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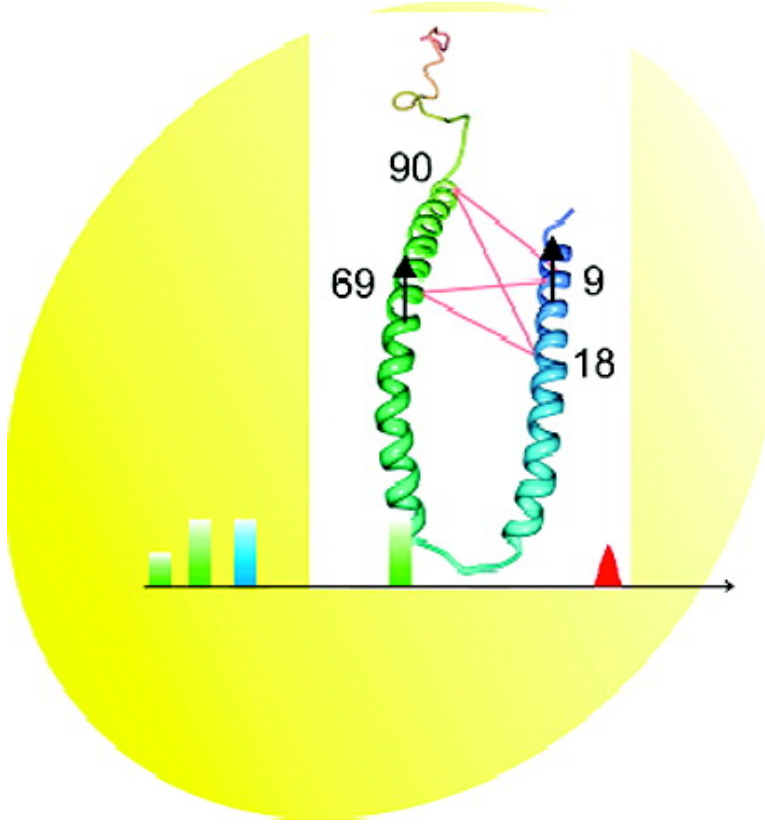

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Antiparallel Arrangement of the Helices of Vesicle-Bound α-Synuclein

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α-Synuclein (αS) is a 140 residue protein, constituting the major component of Lewy bodies found in Parkinson's disease.[1,2] Although the exact function of αS remains obscure, interaction of αS with synaptic vesicles has been suggested to be important for its physiological role, fostering interest in the structural properties of αS upon binding to membranes. Although αS has a random-coil configuration in solution,[3] upon binding to membranes it adopts an amphipathic, α-helical structure involving residues 1−100.[4−11] It was proposed that αS binds either as an extended, continuous helix (residues 1−100)[11−13] or in a horseshoe-like conformation, consisting of helix 1 (residues 3−37), a bend around residue 42, and helix 2 (residues 45−92),[14] with the two helices in an antiparallel arrangement but not touching.[15,16] Most of the structural information available concerns studies of αS on micelles.[14,17] Micelles, however, differ in important aspects from biological membranes. Micelles have typical diameters[18] of 5 nm and therefore may be too small to accommodate αS in the extended conformation (around 15 nm for an extended helix of 100 residues).[19] Therefore, micelles could enforce an antiparallel configuration of the helices even if that is not the preferred state of αS on a larger surface such as that of a biological membrane. In contrast, even small unilamellar vesicles (SUVs) have diameters[4] in the order of 20 to 30 nm, providing a surface area that is large compared to the dimension of αS[19] and a larger radius of curvature than micelles. Therefore, SUVs are more suitable models for biological membranes than micelles.

We present an investigation on the structure of αS on SUVs using a two-frequency, pulsed EPR method (double electron−electron resonance DEER or electron−electron double resonance ELDOR).[20−24] To obtain long-range information on the conformation of αS bound to the vesicle surface, the distances between pairs of spin-labels introduced by site-selective cysteine mutagenesis were measured. The DEER method was chosen, because it allows the measurement of distances up to 8 nm in disordered materials[22] and is not subject to limitations in the tumbling time and thereby the size of the complex. Four double-cysteine mutants, alkylated with MTSL (1-oxy-2,2,5,5-tetramethylpyrroline-3-methyl-methanethiosulfonate), were investigated: αS9/90, αS18/90, αS18/69, and αS9/69, each containing one label in the proposed helix 1, and a second label in helix 2 (see Figure 1). Distances were measured for αS mutants bound to vesicles and compared to those in the absence of vesicles, that is, for αS free in solution.

We show that αS adopts a two-helix, antiparallel arrangement on vesicles that are large enough to accommodate an extended helix of αS. This suggests that the bent structure is the preferred conformation of αS also on membranes, an indication that the horseshoe conformation is the physiologically relevant one.

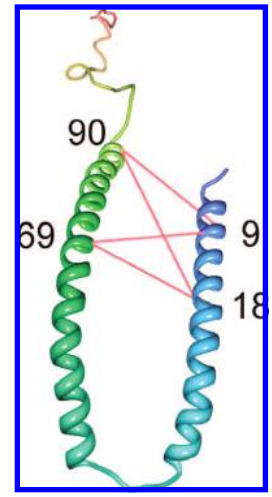

Figure 1. NMR-structure (1XQ8 of RCSB protein database)[14] of αS bound to SDS micelles. Red lines indicate distances measured by DEER for αS bound to SUVs. Visualized by PDB Protein Workshop.[25]

Double-cysteine mutants were incubated with a 6-fold excess per cysteine of MTSL. Free label was subsequently removed using size-exclusion chromatography. The SUVs of POPG lipids [1-palmitoyl-2-oleoyl-*sn*-glycero-3-(phosphorac-(1-glycerol))] were produced by sonication in 10 mM Tris-HCl, pH 7.4, and mixed to obtain an αS/lipid ratio of 1:250.

DEER experiments were performed at $T = 40$ K using a Bruker Elexsys E680 X-band spectrometer modified as described previously.[26] Data were analyzed using DEERAnalysis 2006.1.[27−29] Model free analysis revealed that the distance distributions could be well fitted by Gaussians. For details of sample preparation, measurements, and analysis, see Supporting Information.

In the presence of vesicles, the DEER responses of all four mutants differed significantly from those in the absence of vesicles. This is illustrated in Figure 2, where DEER results are shown for αS18/69 (remaining mutants, see Supporting Information). In Figure 2a, the time traces are shown. The shape of the decays could be well simulated by the Gaussian distance distributions shown in Figure 2b. In the absence of vesicles the distribution was broad. Upon addition of vesicles, the distance distribution became much narrower and shifted by 0.4 nm to shorter distances. Table 1 lists the parameters of the distance distributions for all mutants.

In the absence of vesicles, the widths of the distributions were large (≥1.8 nm), as expected from the unstructured nature of αS in solution.[3] Narrower distributions and a more differentiated set of distances in the presence of vesicles reveal that αS undergoes a

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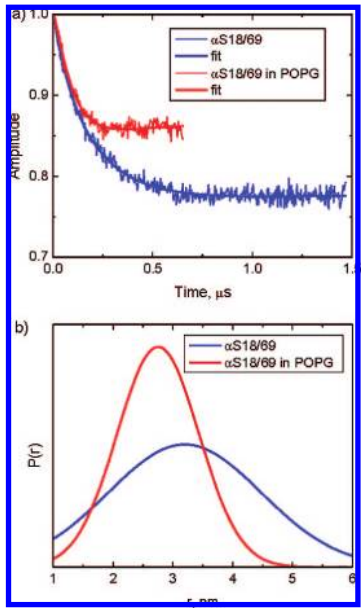

Figure 2. (a) DEER traces of αS18/69 after background correction in the presence (red, top trace) and absence (blue) of POPG vesicles and corresponding fits (solid lines) assuming a Gaussian distance distribution. (b) Distance distributions corresponding to the fits in panel a.

Table 1. Parameters of Gaussian Distance Distributions Obtained by DEER[a]

	in solution		with vesicles	
αS double Cys mutant	distance (nm)	width of distribution (nm)	distance (nm)	width of distribution (nm)
9/90	3.1 ± 0.1	2.1 ± 0.2	3.2 ± 0.2	0.9 ± 0.3
18/90	3.1 ± 0.05	1.8 ± 0.15	3.4 ± 0.1	1.5 ± 0.3
18/69	3.2 ± 0.1	1.8 ± 0.2	2.8 ± 0.05	1.0 ± 0.3
9/69	2.8 ± 0.1	2.0 ± 0.2	3.2 ± 0.2	1.3 ± 0.3

[a] Determination of errors: see Supporting Information.

structural transition to a more ordered state upon binding to the membrane, in agreement with the known transition of αS from randomly disordered to α-helical upon vesicle binding.[4,6] The width of these distributions, although narrower than in the absence of vesicles, is still relatively large, indicating that there is a large range of conformations for the individual molecules. For vesicle-bound αS, the distances measured between strategically chosen points on the two proposed helices span 2.8−3.4 nm, which is consistent with the distances derived from the NMR structure on micelles.[8,14] The four distances determined provide a redundant set that proves that the arrangement of the two helices must be antiparallel, as shown in Figure 1. A more refined model, along the lines of the study of Borbat et al. for αS on micelles,[17] where triangulation in conjunction with rigid body refinement was possible using an extensive set of mutants,[30] is work in progress. The distance constraints obtained in the present study enable us to unambiguously determine that the vesicle-bound αS fits the "broken helix" model, where the first two-thirds of αS loop back upon themselves. We conclude that the horseshoe type conformation is the most likely conformation of αS in the membrane-bound state. This result has implications for the conformation of αS in vivo, where αS interacts with synaptic vesicles with diameters around 40 nm.

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Supporting Information Available: Details of protein expression, sample preparation, measurements, and data analysis. DEER traces before background correction as well as corrected DEER traces for all double mutants. This material is available free of charge via the Internet at http://pubs.acs.org.

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