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Communication

Remodeling of the Conformational Dynamics of Noncanonical DNA Structures by Monomeric and Aggregated α-Synuclein

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ABSTRACT: Research on Parkinson's disease most often focuses on the ability of the protein α-synuclein (α-syn) to form oligomers and amyloid fibrils, and how such species promote brain death. However, there are indications that α-syn also plays a gene-regulatory role in the cell nucleus. Noncanonical tetrahelical nucleic acids, G-quadruplexes (G4Q), and i-motifs have been shown to play an important role in the control of genomic events. Using the conformation-sensitive single-molecule Förster resonance energy transfer technique we show that monomeric and oligomeric α-syn affect G4Qs and i-motifs in a different way and lead to remodeling of their conformational substates. Aggregated α-syn destabilizes the G4Q leading to unfolding. In contrast, both monomeric and aggregated α-syn enhance folding of the i-motif sequence of telomeric DNA. Importantly, macromolecular crowding is able to partially rescue G4Q from unfolding.

Aggregation and fibrillation of neuronal proteins are one of the hallmarks of neurodegenerative diseases.[1,2] The formation of such aggregates induces Parkinson's disease (PD), Alzheimer's disease, and multiple system atrophy.[3−6] Proteins playing a role in these diseases are prion proteins, tau as well as intrinsically disordered proteins (IDPs) like the small 14 kDa α-synuclein (α-syn), which can induce formation of neurotoxic Lewy bodies or fibrils.[7,8] Besides apoptosis, Lewy body aggregates can also reduce formation of dopamine containing vesicles, in that way changing brain activity on a neuronal level.[1,9] Nuclear fractions of human dopaminergic neuroblastoma cells contain monomeric and oligomeric forms of α-syn, implicating its significant biochemical role in the nucleus.[10−13] Furthermore, monomeric α-syn is known to enhance repairing of double-strand breakage of DNA, potentially rescuing neurons from an apoptotic pathway.[7] An aggregation-induced decrease of the α-syn monomer concentration is suspected to raise the death rate of neurons.[7] The toxic aggregated form of α-syn detected in PD affected brain was also found to have a higher propensity to interact with DNA.[12,14,15] Cherny et al. showed that α-syn interacts with histone-free transcriptionally active DNA segments and reduces transcriptional activity of some genes which respond to environmental stimuli.[16] Therefore, studying the conformation of DNAs in the presence of aggregated α-syn has immense importance as the interaction with aggregated α-syn forms can significantly affect DNA replication and transcription along with hastening accumulation of DNA damage.

Noncanonical DNAs play important roles in many biological processes like transcription, translation, and replication.[17−19] Among different noncanonical DNAs, guanine-rich (G-rich) sequences, which fold into tetrameric structures, denoted G-quadruplexes (G4Q), have been well characterized in recent years.[20−25] On the contrary, cytosine-rich (C-rich) sequences, which form intercalated structures known as i-motifs, are still less explored (Figure 1).[21,26] Both structures are observed in the telomeric region of chromosomes.[27] Such noncanonical DNA motifs have also been recognized in connection with

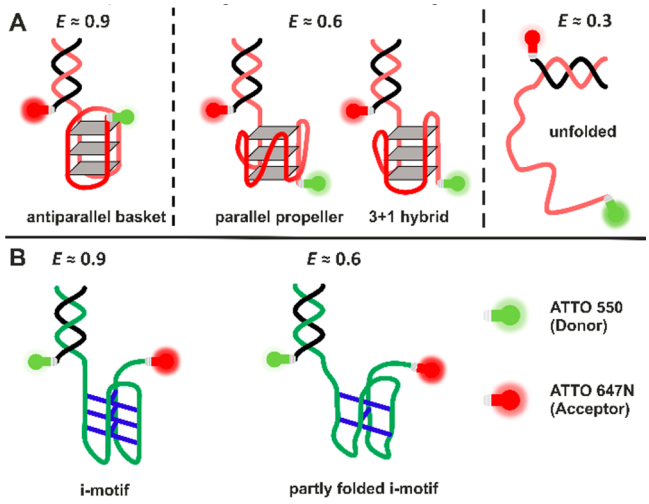

Figure 1. Schematic representation of the different conformations observed for the telomeric G4Q (A) and i-motif (B). G4Qs are stabilized through cyclic Hoogsteen hydrogen-bonding arrangement of 4 guanines with each other and by interactions between cations and the O-6 lone-pair electrons of each guanine. Formation of i-motifs is facilitated by hemiprotonation of cytosines and formation of cytosine−cytosine$^+$ base pairs.[17−19]

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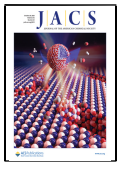

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tumor formation due to their role in promoter regions of different oncogenes.[28−31]

Owing to the lack of a molecular-level understanding of the interaction of α-syn in its native and aggregated state with such noncanonical chromosomal DNA sequences, we carried out corresponding conformation-sensitive single-molecule Förster resonance energy transfer (smFRET) experiments. This method avoids ensemble averaging, which enabled us to elucidate the conformational dynamics of noncanonical DNA structures and how they are affected by the interaction with monomeric and aggregated α-syn. We utilized two different DNA constructs, a telomeric G-quadruplex (hTel G4Q)[24,32] of sequence $(GGGTTA)_3GGG$ combined with a short double-strand part for the second FRET label, and its complementary i-motif (hTel-i-Mot) of sequence $(CCCAAT)_3CCC$ and the same double-strand part for the second FRET label (Figure 1). The α-syn was used at concentrations estimated to be present in brain cells.[33]

G4Q structures, including hTel, are known to take up several conformations. Depending on monovalent cation type, salt, and osmolyte concentration, as well as on physical parameters like temperature and pressure, antiparallel, parallel, and hybrid conformations have been observed (Figure 1A).[24,34,35] Similar studies have been carried out to uncover the conformational space of the hTel-i-Mot sequence, finding a folded i-motif at a low pH of 5, while at neutral pH either random coil or intermediate i-motif structures are suggested (Figure 1B). To mimic cellular crowding effects, measurements have also been carried out in the presence of a macromolecular crowding agent, Ficoll PM 70. For details of sample preparation and technical aspects, please refer to the Supporting Information (SI).

At pH 7.5, the FRET efficiency histograms of the G4Q display two FRET distribution peaks. We assigned the peak at $E \approx 0.9$ in the *E*-histograms to an antiparallel conformation and that at $E \approx 0.6$ to a parallel or hybrid conformational state. In the presence of α-syn aggregates, a low FRET efficiency peak emerges at $E \approx 0.3$, which corresponds to the unfolded conformation of the G4Q.[36,37] Figure S1 shows that the conformational states of the G4Q remain unaltered in the presence of monomeric α-syn even up to a concentration as high as 200 μM. On the contrary, in the presence of aggregated α-syn, a drastic change of the *E*-histogram is observed (Figure 2A), reflecting marked changes in the population of conformational states. Analogous measurements were carried out with a G-quadruplex construct of the c-Myc oncogene promoter region (c-MycG4).[21] The effects of monomeric and aggregated α-syn on the parallel c-MycG4 structure were similar, but less prominent, probably owing to its higher stability (Figure S9; for details please refer to the paragraph in the SI). From dynamic light scattering experiments, the size of the oligomers of α-syn added has been determined to vary between about 20 and 400 nm with a maximum at about 130 nm (Figure S3). With increasing concentration of aggregated α-syn, the population of unfolded states ($E \approx 0.3$) increases gradually. From 0 to 100 μM aggregated α-syn, the population of unfolded states increases from ∼5% to ∼60% at the expense of the antiparallel conformation whose population is decreasing from ∼80% to ∼20%, keeping the minor population of the parallel conformation (∼25%) essentially unchanged (Figure 2B).

As the conformational dynamics of biomolecules may get significantly altered in the crowded *in vivo* environment of the

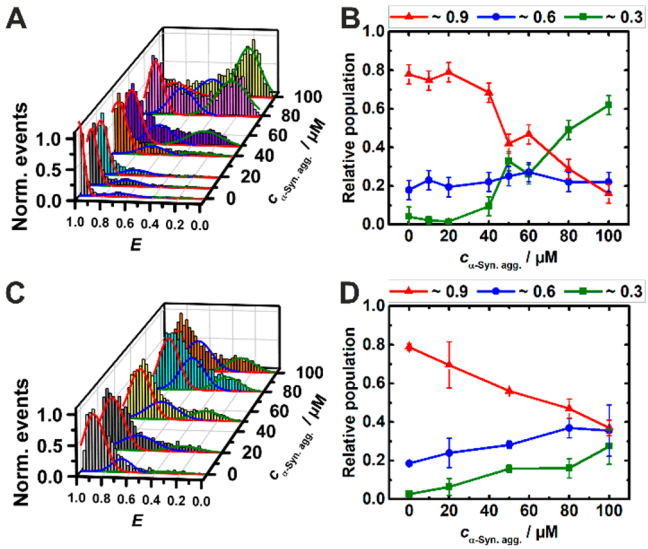

Figure 2. FRET efficiency (*E*) histogram (A) and relative population (B) of G4Q in buffer with increasing concentration of α-syn aggregates. The G4Q concentration was ∼50 pM. Solutions measured in (C) and (D) contained additional 30 wt % Ficoll. The colors of the relative population of conformers correspond to the Gaussian peaks in the *E*-histograms (red: antiparallel, blue: parallel, green: unfolded).

biological cell when compared to that in dilute buffer medium,[37−42] we have also studied the effect of macromolecular crowding on the conformational landscape of these two noncanonical DNA structures in the absence and presence of monomeric and oligomeric α-syn. Figure 2C reveals that the population of conformational states of the G4Q is significantly modulated in the presence of the crowding agent. Addition of 30 wt % Ficoll in the 100 μM α-syn aggregate solution markedly decreases the population of unfolded states of the G4Q, from ∼60% to ∼25%, which is accompanied by a concomitant enrichment of the antiparallel and parallel conformations (Figure 2B and D). This is to say, the crowder is able to largely rescue the folded conformations of the G4Q in the presence of toxic α-syn aggregates.

Generally, telomeric DNA duplexes are found to coexist with G-quaduplexes and i-motifs at acidic pH, and with G-quadruplexes and a (partially or fully) unfolded, random coil-like C-rich strand (C-coil) at neutral pH.[42] However, C-rich DNA sequences may also form stable i-motifs at neutral pH in the nuclei and in crowded environments. Quite unexpectedly, compared to the G4Q, the effect of α-syn on the structure of the complementary i-motif sequence differs entirely for both the monomeric and the aggregated state of the protein. Monomeric α-syn induces formation of the i-motif in its folded conformation at pH 7.5, whereas the i-motif is essentially partially folded only in pure buffer at this pH (Figure 3A). The relative population of the folded conformation increases from ∼5% to ∼30% upon addition up to 200 μM monomeric α-syn (Figure 3B). A similar scenario is observed upon addition of the oligomeric protein; here, the conformational transition occurs at much lower concentration (∼100 μM), however (Figure 3C and D). In the presence of the crowding agent, a similar behavior is observed (Figure S2), which implies that α-syn and Ficoll act synergistically in stabilizing the folded structure of the i-motif. Physiological NaCl concentrations lead to no signifiacnt changes of this scenario (see Figures S7 and S8).

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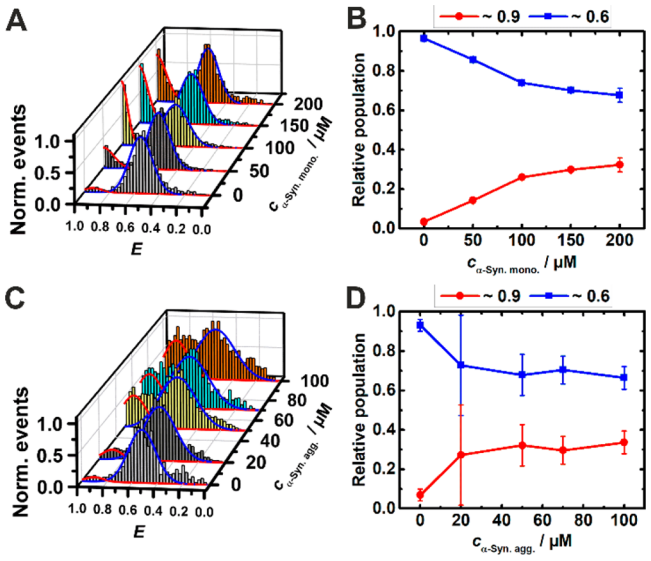

Figure 3. FRET efficiency (E) histogram (A) and relative population (B) of i-motif conformers in buffer with increasing concentration of α-syn monomers. Histogram (C) and relative population (D) of i-motif conformers in buffer with increasing concentration of α-syn aggregates. The i-motif concentration was ~50 pM. The colors of the relative population of conformers correspond to the Gaussian peaks in the E- histograms (red: fully folded, blue: partly folded).

There is clear evidence that positively charged intrinsically disordered proteins like α-syn can form high-affinity complexes with nucleic acids.[44−46] α-Syn is a natively disordered protein under physiological conditions and composed of three distinct regions: an N-terminus that is overall positively charged (+4), a central hydrophobic region so-called NAC (non-Aβ component), which confers the fibrillation potential, and a carboxyl terminus that is highly negatively charged (−12) due to the predominance of negatively charged Asp and Glu residues.[47,48] DNA molecules are highly negatively charged on their surface as they are laced with phosphate groups. They can electrostatically interact with the positively charged epsilon amino group of lysine residues located predominantly in the N-terminal and partly in the central region of the α-syn sequence in a nonspecific manner.[49] α-Syn aggregates, stabilized by intermolecular β-sheet formation, may have a higher exposed net positive charge, fostering the interaction with DNA.[50] Such a view would also be in accordance with the differential binding pattern of HEWL (hen egg white lysozyme) and fibrillar HEWL toward alteration of both DNA and RNA conformations, suggesting also that these nucleic acid−protein interactions are nonspecific in nature.[50] Control measurements using monomeric and aggregated HEWL (Figures S7 and S8) are in line with this interpretation. However, the molecular picture of the nature of interactions between such protein aggregates and polyanions is still uncertain.

In the present study, we have seen that monomeric and aggregated α-syn interact quite differently with the different noncanonical DNA structures. At neutral solution conditions, the G4Q is structurally more stable than the i-motif form, which essentially takes on the partially folded state at neutral pH. Thus, monomeric α-syn is unable to alter the conformation of the G4Q but remodels the conformation of the i-motif. However, the codeposition of protein molecules in the form of oligomeric species with a tentatively higher exposed net charge of the aggregated protein ensemble is able to perturb the conformation of G4Q, leading to unfolding of the quadruplex (Figure 4). For such rigid oligomers, the

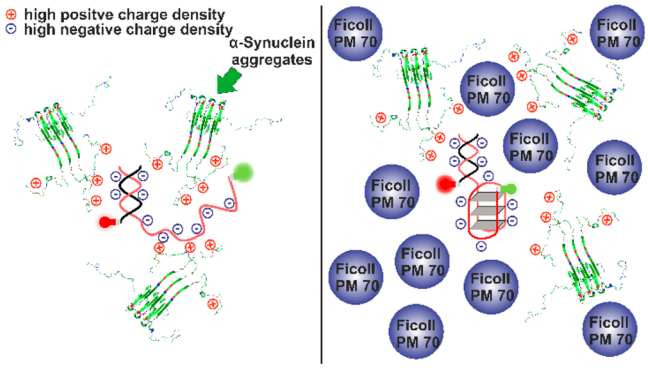

Figure 4. Schematic representation of the interaction of oligomeric α-syn with the telomeric G4Q in the absence (left) and presence (right) of the polymeric crowding agent Ficoll PM 70.

entropy penalty to pay for the interaction with the DNA is also expected to be lower compared to the monomeric α-syn with its random coil-like structure. On the contrary, in the case of the i-motif sequence, α-syn reduces the folding free-energy barrier and shifts the equilibrium toward the folded conformation of the i-motif sequence.

Macromolecular crowding is expected to modulate the energy and conformational landscape of biomolecules, generally favoring more compact conformations by the excluded volume effect.[39−41] The extent of exposed solvent accessible surface area is reduced as compared to that in buffer, thereby entropically stabilizing the compact state and shifting the equilibrium toward the folded conformation. High crowder concentrations (30 wt % Ficoll), mimicking the intracellular milieu, result in a strong excluded volume effect, which impacts the conformational dynamics of the noncanonical DNA structures and leads to stabilization of the compact conformation of the G4Q even in the presence of the deteriorating effect of aggregated α-syn. On the other hand, it drives the coiled C-motif to its folded i-motif conformation.

To conclude, for a long time overlooked, α-syn has been found to be present in the cell nucleus, suggesting that it may have additional roles in PD that are related to nuclear gene regulation. Noncanonical DNA structures like G4Qs work closely with several proteins to modulate cellular processes and are known to strongly affect gene expression. Through single-molecule FRET experiments we could show that α-syn at physiologically relevant local concentrations affect single noncanonical DNA structures in a sequence-specific way. Oligomeric α-syn destabilizes the G-quadruplex leading to unfolding. Conversely, the conformational landscape of the i-motif is remodeled, leading to an increase of folded i-motif structures. As G-quadruplexes play a major role as on-and-off switches that modulate polymerase activity, interaction with aggregated α-syn may alter expression profiles of disease-modifying genes with severe pathological consequences.

■ ASSOCIATED CONTENT

Supporting Information

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Experimental procedures of the smFRET, DLS and CD measurements, sample preparation, and further data (PDF)

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Notes

The authors declare no competing financial interest.

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