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COMMUNICATIONS

Published on Web 12/21/2004

Mapping Long-Range Interactions in α-Synuclein using Spin-Label NMR and Ensemble Molecular Dynamics Simulations

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Intrinsically disordered proteins play key roles in a range of biological processes, including cellular signaling, molecular recognition, and transcriptional regulation.[1−4] Several members of this protein class have also been implicated in debilitating human diseases, such as the Aβ peptides in Alzheimer's disease (AD) and α-synuclein (αSyn) in Parkinson's disease (PD).[5] These proteins appear to lack both secondary and tertiary structure, at least in the absence of binding partners, when studied under physiological conditions. However, the absence of fully formed α-helices and β-sheets does not preclude a rudimentary protein architecture, as demonstrated by the discovery of long-range contacts in globular proteins studied under denaturing conditions,[6−11] and such interactions could play a key role in the pathogenic nature of these proteins. Here, a combination of paramagnetic relaxation enhancement (PRE) NMR spectroscopy and ensemble molecular dynamics (MD) simulations[11] are used to probe the topology of native αSyn.

The high-resolution characterization of disordered protein states is challenging due to their intrinsic heterogeneity.[12] Such states are composed of dynamic ensembles rather than singular structures, and additionally, many important protein contacts in disordered states tend toward distances far exceeding the useful range of conventional NOE effects (≤5 Å).[13] The PRE method overcomes these difficulties by probing ensemble-averaged, transient contacts over distances as great as 20 Å. As previously described,[6,7,9−11] a nitroxide spin-label is attached to a region of a protein, and in its oxidized (paramagnetic) state, enhances relaxation of heteronuclear coherences observed in a ^{1}H−^{15}N correlation (HSQC) experiment; no effects are observed when the label is in a reduced (diamagnetic) state. Relaxation enhancement scales as r^{-6} with label proximity, allowing the computation of distance restraints from ratios of HSQC cross-peak intensities acquired for both oxidized and reduced label states. Ensembles of structures can then be calculated by restraining MD simulations using such PRE-derived distance thresholds. Applying PRE restraints on a single protein molecule results in anomalously compact structures due to the bias inherent in the r^{-6} weighting function.[6,11] This problem can be alleviated by the parallel simulation of multiple copies, or replicas, of the protein chain, whereby the distance restraints are enforced on the simulated ensemble average rather than on the individual structures themselves.[11]

We describe here a PRE study of the intrinsically disordered protein αSyn. Cysteine mutations facilitating attachment of the spin-label, MTSL, were made at positions Q24, S42, Q62, S87, and N103 along the 140-residue sequence of αSyn as described in the Supporting Information (SI). Intensity ratios were obtained from HSQC spectra acquired for each mutant with the spin-label in either its oxidized or reduced state. In addition to the local effects of the oxidized spin-label, which reduce strongly the cross-peak intensity of adjacent residues, long-range contacts between the C-terminal tail and the central region of the protein were also observed, indicating the presence of nonrandom structure (see Figure 1 in the SI).

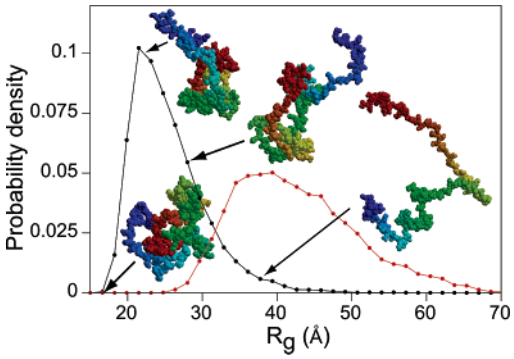

Figure 1. Radius of gyration (R_g) probability distributions calculated as described in the text for native (black) and random coil (red) models of αSyn. Representative structures are shown with arrows pointing to their corresponding R_g values. The structures are color-coded according to sequence, ranging from dark blue to red at the N- and C-termini, respectively.

Control HSQC experiments confirmed that the observed intensity decreases were due to intramolecular long-range contacts, rather than from aggregation (see Figure 1 in the SI).

The presence of long-range contacts suggests that the native state of αSyn is composed of a more compact ensemble of species than would be expected for a random coil state. To test this hypothesis, PRE-derived distance restraints were incorporated into ensemble MD simulations using the CHARMM force-field and 20 protein replicas. The radius of gyration (R_g) probability distribution for native αSyn was calculated from 40,000 computed structures and is shown in Figure 1 (black line). The distribution appears quite broad with a mean R_g of 24.7 Å, which corresponds to an average hydrodynamic radius (R_h) of 27.2 Å, according to a phenomenological relationship between R_g and R_h.[11] The calculated R_h value closely matches the experimental R_h of 26.6 ± 0.5 Å determined previously for αSyn.[14]

For comparison, the R_g distribution of a fully unfolded model of αSyn was generated by MD simulations in the absence of PRE restraints and using only the repulsive part of the Lennard-Jones potential, effectively simulating an excluded volume model of a random coil. Figure 1 shows the R_g distribution for the random coil model of αSyn (red line), which is extremely broad with a mean R_g of 41.9 Å. Nevertheless, the corresponding R_h value of 34.5 Å[11] agrees closely with the value of 33.3−36.9 Å expected for a highly denatured 140-residue polypeptide.[15]

Although the native distribution of αSyn conformers is significantly narrower than that of a random coil, it is still composed of numerous structures with widely varying radii, as demonstrated by those shown for specific values of R_g (Figure 1). The use of sufficient replicas (≥10) is critical in capturing the ensemble-like nature of the native state of αSyn since simulations incorporating one or very few replicas (<10) yield abnormally compact distributions (see Figure 2 in the SI). In the present study, parallel

10.1021/ja044834j CCC: $30.25

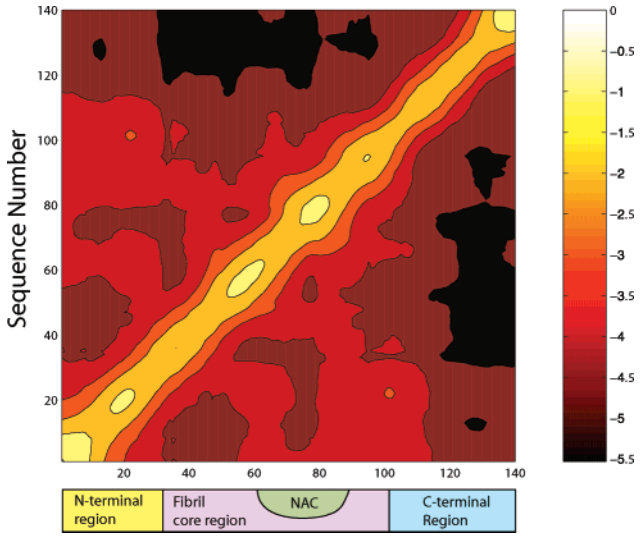

Figure 2. Residual contact plot for native αSyn. Darker colors correspond to decreasing values of $-\ln(p_{ij}/p_{ij}^{\mathrm{ref}})$, indicating contacts that are more probable than in a random coil model of αSyn. The residual contacts have been averaged locally over ±7 residues. The different regions of the αSyn sequence, as defined previously (refs 16, 17), are shown in the lower bar.

simulation of 20 protein replicas was used to generate expanded ensembles that fully satisfied both the PRE distance restraints and the experimentally determined R_h.

The extent of structure in native αSyn was quantified by analyzing residue−residue contacts in 4000 structures obtained as described above. Contacts in native αSyn were evaluated with respect to contacts in the random coil ensemble of αSyn, with nonrandom structure being designated as a preference for specific interactions relative to their probability of formation in the random coil state.[11] Although the native state of αSyn is a highly heterogeneous ensemble, structural analysis reveals the presence of a number of significant long-range interactions. The nonrandom structure in native αSyn is shown in Figure 2 as a topological contact map of $-\ln(p_{ij}/p_{ij}^{\mathrm{ref}})$, where $(p_{ij}/p_{ij}^{\mathrm{ref}})$ is the contact probability ratio between the restrained and reference simulations. Darker coloring indicates an increased probability for interactions between two regions of sequence. It is evident that the native αSyn ensemble samples nonrandom conformations involving, in particular, contacts between residues ∼120−140 of the C-terminus and residues ∼30−100 in the central region of the protein sequence. These results were reproduced consistently in ensemble calculations utilizing only a subset of PRE restraints and also for shorter simulation times. Due to the discrete position of spin-labels in PRE experiments, more distance restraints are available at spin-labeling sites relative to unlabeled sequence locations. While this can potentially result in a calculated structural compaction surrounding the site of labeling, long-range interactions observed here for αSyn do not arise from effects of this type. For example, the most probable contacts occur at positions ∼120−140, whereas the nearest site of labeling is residue 103; the large separation in sequence of these regions supports strongly the validity of the PRE method in this study. Considering the broad nature of the native αSyn ensemble (Figure 1) and the absence of any significant secondary structure, the presence of long-range contacts that are ∼200 times more probable than in a random coil is a striking topological feature.

We have shown therefore that the native state of αSyn is composed of an ensemble of structures that are, on average, significantly more compact than a random coil. This partial condensation is driven by long-range contacts between residues 120−140 in the negatively charged C-terminal tail and residues 30−100 in the center of the protein. The NAC region (61−95)[16] is the most hydrophobic part of αSyn, and since it is known to form amyloid fibrils both in vitro and in vivo,[16] the proximity of the highly charged C-terminus may shield this region from aggregation. Indeed, truncation of the C-terminal region greatly accelerates fibril formation in vitro,[18] and a large proportion of aggregated αSyn found in vivo in Lewy bodies is truncated in this manner.[19] Furthermore, EPR studies[17] of αSyn fibrils suggest that the highly ordered amyloid fibril core region is composed of residues ∼30−100, precisely the region that we observe to interact with the C-terminal residues. The results presented here are therefore consistent with the idea that the aggregation of αSyn is inhibited by the existence of long-range interactions within the native structure. Moreover, the C-terminal region also contains binding sites for Ca^{2+} ions, heavy metals, and polyamines, all of which increase the rate of αSyn aggregation.[20−22] The binding of ligands to the C-terminal region may therefore act to modulate the function and/or aggregational propensity of αSyn. The long-range contacts observed here may therefore affect substantially the role of αSyn in the pathogenesis of PD, and their detection represents a significant step toward an increased understanding of the consequences of intrinsic disorder in proteins.

Acknowledgment. We thank A. Olteanu and G. Pielak (UNC-Chapel Hill) for the αSyn plasmid, and C. Griesinger and M. Zweckstetter (Max Planck Institute, Göttingen) for valuable discussions. This work was supported by the Wellcome and Leverhulme Trusts.

Supporting Information Available: Experimental and computational details, and Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA044834J