in the Guinier coordinates is nonlinear and it is difficult to evaluate radius of gyration (R_g) and

molecular mass (M_w) from I(0). Therefore we evaluated the above values from the distance distribution function P(r) calculated by GNOM program in the range of Q=0.02-0.25 Å⁻¹. It was found that R_g=(22,7±0,12) Å. It is too large value for the globular protein with M_w=15.3 kDa. We evaluated the protein volume from the Porod invariant and found V=38040 ±200 Å³ which is about twice larger V=18670 Å³ calculated from the partial specific value of EndoT5. It means that protein is preferentially in dimeric form in solution. The binding of Zn²⁺ practically does not change the SAXS pattern (see Fig. 1). Ca²⁺ ions possibly cause some dissociation of dimers.

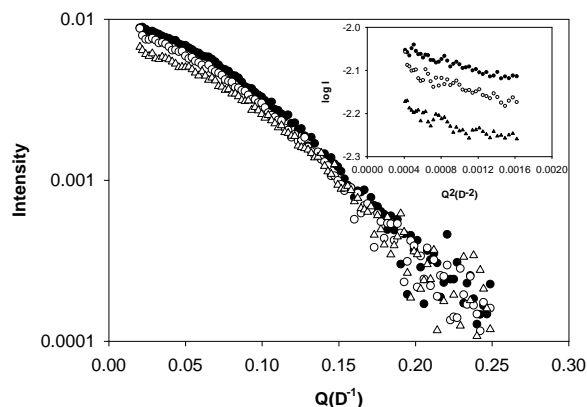


Fig.1: The dependence log I versus Q for EndoT5 with EDTA (filled circles), with ZnSO₄ (open circles) and with CaCl₂ (triangles). Inset: Guinier plot of initial part of SAXS patterns.

References

[1] G.V. Mikoulinskaia *et al.*, *FEBS J.* **276**, 7329 (2009).

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