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C-terminal truncation of α -synuclein alters DNA structure from extension to compaction

Kai Jiang, Sandra Rocha, Ranjeet Kumar, Fredrik Westerlund^{**}, Pernilla Wittung-Stafshede^{*}

Department of Biology and Biological Engineering, Chalmers University of Technology, 412 96, Gothenburg, Sweden



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ABSTRACT

Parkinson's disease (PD) is linked to aggregation of the protein α -synuclein (aS) into amyloid fibers. aS is proposed to regulate synaptic activity and may also play a role in gene regulation via interaction with DNA in the cell nucleus. Here, we address the role of the negatively-charged C-terminus in the interaction between aS and DNA using single-molecule techniques. Using nanofluidic channels, we demonstrate that truncation of the C-terminus of aS induces differential effects on DNA depending on the extent of the truncation. The DNA extension increases for full-length aS and the (1–119)aS variant, but decreases about 25% upon binding to the (1–97)aS variant. Atomic force microscopy imaging showed full protein coverage of the DNA at high aS concentration. The characterization of biophysical properties of DNA when in complex with aS variants may provide important insights into the role of such interactions in PD, especially since C-terminal aS truncations have been found in clinical samples from PD patients.

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1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease that is estimated to affect 2% of the population older than 60 years. The main component of the intracellular inclusions (Lewy bodies and neurites) found in the brain of PD patients is α -synuclein (aS) protein in an aggregated form (amyloids) [1,2]. The protein is present mainly at synaptic terminals of the central nervous system [3,4] and is proposed to modulate neurotransmitter vesicle recycling [5–7]. The protein is monomeric at physiological conditions, but can aggregate into amyloid fibrils at pathological conditions, resulting in deposits as well as neuronal cell death. Mostly overlooked, aS is also present in the cell nucleus, which appears to be regulated by different factors, including post-translational modifications [8,9]. aS protein positioned in the nucleus can modulate the transcription level of a master mitochondrial transcription activator called PGC1 α [10], and aS may also affect repair of DNA [11]. When oxidized and bound to chromatin, aS is able to promote double-strand breaks in DNA and thereby induce cell death [12].

Since aS's physiological function(s) in the nucleus is not known, and whether aS binding to DNA is related to PD initiation, more investigations of aS interacting with DNA are needed.

We recently used nanofluidic channels to investigate how aS binding alters the physical properties of DNA [13]. Nanofluidic channels allow stretching of single DNA molecules by confinement and are perfectly suited for studying how proteins affect the physical properties of DNA [14]. We demonstrated that aS binding to DNA increases the persistence length of DNA [13]. The DNA-protein interaction appears driven by electrostatics and, since DNA is negatively charged, the interaction with the positively charged N-terminal domain of aS will determine the binding to DNA. The sequence of aS can be divided into three domains [15]: the N-terminal region, rich in positively charged residues (12 of a total of 16 positively charged residues) [15,16], the non-amyloid- β component (NAC) domain (residues 61–95), which forms the core of the amyloid fibril [4], and the C-terminus, which is rich in negatively charged residues (15 of a total of 24 negatively charged residues) and is proposed to be involved in protein-protein interactions (Fig. 1). C-terminal truncated variants are more prone to aggregation than full-length aS [17–21]. Importantly, C-terminal truncated variants of aS are found in the brain of both patients with PD and healthy individuals [22,23].

To address the role of C-terminal truncations on aS interaction with DNA, and based on our previous study [13], we applied the

^{*} Corresponding author.

^{**} Corresponding author;

E-mail addresses: fredrik.westerlund@chalmers.se (F. Westerlund), pernilla.wittung@chalmers.se (P. Wittung-Stafshede).

3. Results

Since aS binding to DNA is proposed to be governed in part by charge-charge interactions [13], we investigated whether the protein binding was affected by truncation of the aS C-terminal domain, which would remove negatively charged residues that are unfavorable for binding to the negatively charged backbone of the DNA. We studied two C-terminal truncated aS variants: one containing residues 1–97 (aS (1–97)) and another containing residues 1–119 (aS (1–119)), thereby removing 15 and 8 negatively charged residues from the full-length polypeptide, respectively.

First, we tested that the purified aS variants formed amyloids as expected. The aggregation kinetics of the two aS variants and full-length aS (aS (1–140)) were monitored immediately after gel filtration chromatography to ensure that only protein monomers were present at the starting point. The aggregation experiments were performed under agitation in the presence of a glass bead to increase the reproducibility [28]. Sigmoidal aggregation profiles were obtained for all variants (30 μ M, 37 $^{\circ}$ C, pH 7.4) in the presence of 150 mM NaCl with a lag phase of around 8 h for aS (1–140), 6 h for aS (1–119), and 3 h for aS (1–97) (Fig. 2A). Decreasing the ionic strength (thereby enhancing electrostatic effects) significantly slowed down the aggregation rate of aS (1–140) (Fig. 2B), but had no effect on the aggregation of aS (1–97) (Fig. 2C), supporting that the presence of negatively charged residues in the C-terminal domain delays aS (1–140) amyloid fibril formation. Importantly, these experiments showed that aggregation is slow (several hours of lag phase), even at conditions (glass bead and agitation) that favor aggregation. aS incubated without agitation does not aggregate at the conditions used here. Therefore, in the nanofluidic channel experiments, where the samples were mixed without agitation and measurements were performed within 2 h of sample preparation, amyloid formation of the aS variants is not expected.

To investigate individual DNA molecules upon binding of the aS variants, the experimental set up included 48.5 kbp λ -DNA (5 μ M base pairs) mixed with a range of aS concentrations (up to 40 μ M; providing 8:1 aS-to-base pair ratio at the highest protein concentration). For such mixtures, we determined the extension of individual DNA molecules in nanofluidic channels having a $100 \times 150 \text{ nm}^2$ geometry (Fig. 3A). To visualize the DNA, the well-characterized fluorophore YOYO-1 was used: it binds to DNA via intercalation between DNA base pairs [29]. The relative extension R_{II}/L (extension R_{II} normalized to the DNA contour length L) of DNA molecules increased with the protein:base pair ratio for full length aS (1–140) (as reported before [13]) and for aS (1119), but decreased in the case of aS (1–97) (Fig. 3B; examples in Fig. 3C). Importantly,

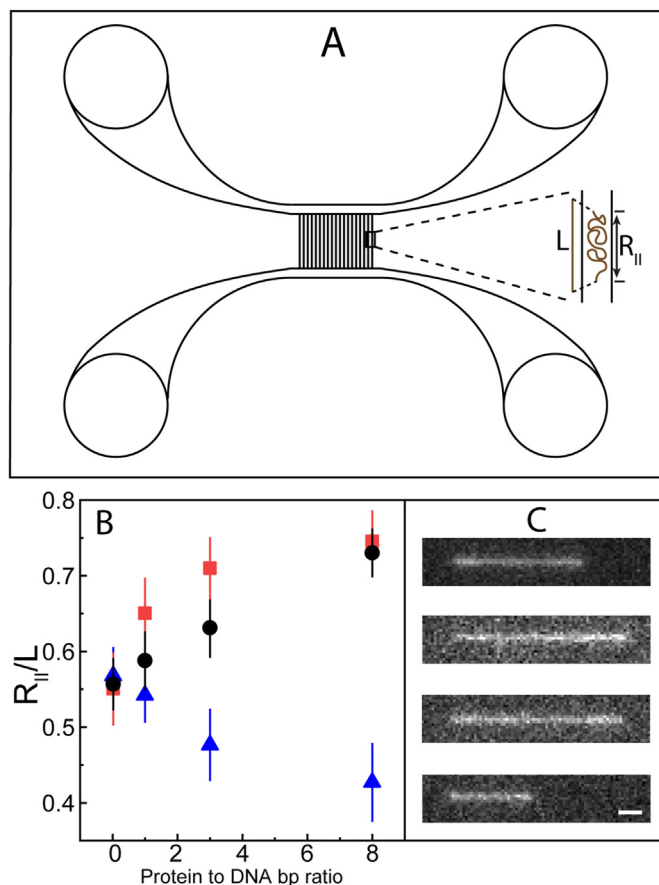


Fig. 3. Nanofluidic set up and results upon aS addition to DNA. (A) Schematic illustration of the nanofluidic chip design. The channel system comprises pairs of micro-channels, spanned by an array of nanochannels ($100 \times 150 \text{ nm}^2$). The cartoon shows a DNA molecule confined inside a nanochannel. DNA will be partially stretched along the nanochannel with an extension R_{II} shorter than its contour length L . (B) Relative extension R_{II}/L of λ -DNA in the presence of aS (1–140) (\bullet , black), aS (1–119) (\blacksquare , red) and aS (1–97) (\triangle , blue) in $1 \times \text{TE}$ buffer in $150 \times 100 \text{ nm}^2$ channels, versus the protein to DNA base pair (bp) ratio. (C) Representative fluorescence images of λ -DNA with aS variants. Top to bottom: bare DNA, DNA with aS (1–140), DNA with aS (1–119), and DNA with aS (1–97), each at a protein to base pair ratio of 8:1. The scale bar is 1 μm . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the changes induced by aS variants ($\sim 25\%$ compaction by aS (1–97) or $\sim 30\%$ extension by aS (1–140) and aS (1–119)) are minor when

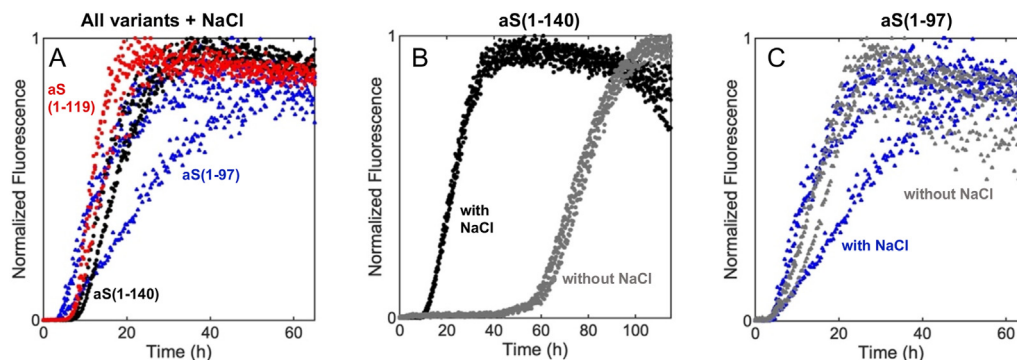


Fig. 2. Aggregation kinetics of aS variants incubated under agitation in the presence of a glass bead (37 $^{\circ}$ C, pH 7.4). (A) aS (1–140) (\bullet , black), aS (1–119) (\blacksquare , red) and aS (1–97) (\triangle , blue) samples with 150 mM NaCl. (B) aS (1–140) incubated in the absence (gray) and presence of 150 mM NaCl (black); (C) aS (1–97) incubated in the absence (gray) and presence (blue) of 150 mM NaCl. Three replicates are shown and the protein concentration was 30 μ M. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

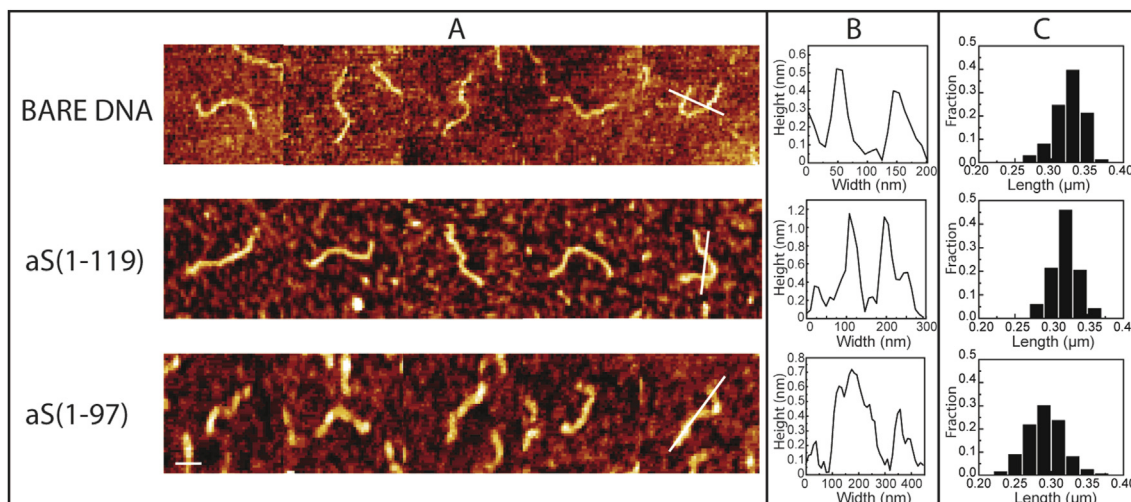


Fig. 4. AFM analysis of the binding of aS variants to DNA. (A) Tapping mode AFM images of bare DNA (1kbp, 5 μ M base pairs) and DNA with aS (1–119) or aS (1–97) at a protein concentration of 15 μ M (3:1 protein:DNA). The scale bar is 100 nm. The line in each panel corresponds to the height profile of the molecule shown in (B). The corresponding distributions of DNA contour length are shown in (C).

compared to other proteins that can induce full compaction of DNA [30,31]. Another observation is that, for all aS variants, it is possible to stain the DNA with YOYO-1 even though the DNA is fully coated with protein, suggesting that binding sites for YOYO-1 are still available on DNA.

As a complement to the nanochannel data, we turned to atomic force microscopy (AFM) analysis using shorter DNA (1kbp, 5 μ M base pairs) and the aS (1–97) and aS (1–119) variants at a protein:base pair ratio of 3:1 (Fig. 4A). We found that the DNA height increased in the presence of both aS variants, from approximately 0.5 nm for bare DNA to 0.7–1.0 nm in the presence of the protein and the DNA appears to be fully covered by the proteins (Fig. 4B). This is similar to what we found earlier for aS (1–140) [13]. Whereas the DNA contour length did not change upon aS (1–119) binding as compared to bare DNA, the DNA contour length decreased for aS (1–97) (Fig. 4C), which parallels the result of DNA compaction in the nanochannel.

4. Discussion

We here show that C-terminal truncation of aS can induce changes from extension (over 30% DNA length increase for aS (1–140) binding and aS (1–119)) to compaction (more than 20% DNA length decrease for aS (197) binding). Clearly, sufficient removal of negatively charged residues from aS changes the resulting structural consequences for DNA upon protein binding. The most important residues appear to be located between residues 97 and 119 in the sequence. It appears that upon full C-terminal truncation (43 residues), aS behaves as one expects for multivalent cation interactions, which often lead to compaction of DNA. However, we do not observe full compaction of DNA [30,31] suggesting that the aS-DNA interaction is relatively weak, as is also reflected by the high concentration of aS needed to saturate the effect on DNA. The differential result with aS (1–97) reinforces that the interaction between full-length aS and DNA includes other forces, such as hydrophobic and/or specific interactions. For a fully coated DNA it is also possible that protein-protein interactions between peptides next to each other affect the binding. For full length aS we concluded that the increase in DNA extension was due to an increase in DNA persistence length [13] and we propose a similar mechanism for aS (1–119).

Several proteins bind to DNA and affect its structure as a way to modulate activity, such as gene replication and transcription. For example, different bacterial nucleoid proteins (e.g., H-NS, Hfq, and HU) have been shown to specifically compact or extend DNA [32–34]. To better understand the role of aS-DNA interactions in PD, underlying molecular principles of such complexes need to be characterized. Several proteins and short peptides linked to other amyloidogenic disorders, including Alzheimer's, Amyotrophic lateral sclerosis (ALS) and prion diseases, exhibit nucleic acid binding abilities that may be of functional relevance [35]. Moreover, liquid-liquid phase separated biological systems often involve loosely associated -complexes (involving multivalent often electrostatic interactions) between amyloidogenic proteins and RNA [36,37]. Single DNA molecule techniques, such as the approach used here, may be useful to characterize putative nucleic-acid binding of many other proteins involved in both function and disease.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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