LS-4-P-1453 Atomic force microscopy investigation of amyloid fibril formation of Escherichia coli RNA polymerase σ(70) subunit

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σ70 subunit is a part of Escherichia coli RNA polymerase holoenzyme and plays a key role in transcription initiation. Using atomic force microscopy (AFM) imaging in different conditions (both in air and in liquid), we have found that this protein forms amyloid fibrils under a wide range of cationic concentrations including physiological ones. These fibrils have straight cylindrical shape based on a helical structure with diameter 5.4 nm and length from several tens nanometers up to several microns (figure 1). By the utilization of ultrafilters, which either allowed only σ70 monomers passing through or, oppositely, retaining them, we have proved that σ70 subunit aggregation is a spontaneous process and does not require any additional catalyst. To understand the mechanism of aggregation we have studied three mutant variants of σ70 subunit devoid the whole 1.1 region (N-terminus) or its part. All studied mutant proteins showed either the same or better ability to aggregate compared to the wild type of σ70 subunit. The obtained data allowed us proposing a model of σ70 subunit aggregation, which is based on the domain swapping mechanism accompanied by partial rearrangement of protein structure with subsequent intermolecular β-sheets formation due to the exposure of amyloidogenic regions. σ70 subunit of Escherichia coli RNA polymerase may serve as a good model object for studying amyloid fibril formation and searching factors influencing this process. Directed fibril formation can be also utilized in molecular architecture and other nanobiotechnology applications.

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Fig. 1: AFM height image of a σ\textsubscript{70} linear aggregate.