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SAXS study of Y187N mutant of Bence-Jones protein BIF

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1 Introduction

Multiple myeloma nephropathy occurs due to the formation of different aggregates by immunoglobulin light chains (Bence-Jones protein) in kidneys. The mechanism of amyloid deposits formation is still unclear. Solomon et al. revealed in one of the patients amyloids consisted of constant domain (CL) of light chains. The analysis of aminoacid sequence of such protein (BIF) showed the change of Ser 177 to Asn. It was proposed that in many neurodegenerative diseases the key role in amyloidogenesis play proteins with Asn-rich regions. We decided to check this hypothesis and made the additional mutation Y187N. Here we present SAXS pattern of Y187N mutant of BIF at different ionic conditions simulating native pathway of this protein.

2 Experimental

We obtained recombinant Y187N mutant of BIF (M_w=25 kDa) and studied the process of fibril formation in two buffer systems reflecting environments within the nephron. Buffer 1: 50 mM Na-P, pH 7.2, 0.1 M NaCl. Buffer 2: 25 mM $NaHCO_3$+NaOH pH 10.1. The protein concentration was around 0.25 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 $Å^{-1}$. For better accuracy we evaluated radius of gyration (R_g) from the distance distribution function P(r) calculated by GNOM program [1] in the range of Q=0.01-0.15 $Å^{-1}$.

3 Results

SAXS experiments were carried out at small protein concentration due to high amyloidogenic properties of BIF and its mutants. Therefore one can see only overall dimensions and shape of particles. The obtained radii of gyration for Y187N in buffer 1 was Rg=(83.6±1.0) Å and in buffer 2 - Rg=(77.8±1.0) Å. It means that in both cases protein was oligomeric. We investigated the shape of oligomers plotting dependence logI-logQ (Fig. 1). One can see that in both cases we have linear dependence with incline 1.66 (correlation coefficient r=0.928) for buffer 1 and incline 1.04 (r=0.943) for buffer 2. It means that in buffer 2 the shape of oligomer is rod-like one and dendric elongated one in buffer 2.

These data are in agreement with our atomic force microscopy observations where we registered rod-like structure in buffer 2, and dendric elongated shape in buffer 1.

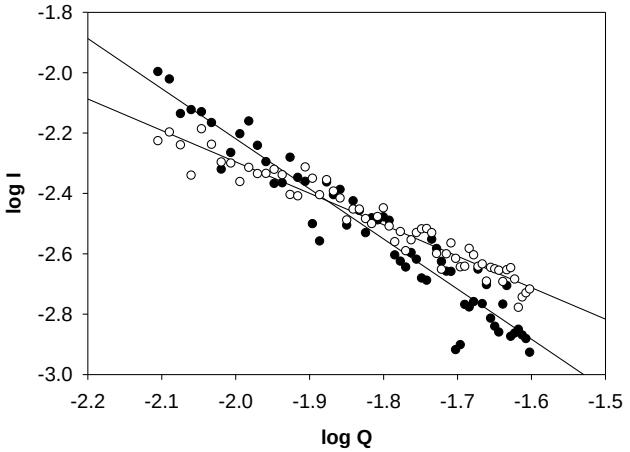

Fig.1 The dependence log I versus log Q for Y187N in buffer 1 (filled circles) and in buffer 2 (open circles).

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References

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