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SAXS study of N177S mutant of Bence-Jones protein BIF

Alexander Timchenko[1], Maria Timchenko[2], Masaji Shinjo[4], Hiroshi Kihara[4-6*]

[1] Institute of Protein Research, Pushchino , Russia, 142290;
Institute of Theoretical and Experimental Biophysics, Pushchino, Russia, 142290;
[3]Department of Physics, Kansai Medical University, Hirakata, Osaka 573-1010, Japan
[4]SR Center, Ritsumeikan University, 1-1-1 Noji-Higashi, Kusatsu 525-8577, Japan
[5]Nagoya University SR Ressearch Center, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan
[6] Himeji-Hinomoto College, 890 Koro, Kodera-cho, Himeji 679-2151, Japan

Introduction

Multiple myeloma (MM) is one of the incurable diseases. One of the severe complication 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 a 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 amyloidogenity. Here we present SAXS pattern of N177S mutant of BIF proposed to be not amyloidogenic.

Experimental

We obtained recombinant protein BIF (M_w=25 kDa) and its analogue (N177S) with the substitution of Asn177 to Ser and studied the process of fibril formation in three buffer systems which were chosen to reflect environments within the nephron. Buffer 1: 50 mM Na-P, pH 7.2, 0.1 M NaCl. Buffer 2: 50 mM Na-P, pH 6.5, 0.4 M NaCl, 0.4 M urea. Buffer 3: buffer 1+ 20 mM DTT. The protein concentration was 0.37 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) and molecular mass (M_w) of protein from the distance distribution function P(r) calculated by GNOM program [1] in the range of Q=0.015-0.15 Å^{-1}.

Results

Unfortunately, due to highly amyloidogenic properties of BIF it is practically impossible to carry out SAXS experiments with it. Therefore we studied N177S in details. It was obtained for N177S in buffer 1 Rg=(62.8±0.6) Å, M_w=(1100±10) kDa; in buffer 2 Rg=(39.9±1.0) Å, M_w=(32±2) kDa; in buffer 3 Rg=(55.6±1.8) Å, M_w=(112±5) kDa. It means that in buffer 1 protein is oligomeric one. The addition of urea (buffer 2) causes breakdown of oligomers. The addition of DTT (buffer 3) greatly diminishes the oligomerization. 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 1.1 (correlation coefficient r=0.941) for buffer 3 and incline 2.0 (r=0.946) for buffer 1. It means that in buffer 1 shape of oligomer is flat-like one and rod-like one in buffer 3. These data are in agreement with our atomic force microscopy observations where we registered multimeric plane structure in buffer 1, and dendritic multimers in buffer 3 and small particles in buffer 2.

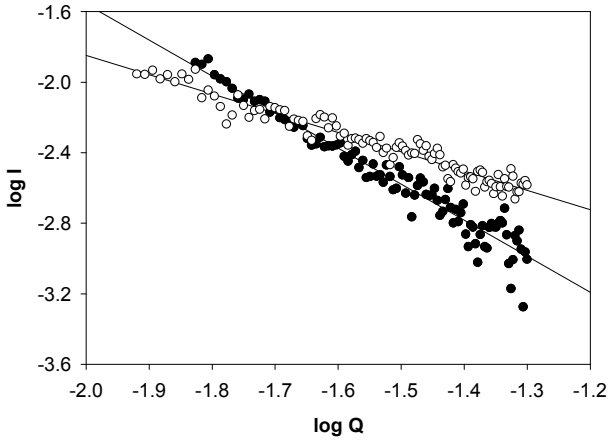

Fig.1 The dependence log I versus log Q for N177S in buffer 1 (filled circles) and in buffer 3 (open circles).

References

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*** E-mail:kiharah1234@gmail.com**