

SAXS study of Bence-Jones protein BIF at different ionic conditions.

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Introduction

Multiple myeloma (MM) is one of the incurable diseases. One of the severe complications of MM is a renal amyloidosis caused by Bence-Jones proteins fibril formation. Earlier it was thought that the key role in amyloid deposits formation belongs to the variable domain (VL) of light chains. Several years ago Bence-Jones protein BIF was found in the urine of one patient which amyloidogenity was due to the single change of Ser177 to Asn in a constant domain (CL) of light chains [1]. It was the first case of participation of CL of light chain in amyloid deposits formation. Here we present SAXS pattern of BIF at different ionic conditions simulating native pathway of this protein in kidney.

Experimental

We obtained recombinant protein 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: buffer 1+ 20 mM DTT (dithiothreitol). The protein concentration was around 0.35 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.

Results

SAXS experiments were carried out at small protein concentration due to high amyloidogenic properties of BIF. Therefore one can see the overall dimensions and shape of molecules. The obtained radii of gyration were $R_g=(55.2\pm 1.0)$ Å (for BIF in buffer 1) and $R_g=(114.7\pm 1.0)$ Å (for BIF in buffer 2). After incubation of BIF during 1 hour in buffer 2 at 37°C the value of R_g was (121.3 ± 1.8) Å. It means that in both cases protein is oligomeric. We investigated the shape of oligomers plotting dependence $\log I$ - $\log Q$ (Fig. 1). One can see that

in both cases we have linear dependence with incline 1.02 (correlation coefficient $r=0.978$) for buffer 1 and incline 3.42 ($r=0.916$) for buffer 2 at room temperature and 3.68 ($r=0.965$) at 37°C. It means that in buffer 1 shape of oligomer is rod-like one and granular one in buffer 2.

Perhaps the presence of DTT results in the destruction of filaments into granules. Incubation of protein with DTT at 37°C practically does not change the dimensions and shape of granular particles. These data are in agreement with our atomic force microscopy observations where we registered rod-like structure in buffer 1, and granular multimers in buffer 2.

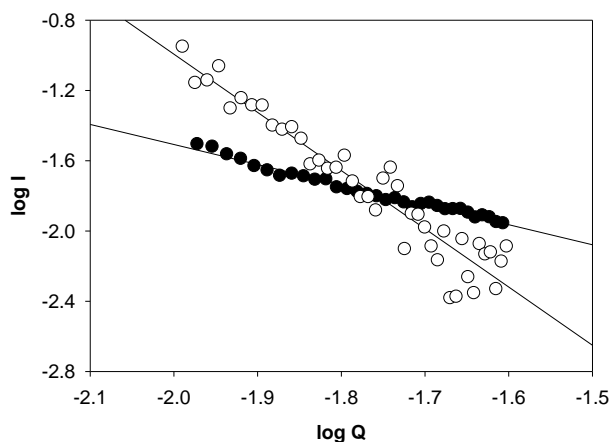


Fig.1 The dependence $\log I$ versus $\log Q$ for BIF in buffer 1 (filled circles) and in buffer 2 (open circles).

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References

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