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

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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 (α S), a 140-amino acid protein, is the main fibrillar component of Lewy bodies, a pathological hallmark of Parkinson's disease (PD).^{1–3} α S 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 α S is correlated with the binding to membranes.^{7–10} α S-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 α S function.¹⁸ Several reports have suggested a role for endogenously expressed α S, e.g., in maintaining the integrity of mitochondrial complexes.^{19–21} Binding of α S 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 α S-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 α S may influence the SUV membrane structure or even disrupt SUVs, causing membrane leakage.^{26,27}

In this work, α S-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 α S. Prior SDSL EPR studies have shown that α S 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 α S 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 α S derivatives labeled at single cysteines introduced at positions 9, 18, 27, 56, 69, 90, and 140 (α S9, α S18, 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 α S (α Swt) and an α S variant lacking amino acids 2–11 of the N-terminal region (α S Δ 2–11) for mitochondria support this result.^{29–31}

The EPR spectra of singly labeled α S 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 α S9 and α S90 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 α S 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 :

Figure 1. Experimental spectra of representative singly labeled mutants α S9 (A) and α S90 (B) in the absence of LUVs (○) and their spectral simulation (fit, red line).

Table 1. Simulation Parameters Describing the Rotational Mobility for α S9 and α S90 in Solution and in the Presence of LUVs^a

	$S_A \tau_r$ (ns)	$S_B \tau_r$ (ns)	b
α S9	0.44	—	—
α S90	0.36	—	—
α S9/LUV ($\rho = 0$)	0.44	—	0.00(2)
α S90/LUV ($\rho = 0$)	0.36	—	0.00(2)
α S9/LUV ($\rho = 0.4$)	0.44	2.39	0.92(5)
α S90/LUV ($\rho = 0.4$)	0.36	3.16	0.13(5)
α S9/LUV ($\rho = 1.0$)	0.44	2.56	0.99(2)
α S90/LUV ($\rho = 1.0$)	0.36	2.45	0.80(5)

^aRotational 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 α S9 (red line) and α S90 (blue line) in the presence of POPC (A and B), POPG/POPC ($\rho = 0.4$) (C and D), and POPG (E and F) LUVs.

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

The simulation parameters obtained for α S 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 α S 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 α S in solution. This finding suggests that there is no significant binding of α S 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 α S140 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 α S 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 α S with the LUVs. Hence, the slow component S_B can be attributed to binding of α S 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 α S. The sigmoidal curves serve as guides to the eye.

For α S140, 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 α S140). From $\rho = 1.0$ to $\rho = 0.3$, 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 α S for the membrane surface. For instance, fraction b of α S9 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 α S binding behavior,³² the binding of α S wild type and α SΔ2–11 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 kDa³³ of mitochondria bound α Swt increases with an increasing α Swt concentration in the range of

Figure 4. Mitochondria corresponding to a total protein concentration of 1 mg/mL were incubated with α Swt and α S Δ 2–11 at different concentrations. Quantitative data were obtained from three independent experiments and expressed as means $\pm$ the standard deviation.

0.125–5 μ g/mL α S. The absence of significant binding of α S Δ 2–11 to the native mitochondrial membrane shows that the N-terminal region of α S 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 α S with the membrane than a global binding affinity measurement. These results not only imply that the binding of α S to membranes could be initiated in the N-terminal part of α S 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, α S 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 $\rho = 0.5$, we measure fractions binding with residues ~9–69 and ~9–27).

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Notes

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