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Single-Molecule FRET Reveals Structural Heterogeneity of SDS-Bound α-Synuclein

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The intrinsically disordered protein α-synuclein (αSyn), involved in the etiology of Parkinson's disease, adopts multiple conformations depending on the environment, binding to targets, and aggregation state. Free in solution, it has random-coil-like conformations,[1] but adopts α-helical structures upon binding to negatively charged membranes.[2,3] When bound to SDS micelles, αSyn folds into a horseshoe conformation with two antiparallel helices,[4] a conformation that has been confirmed in the vesicle-bound state.[5,6] In the fibrillar state, a rigid cross-β-structure is prominent,[7] likely lining up the fibril core. The versatility of conformational plasticity might reflect an important biological role. Although its exact function remains obscure, αSyn has been associated with dopamine neurotransmission and regulation of the synaptic vesicular pool.[8] Furthermore, it has been suggested that it acts at the presynaptic membrane interface.[9] Recently, it has been shown that αSyn can adopt multiple folded states with different fractions of α-helical content upon interaction with SDS molecules of either a monomeric or micellar nature.[10]

We have used single-molecule Förster resonance energy transfer (SM-FRET) to investigate the structural architecture of αSyn along the trajectory of SDS-induced partially folded conformational species. The FRET-efficiency distributions obtained reflect the conformational heterogeneity of the resulting species. Analysis of the SM-FRET data reveals the existence of two distinct subpopulations within the range of SDS-induced conformations, suggesting an all-or-none folding transition. The N-terminal domain of αSyn, containing several imperfect repeats, is involved in the membrane binding process.[2] We therefore engineered a variant of αSyn with two cysteines in the putative membrane-binding domain (amino acids 9 and 69). The cysteines were labeled with donor and acceptor dyes suitable for SM-FRET (see the Supporting Information). FRET-efficiency (E_{obs}) histograms of 100 pM Alexa Fluor 488- and Alexa Fluor 568-labeled αSyn-9C/69C at increasing concentrations of SDS are presented in Figure 1. Initially, without SDS, αSyn adopts conformers that result in an E_{obs} centered at 0.54 (first panel, Figure 1). At low SDS concentrations (up to ~0.5 mM) no apparent changes in the histograms were observed. However, upon increasing the SDS concentration from 0.5 to 1.0 mM, a clear second distribution centered at E_{obs} ~0.82 and of smaller width appeared (peak 2). The higher E_{obs} value is indicative of a population in which positions 9 and 69 are closer together. The area of the first peak decreased concomitantly with the rease in area of the second. This observation suggests that SDS-induced structural changes in αSyn result in one or other of the conformers, at least within the 1 ms timeframe of the experiment. At even higher SDS concentrations (1.5–10.0 mM), the first distribution completely disappeared. Remarkably, above 1.5 mM SDS, although the mean value of the peak did not alter significantly, the width decreased further by ~15% (with an error in the FWHM below 5%; Figure 2A); this suggests a further stabilization of the structure resembling the horseshoe conformation that has been structurally resolved with NMR[4] (see Figure 3).

The peak positions and relative areas obtained with SM-FRET (Figure 2) corresponded very well with the increase in α-helix content, as judged from CD measurements with a protein concentration 5 orders of magnitude higher (Figures 4A and S3), thus confirming the findings for SDS-induced structural alterations in wild-type αSyn.[10]

Taking into account the reported value (62 Å) for the Förster distance of the dye-pair used and the E_{obs} value at [SDS] > 1.5 mM, the most frequently found distance between the two dyes in the SDS-bound state can be estimated at 45 Å, as obtained from the mean E_{obs} value for peak 2 in the histogram. This value is higher than the 32 Å distance between amino acid positions 9 and 69 obtained from either NMR[4] or EPR.[5] One should keep in mind, however, that the labels have ~10 Å linkers between the maleimide and fluorophore moieties (Figure 3), and that the observed distance is the distance between the centers of the two fluorophores. Since the exact orientation of the dyes with respect to αSyn bound to the SDS micelle is not known, it is in this case not possible to translate the observed distance to topological distance information within the αSyn molecule.

It has been shown that αSyn in solution does not behave as a fully random-coil protein, that is, residual structure appears to be present in the polypeptide chain.[2,11] Considering the histograms of αSyn free in solution without SDS, one would perhaps expect a more narrow distribution for an unfolded, random-coil-like protein with very fast folding transitions.[12] The width of the histograms in the case of αSyn could point to the presence of some residual structure within αSyn, with a limited set of conformers. However, as has been remarked, care should be taken in interpreting the distribution widths as they are highly dependent on the timescale of chain motions relative to the observation time of each molecule.[13–17] A similar explanation might hold for the apparent broadening of the first peak in the histograms at lower SDS concentrations. The overall trend in broadening (an increase in the FWHM of 35%) holds up to at least 0.8 mM SDS, at which point a significant

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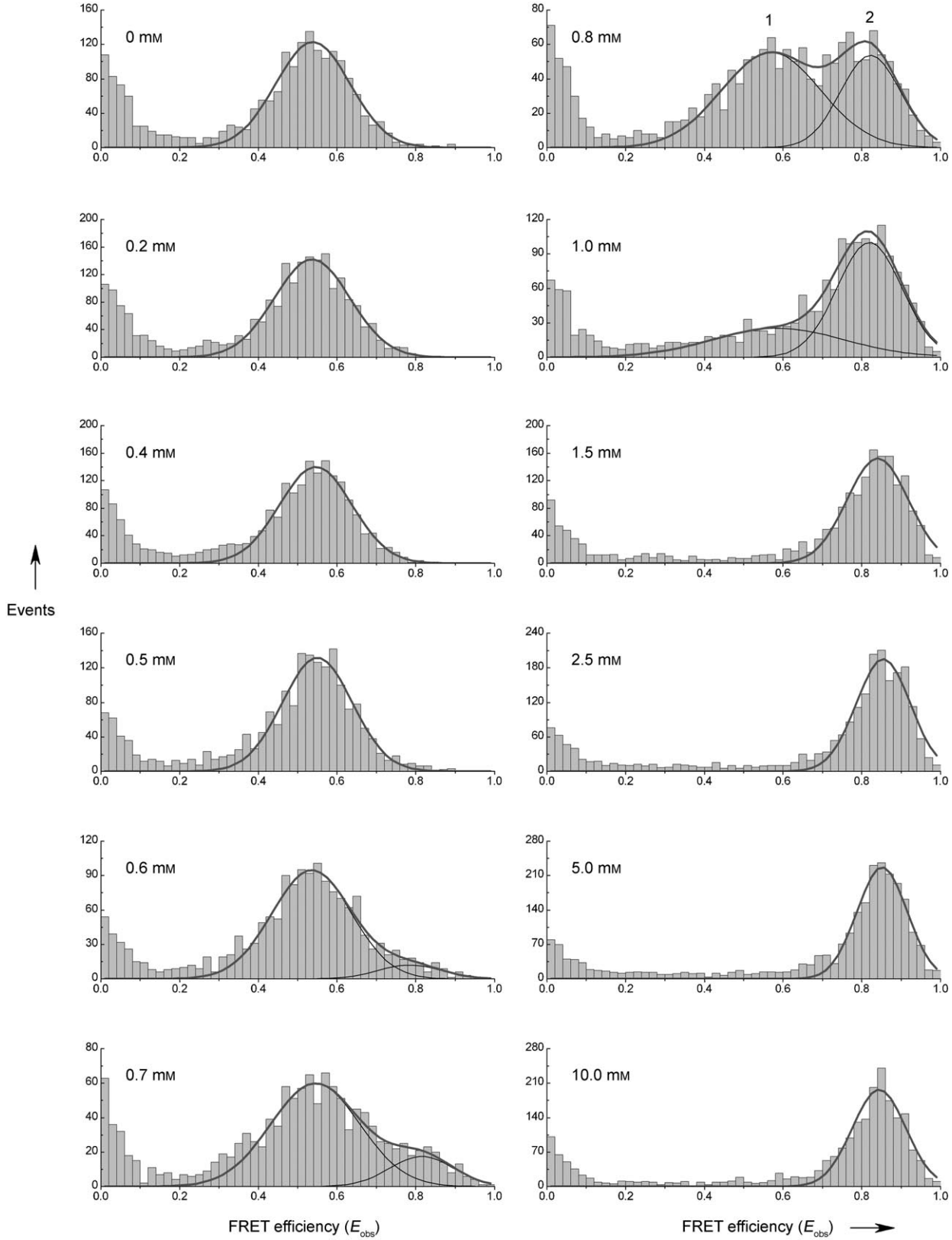

Figure 1. FRET-efficiency histograms of αSyn-9C/69C as a function of SDS concentration. Solid lines represent Gaussian fits; where applicable individual Gaussians are shown for peaks 1 and 2.

portion of the data points still originate from peak 1, and the errors in the FWHM of peak 1 are below 16%. (For higher SDS concentrations, the errors in FWHM of peak 1 were too high to allow a statistically valid conclusion about peak broadening.)

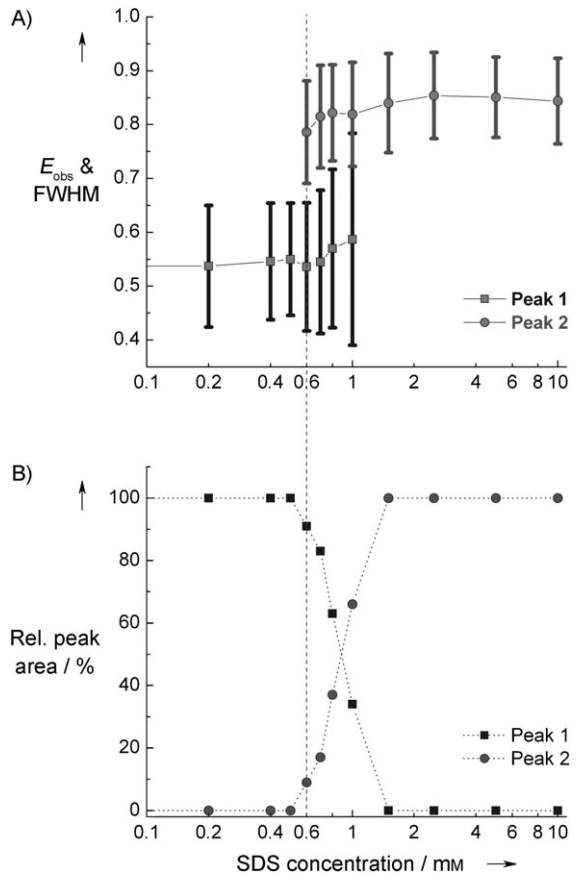

Figure 2. SM-FRET fitted parameters as a function of SDS concentration. A) E_{obs} values for peak 1 (■) and peak 2 (●), FWHM values (vertical bars) and B) relative peak areas of the Gaussian fits for peak 1 (■) and peak 2 (●).

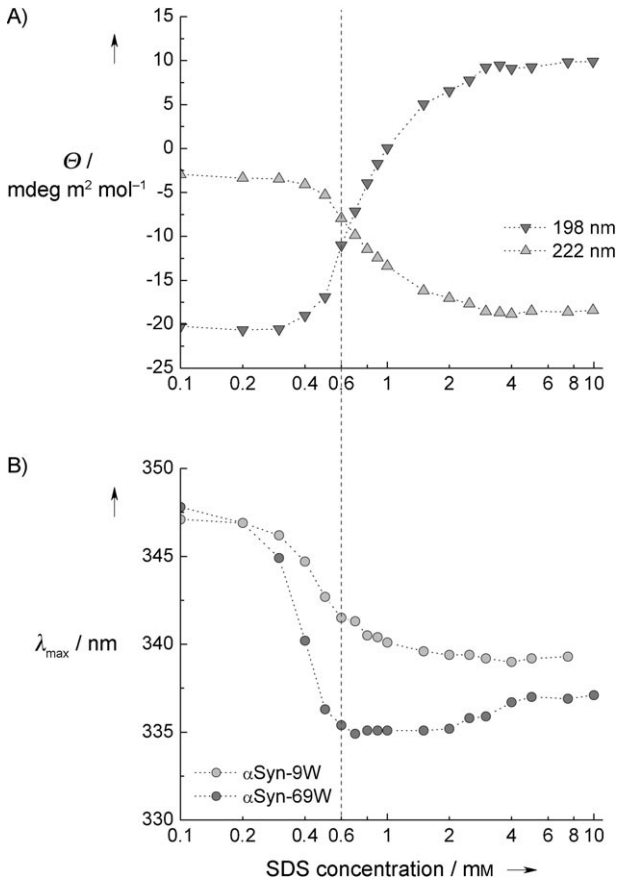

Figure 4. CD and tryptophan fluorescence emission as functions of SDS concentration. A) Ellipticity of αSyn-9C/69C at 198 (▼) and 222 nm (▲). B) Fluorescence emission maxima, λ_{max}, of single-Trp mutants αSyn-9W (●) and αSyn-69W (●).

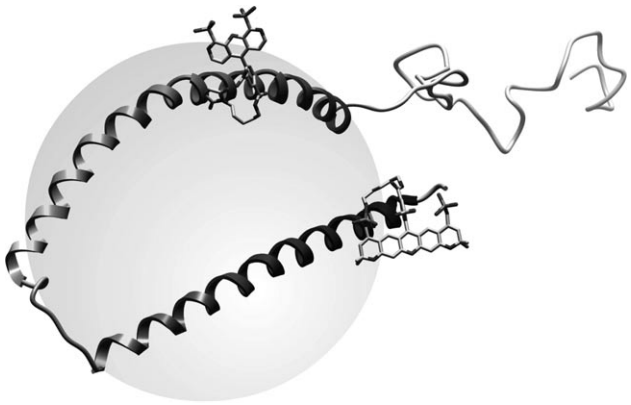

Figure 3. Representation of AF488- and AF568-labeled αSyn-9C/69C bound to a SDS micelle. The two putative membrane-binding helices are shown together with the unstructured C-terminal tail.

Furthermore, the average E_{obs} values did not shift significantly in this SDS concentration regime. Thus, broadening could point to an increased heterogeneity and/or altered flexibility within this population by binding of SDS monomers to the polypeptide chain. However, the broadening could also arise from slower conformer interconversion or chain stiffening caused by SDS binding, especially since the average E_{obs} value did not shift. Although techniques that can resolve faster timescales will be necessary to elucidate the detailed mechanism, the observed broadening does indicate dynamic structural alterations within αSyn induced by selective SDS binding.

Although SDS is not a chaotropic salt, it is well known for its ability to disrupt structure within globular proteins by the addition of negative charges to the polypeptide that induce unfolding by electrostatic repulsion. αSyn, on the other hand, with its amphipathic motif in the N-terminal region,[1] adopts a horseshoe-like structure upon binding to SDS micelles[4] and large unilamellar vesicles.[5] Closer inspection of the data in Figures 2 and 4 suggests that the appearance of peak 2 in SM-FRET occurs just at the onset of α-helix formation measured with CD. This is a strong indication that peak 2 contains the conformers with high α-helical content, while peak 1 represents largely unstructured conformers. Furthermore, peak 2 very likely represents the horseshoe conformation,[4] as judged from the high mean E_{obs} and the width corresponding to the state bound to fully formed micelles at much higher SDS concentrations.

The existence of two peaks within the narrow SDS-concentration regime (~0.5–1.0 mM) suggests that αSyn is able to adopt metastable structures through some sort of all-or-none mechanism for structural rearrangement (on the timescale of

the experiment). Even more surprising is that these transitions occur below the critical micelle concentration (CMC) of SDS (6.5 mM, as determined with isothermal titration calorimetry (ITC), Figure S4), as has been reported before.[10] Although ITC indicated that the CMC is unaffected by small additions of the protein,[10] we hypothesize that αSyn may still locally induce micelle formation at such low amounts that it is not obvious from the ITC measurements. Interestingly, tryptophan residues engineered at positions 9 and 69, probing local polarity, also displayed a dependence on the SDS concentration. However, the blue-shifts of the Trp fluorescence approached their maxima at ~0.6 mM SDS (Figure 4B), just at the onset of α-helix formation measured with CD and the appearance of peak 2 in SM-FRET (dashed vertical lines). Thus, below the apparent CMC of SDS, Trp residues report apolar environments very likely arising from the apolar hydrocarbon tails of SDS and suggesting either micelle formation or at least some sort of SDS encapsulation or binding around the Trp. It is interesting to speculate whether this putative micelle formation is induced by αSyn. Once SDS monomers were bound (blue-shift) and when α-helical structure formation was induced (increase in ellipticity), the second distribution became prominent (SM-FRET). Although analysis of more double-cysteine and single-Trp mutants will be necessary to probe if these transitions occur along the whole peptide, it is interesting to speculate whether these apparent sharp transitions also occur in vivo and what their role may be.

In conclusion, the SM-FRET approach in combination with ensemble CD and Trp fluorescence spectroscopy enabled us to discriminate two apparent states of αSyn and to analyze these conformers in terms of distribution and heterogeneity, information that is otherwise difficult, if not impossible, to extract solely from ensemble measurements.

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