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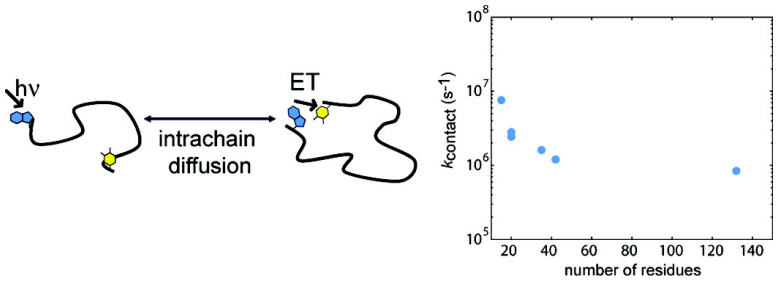

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Tertiary Contact Formation in α-Synuclein Probed by Electron Transfer

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Natively unfolded polypeptides pose tremendous challenges to the traditional structure−function approach of biochemical science.[1,2] The main protein component of the β-sheet-structured amyloid deposits found in Parkinson's disease (PD),[3,4] α-synuclein (α-syn), which appears to be unstructured in vitro,[5] becomes highly helical[6−8] in the presence of acidic micelles or phospholipid vesicles, suggesting that its interactions with membranes may be important for function.[9,10] Large environmentally induced conformational changes may hold the key to α-syn pathology; one or more of these species could disrupt cellular function, which could lead to disease.

Fluorescence energy transfer (FET) kinetics measurements can be used to define distributions of donor−acceptor (***DA***) distances ($P(r)$) in biopolymers.[11−14] Our FET studies of α-syn have revealed that the protein adopts a distribution of conformations with substantial population of extended structures at physiological pH.[15] As the lifetimes of tryptophan singlet excited states (^{1}W*) are substantially shorter than the time scales expected for intrachain diffusion, the data were analyzed in terms of static distributions. Of course, we recognize that it is of interest to elucidate α-syn conformational dynamics, because large amplitude motions of the polypeptide chain could play a central role in the aggregation process that is implicated in pathogenesis.

Electron transfer (ET) reactions are well-suited to probing conformational dynamics of biopolymers.[16,17] We have examined the rates of reaction between a powerful electron donor (the triplet excited state of tryptophan, ^{3}W*) and an acceptor (3-nitro-tyrosine, Y(NO_2)) in six different W−Y(NO_2) α-synucleins,[15] probing loop sizes between 15 and 132 residues. The second-order rate constant for the bimolecular ET quenching reaction is near the diffusion limit (7×10^9 M^{-1} s^{-1}; 20 mM sodium phosphate buffer, pH 7.4).[18] The relatively long-lived ^{3}W* excited state ($\tau = 40-60$ μs) is produced upon 290-nm laser excitation and monitored by transient absorption spectroscopy using an Ar-ion laser probe (457.9 nm). The quenching kinetics show conclusively that the protein is highly dynamic and that conformers are interchanging rapidly on the microsecond time scale (Figure 1).

If the intrachain dynamics in α-syn are diffusive, then the tryptophan excited-state decay kinetics can be described by a modified form of the Smoluchowski equation.[19−22] Energy transfer quenching of ^{1}W* depends primarily on the equilibrium distribution $P(r)$ and weakly on the diffusion coefficient D, whereas ET kinetics of ^{3}W* are quite sensitive to both $P(r)$ and D. Treating the Smoluchowski equation numerically, the ^{1}W* and ^{3}W* decays were simultaneously fit to refine our earlier distance distributions and to define the intrachain diffusion coefficients. We used two different continuous distribution functions based on polymer models to define $P(r)$. With a freely jointed chain (FJC) model, the data were fit by using the mean-squared ***DA*** distance ($\langle r^2 \rangle$) as a parameter.[23] Fits to a wormlike chain (WLC) model optimized the persistence length (l_p) parameter.[24] Finally, we used a model-independent fitting approach to extract discrete distance distributions (Supporting Information).

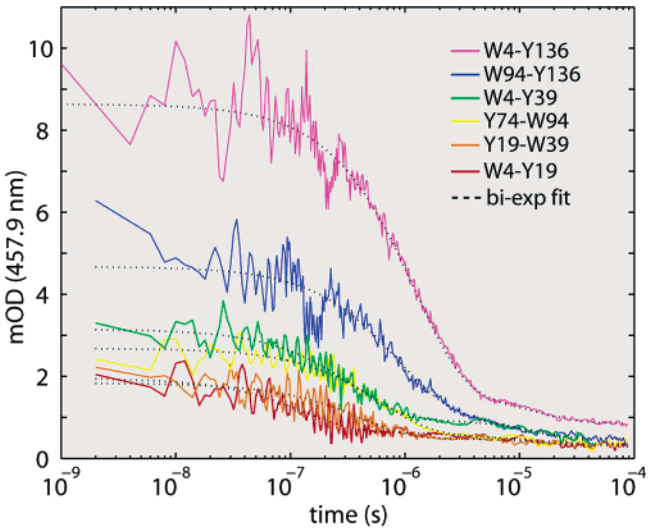

Figure 1. Quenching of ^{3}W* by Y(NO_2) in α-synuclein mutants (20−50 μM) in deoxygenated 20 mM sodium phosphate, pH 7.4 and in the presence of N_2O as a solvated electron scavenger. Bi-exponential functions (dotted) were used to fit the observed ^{3}W* decay kinetics (colored) with a dominant fast phase (>70%; $\tau \approx 0.1-5$ μs) and a minor slow component with time constants of 10−20 μs.

From the combined FET and ET analysis, we have extracted moments (i.e., $\langle r \rangle$ and $\langle r^2 \rangle$) from the experimental $P(r)$, along with empirical estimates of effective diffusion coefficients for α-syn mutants (Table S1). The FJC and WLC models yield comparable values of $\langle r^2 \rangle$, which in turn exhibit an approximate linear dependence on the number of residues in the loop (n) formed in the tertiary contact between W and Y(NO_2) (Figure S1). The l_p values from the WLC fits do not exhibit a clear trend with n, and generally fall in the range 6.0 ± 0.5 Å. For the FJC model, inclusion of the ^{3}W* decay kinetics does not substantially modify the equilibrium ***DA*** distance distributions (Figure 2); although values of $\langle r \rangle$ shorten slightly, particularly in the N- to C-terminal pair (62 to 54 Å).[15] Notably, the W4−Y136 pair exhibited very little FET quenching, so the distances extracted have large errors. By simultaneously fitting the ^{1}W* and ^{3}W* kinetics, we can better describe the conformational heterogeneity of α-syn, especially in the pairs in which little energy transfer was observed.

In our prior study, we used a nonnegative linear least-squares algorithm to extract $P(r)$ from FET kinetics without recourse to any specific model. This fitting procedure produces the narrowest distance distribution required to fit the data. For ***DA*** pairs separated by 15 and 20 residues, the protein ensemble consists of short (15 Å, 10%), intermediate (~20 Å, 10%), and extended (≥30 Å, 10%) conformations. Simultaneous fits to ^{1}W* and ^{3}W* kinetics produced model-independent ***DA*** distance distributions that are significantly

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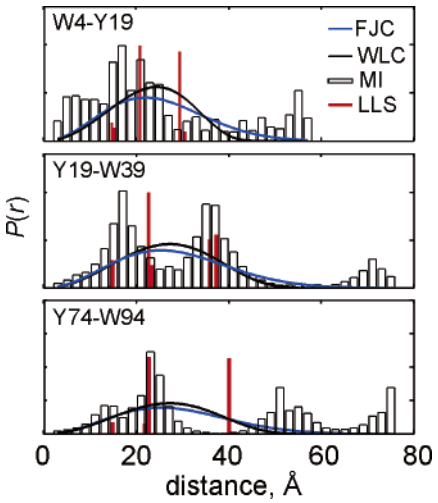

Figure 2. Probability distributions of ***DA*** distances $P(r)$ extracted from simultaneous fits of ^{1}W* and ^{3}W* decay kinetics (WLC, black line; FJC, blue line; MI, bars). Previously obtained $P(r)$ from a linear least-squares (LLS) method[15] without including diffusion also are shown (r values >40 Å produce little fluorescence quenching, and $P(r)$ in this region is not well defined).

broadened and appear to be continuous at our fitting resolution of 2 Å (Figure 2). It should be noted that the previously unobserved shorter ***DA*** distances (<15 Å) are necessary to account for ^{3}W* quenching. Importantly, the multimodality of the ***DA*** distance distributions is preserved. In all cases, the model independent algorithm provides better fits to the data than the two polymer models. Our results establish that α-syn structures are highly dynamic, interchanging between collapsed and extended populations on the microsecond time scale.

The diffusion coefficients extracted using all three fitting methods exhibit a systematic increase with n (Figure S2). Values of D increase from $\sim 2 \times 10^{-6}$ cm^2 s^{-1} for 15-residue loops to $\sim 10^{-5}$ cm^2 s^{-1} for 35-residue loops. The latter value is close to that expected for three-dimensional diffusion of a free amino acid in water ($D_0 \approx 10^{-5}$ cm^2 s^{-1}).[25] The dependence of D on n may be a reflection of greater chain stiffness in the smaller loops.[26] Another factor affecting the values of D could be the drag of the polypeptide external to the tertiary contact loop.[27]

Approximate analytical solutions to the Smoluchowski equation for three-dimensional polymers predict that the rate constant for tertiary contact formation is proportional to $D/\langle r^2 \rangle^{3/2}$.[19] An alternative dimensional analysis predicts that the contact rate will vary as $D_0 G(n)/\langle r^2 \rangle$, where D_0 is the free amino acid diffusion coefficient and $G(n)$ is the loop formation probability.[26] In random polymers, $\langle r^2 \rangle$ is expected to depend almost linearly on n, and $G(n)$ is estimated to exhibit an $n^{-2.2}$ dependence. The models suggest that contact rates will exhibit an n^{-x} ($x = 1.5-3.2$) dependence. In accord with theory, ET rates decrease with loop size, with the fastest contact time of 140 ns for the N-terminal pair and slowest of 1.2 μs for the N- to C-terminal pair. If α-syn is not a random coil, then tertiary contact formation rates will reflect structural preferences. Indeed, our ^{3}W* quenching rates deviate from the power-law dependence predicted by theoretical models for random polymers; in particular, the rates for the shortest (15-residue) and longest (132-residue) loops are higher than expected. It also is plausible that deviations from theoretical predictions are the result of nonrandom structural preferences in the central region of the protein (Y19−W39; Y74−W94; W4−Y39).[15]

It is not possible to obtain reliable distance distributions and diffusion coefficients from analyses of FET or ET kinetics alone.[11,20] We have shown that *both* these structural and dynamical parameters can be extracted from a simultaneous treatment of ^{1}W*/^{3}W* quenching kinetics. In the case of α-syn variants, our analysis reveals highly heterogeneous equilibrium structures and intrachain diffusion coefficients that depend on the number of residues separating W and Y(NO_2). The data indicate that α-syn has a strong preference for extended conformations. We anticipate that parallel ^{1}W*/^{3}W* quenching experiments will be a powerful tool for probing intermediates in the early stages of amyloid formation.

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Supporting Information Available: Details about the analysis of ^{1,3}W* decay kinetics, Table S1, Figures S1 and S2, and plots of distribution functions. This material is available free of charge via the Internet http://pubs.acs.org.

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