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Locally Resolved Membrane Binding Affinity of the N-Terminus of α-Synuclein

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Supporting Information

ABSTRACT: α-Synuclein is abundantly present in Lewy bodies, characteristic of Parkinson's disease. Its exact physiological role has yet to be determined, but mitochondrial membrane binding is suspected to be a key aspect of its function. Electron paramagnetic resonance spectroscopy in combination with site-directed spin labeling allowed for a locally resolved analysis of the protein−membrane binding affinity for artificial phospholipid membranes, supported by a study of binding to isolated mitochondria. The data reveal that the binding affinity of the N-terminus is nonuniform.

Human α-synuclein (αS), a 140-amino acid protein, is the main fibrillar component of Lewy bodies, a pathological hallmark of Parkinson's disease (PD).[1−3] αS is intrinsically disordered and is characterized in vitro by the absence of a well-defined structure and by remarkable conformational flexibility.[4−6] There is strong evidence that the biological function of αS is correlated with the binding to membranes.[7−10] αS−membrane interactions are mediated by the N-terminal region (approximately residues 1−100), forming α-helices upon association, while the negatively charged C-terminal region remains unfolded and potentially available for interactions with other proteins.[11−17] This membrane-bound form has been suggested to play a crucial role in αS function.[18] Several reports have suggested a role for endogenously expressed αS, e.g., in maintaining the integrity of mitochondrial complexes.[19−21] Binding of αS to the outer mitochondrial membrane appears to affect the fusion−fission cycle of mitochondria, a key event in cellular viability in PD, in organelle turnover, and in mitophagy.[22]

Electron paramagnetic resonance (EPR) spectroscopy in combination with site-directed spin labeling (SDSL EPR) has been used to investigate αS−membrane interactions.[12,15,23−25] In this context, different membrane affinities of the two antiparallel α-helices interacting with negatively charged small unilamellar vesicles (SUVs) have been reported.[24] However, there are indications that αS may influence the SUV membrane structure or even disrupt SUVs, causing membrane leakage.[26,27]

In this work, αS−membrane interactions are investigated using large unilamellar vesicles (LUVs) made of mixtures of POPG and POPC. Such vesicles are not damaged by the interaction with αS. Prior SDSL EPR studies have shown that αS bound to LUVs of these lipid compositions coexists in a superposition of both horseshoe and extended forms.[26]

Here, the rotational mobility at room temperature of spin-labels attached to αS has been used to monitor the local degree of membrane binding in the proximity of the labeled sites as a function of membrane composition. The rotational mobility was determined by EPR spectroscopy in X-band, accompanied by spectral simulations performed with EasySpin.[28] We prepared seven different αS derivatives labeled at single cysteines introduced at positions 9, 18, 27, 56, 69, 90, and 140 (αS9, αS18, etc.). We studied the interactions of these labeled proteins with LUVs composed of different ratios of negatively charged {POPG [1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-(1′-glycerol)]} and zwitterionic [POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine)] lipids. We found a nonuniform binding affinity, increasing toward the N-terminus. Experiments comparing the binding affinity of wild type αS (αSwt) and an αS variant lacking amino acids 2−11 of the N-terminal region (αSΔ2−11) for mitochondria support this result.[29−31]

The EPR spectra of singly labeled αS in the absence of LUVs can be described by a one-component spectral simulation S_A in the fast motion regime (Figure 1). The corresponding isotropic rotational correlation times (τ_r) are listed in Table 1. They are consistent with expectations for unstructured peptides in solution.

Representative EPR spectra of αS9 and αS90 upon interaction with 100 nm LUVs of different surface charge densities [ρ = [POPG]/([POPG] + [POPC])] are shown in Figure 2, and the entire set of spectra is displayed in Figure S1 of the Supporting Information. The spectra of αS in the presence of LUVs are described well by a superposition of two components featuring different rotational mobilities, a slow component S_B and a fast component S_A:

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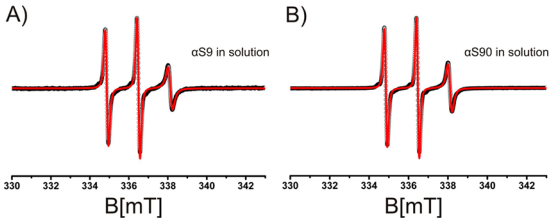

Figure 1. Experimental spectra of representative singly labeled mutants αS9 (A) and αS90 (B) in the absence of LUVs (○) and their spectral simulation (fit, red line).

Table 1. Simulation Parameters Describing the Rotational Mobility for αS9 and αS90 in Solution and in the Presence of LUVs[a]

	S_A τ_r (ns)	S_B τ_r (ns)	b
αS9	0.44	–	–
αS90	0.36	–	–
αS9/LUV (ρ = 0)	0.44	–	0.00(2)
αS90/LUV (ρ = 0)	0.36	–	0.00(2)
αS9/LUV (ρ = 0.4)	0.44	2.39	0.92(5)
αS90/LUV (ρ = 0.4)	0.36	3.16	0.13(5)
αS9/LUV (ρ = 1.0)	0.44	2.56	0.99(2)
αS90/LUV (ρ = 1.0)	0.36	2.45	0.80(5)

[a]Rotational correlation time τ_r and fraction b of spectral component S_B (for values of g and A, see the Supporting Information).

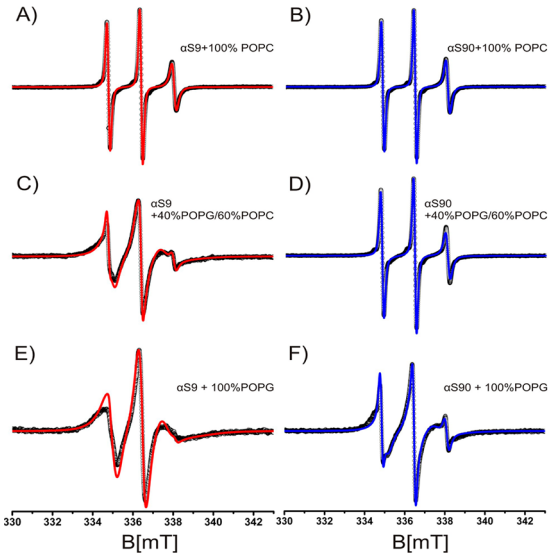

Figure 2. Representative experimental spectra (○) and corresponding fits of αS9 (red line) and αS90 (blue line) in the presence of POPC (A and B), POPG/POPC (ρ = 0.4) (C and D), and POPG (E and F) LUVs.

$$S = (1 - b)S_A + bS_B \quad (1)$$

The simulation parameters obtained for αS in solution were taken as the fast component for S_A, while τ_r of S_B and the fraction b were fitted.

The simulations of the EPR spectra obtained for all spin-labeled αS mutants in the presence of LUVs made solely of zwitterionic POPC lipids show that the spectra contain only the fast component S_A with parameters corresponding to those obtained for αS in solution. This finding suggests that there is no significant binding of αS to these uncharged membranes.

The spectra of singly labeled mutants gradually change with an increasing POPG/POPC ratio (ρ) (Figure 2). Only the spectra of αS140 remained the same irrespective of the LUV lipid composition. The increase in fraction b of the slow component S_B for all other mutants is reflected by line broadening (Figure 2C–F) relative to the spectra of αS both in solution (Figure 1) and in the presence of uncharged POPC LUVs (Figure 2A,B). The spectral simulations for component S_B show that τ_r is much smaller than the rotational correlation time of a LUV ($\tau_r \sim 1$ ms, calculated from the Stokes–Einstein equation). Therefore, τ_r rather reflects the residual mobility of the spin-label. Because component S_B originates from spin-labels with reduced mobility and is observed only when LUVs are present, the restricted mobility of the spin-labels must stem from the interaction of αS with the LUVs. Hence, the slow component S_B can be attributed to binding of αS to the membrane in the vicinity of the labeled residue, so that b reflects the local degree of binding of this region of the protein.

We performed a systematic study to examine the influence of the surface charge density ρ of the LUVs in the range of 0–1 on the local degree of binding b in seven different positions (Figure 3).

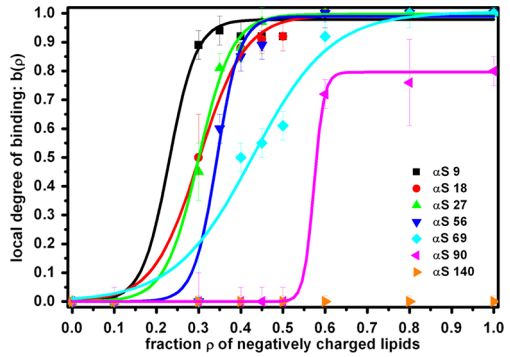

Figure 3. Surface charge density (ρ) of the LUVs that can be controlled by varying the content of negatively charged lipids. The fraction b of the slow component S_B derived from numerical spectral simulations of EPR spectra according to eq 1 reflects the degree of local binding in the proximity of different residues of αS. The sigmoidal curves serve as guides to the eye.

For αS140, the degree of local binding b is zero for all values of ρ. We found the degree of local binding b decreases with decreasing values of ρ for all residues (except αS140). From ρ = 1.0 to ρ = 0.3, the behavior is strongly dependent on the labeled region; i.e., regions close to the N-terminus bind at lower membrane surface charge densities than the regions distal from the N-terminus. We attribute differences in the dependence of the local degree of binding b on ρ to an effect of nonuniform binding affinity of αS for the membrane surface. For instance, fraction b of αS9 reflects the local binding affinity around residue 9, which starts binding at $\rho \approx 0.1$, while the region around residue 90 does not bind until $\rho \approx 0.5$.

Because differences in lipid composition or membrane fluidity may result in different αS binding behavior,[32] the binding of αS wild type and αSΔ2–11 to isolated mitochondria was studied using sodium dodecyl sulfate–polyacrylamide gel electrophoresis experiments (Figure 4). The outer mitochondrial membrane protein TOM20 was used as loading control. The band intensity at 17 kDa[33] of mitochondria bound αSwt increases with an increasing αSwt concentration in the range of

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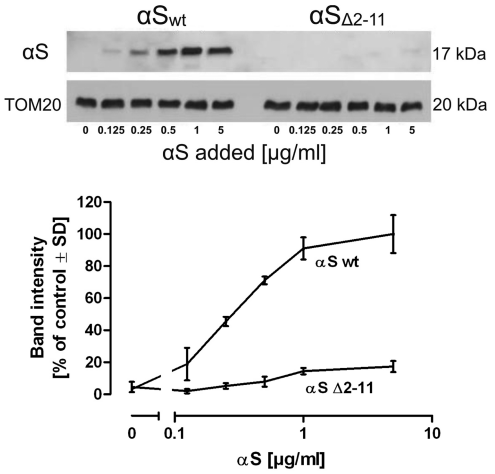

Figure 4. Mitochondria corresponding to a total protein concentration of 1 mg/mL were incubated with αSwt and αSΔ2−11 at different concentrations. Quantitative data were obtained from three independent experiments and expressed as means ± the standard deviation.

0.125−5 μg/mL αS. The absence of significant binding of αSΔ2−11 to the native mitochondrial membrane shows that the N-terminal region of αS is also needed for binding to native membranes, suggesting that under cellular conditions localized interactions are crucial for binding.

The EPR approach gives a more differentiated view of the interaction of αS with the membrane than a global binding affinity measurement. These results not only imply that the binding of αS to membranes could be initiated in the N-terminal part of αS but also suggest that at lower surface charge densities the binding affinity of regions close to the N-terminus is stronger than that of regions distal from the N-terminus in sequence. We have previously shown that on LUVs, αS coexists in the horseshoe and extended helix forms,[26] exhibiting quantitative binding of at least residues 9−69. In this work, we demonstrate that different binding modes exist for different subpopulations, involving different stretches of residues (for example, for ρ = 0.5, we measure fractions binding with residues ∼9−69 and ∼9−27).

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, sample preparation, EPR measurements, and analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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