NMR Observation of α-Synuclein Membrane Interaction by Monitoring Acetylation Reactivity of its Lysine Sidechains

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Supporting Information

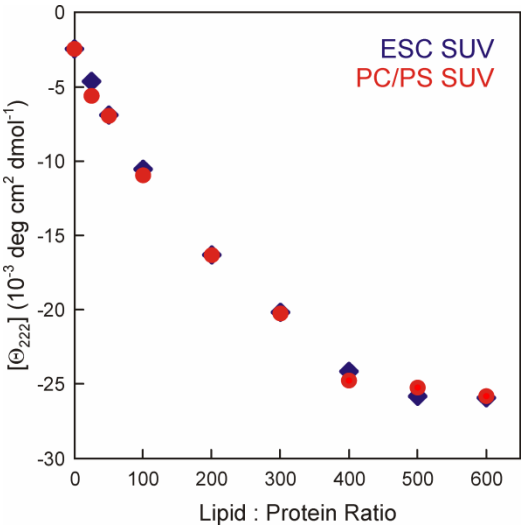

Figure S1. Induced helicity of αS in the presence of different concentrations of ESC and PC/PS SUVs measured by CD. Measurements were performed with 50μM αS as a function of SUV concentration in PBS buffer at 20 °C using 0.2-mm path length cuvette.

Table S1. Chemical Shift Perturbation (CSP) upon Acetylation of Lysine at Position (i). [a, b]

$^{1}H^{N}$

residue i	i-1 (ppb)	i (ppb)	i+1 (ppb)
K6	-27	-69	-35
K10	0	-94	-49
K12	-12	-111	-60
K21	1	-85	-21
K23	21	1	5
K32	-18	-40	15
K34	10	-63	
K43	-17	-85	-20
K45	9	-86	-24
K58	-46	-96	-36
K60	12	-38	-14
K80	-30	-81	-44
K96	-17	-61	-91
K97	-25	-58	-6
K102	-13	-53	-39

^{15}N

residue i	i-1 (ppb)	i (ppb)	i+1 (ppb)
K6	163	166	-289
K10	-125	102	-236
K12	-364	-63	-139
K21	-363	122	-220
K23	161	260	18
K32	-31	200	-335
K34	195	264	
K43	-134	154	-320
K45	99	242	149
K58	-503	-87	-555
K60	110	168	55
K80	-335	-58	-554
K96	248	-49	-829
K97	-319	304	-147
K102	-76	151	-585

$^{13}C^{\alpha}$

residue i	i-1 (ppb)	i (ppb)	i+1 (ppb)
K6	-33	408	122
K10	-31	224	-86
K12	107	196	174
K21	-48	285	-68
K23	-204	206	41
K32	-39	260	-218
K34	-10	76	
K43	-37	261	-169
K45	49	90	30
K58	55	374	-130
K60	-143	144	105
K80	16	287	-133
K96	-36	222	151
K97	-140	183	-4
K102	31	275	-85

$^{13}C'$

residue i	i-1 (ppb)	i (ppb)	i+1 (ppb)
K6	89	330	180
K10	-39	143	-91
K12	85	304	149
K21	-28	192	-13
K23	-33	209	10
K32	-88	145	35
K34	-88		
K43	-74	159	
K45	-49	163	
K58	33		
K60	-35	184	
K80	-21	191	-58
K96	25	336	29
K97	-19	277	
K102	75	246	-3

[a] The data presented here is used for Figure 4.

[b] CSPs are measured for an N-succinimidyl acetate reacted (in PBS, followed by methanol precipitation) $^{13}C/^{15}N$-enriched α-synuclein sample in 10 mM sodium phosphate, 10 mM NaCl, and 5% D_2O at pH 6, 283 K, and 700 MHz ^{1}H frequency.

[c] Cells are left blank when backbone chemical shifts of acetylated lysines or their neighboring residues cannot be determined accurately due to resonance overlap.

Table S2. Second-order rate constants for the reaction of each αS lysine sidechain with N-succinimidyl acetate in the presence of indicated amount of ESC and PC/PS SUVs. [a, b, c]

residue number	αS only (no SUV) ($M^{-1} s^{-1}$)	αS : ESC = 1 : 200 ($M^{-1} s^{-1}$)	αS : ESC = 1 : 500 ($M^{-1} s^{-1}$)	αS : PC/PS = 1 : 200 ($M^{-1} s^{-1}$)	αS : PC/PS = 1 : 500 ($M^{-1} s^{-1}$)
K6	0.54	0.13	0.078	0.20	0.11
K10	0.45	0.10	0.057	0.19	0.11
K12	0.47	0.14	0.062	0.18	0.10
K21	0.46	0.11	0.055	0.18	0.077
K23	0.42	0.12	0.053	0.21	0.087
K32	0.35	0.073	0.027	0.14	0.046
K34	0.46	0.13	0.050	0.19	0.064
K43	0.47	0.11	0.043	0.19	0.071
K45	0.50	0.15	0.064	0.24	0.108
K58	0.40	0.11	0.044	0.19	0.069
K60	0.42	0.10	0.040	0.16	0.066
K80	0.44	0.14	0.074	0.24	0.107
K96	0.41	0.16	0.088	0.22	0.114
K97	0.36	0.18	0.121	0.24	0.143
K102	0.22	0.17	0.142	0.18	0.158

[a] The data presented here is used for Figure 9.

[b] Reactions were performed as described in the Methods section. Briefly, 50 μM of N-terminally acetylated ^{15}N-enriched αS was reacted with 250 μM of N-succinimidyl acetate in the presence of indicated molar-excess of lipids for 5 mins in PBS buffer. L-lysine was used to stop the reaction and αS was precipitated by methanol. The precipitated αS was resuspended in 10 mM sodium phosphate, 10 mM NaCl, 5% D_2O, and pH 6 buffer, and high resolution 2D ^{1}H-^{15}N HSQC data was collected on the sample at 283 K at 900 MHz ^{1}H frequency.

[c] The rate constants are derived from fitting the decay of reactant (N-succinimidyl acetate) and buildup of product (N-hydroxysuccinimide) (Figure 1) for 5-25 mins time interval. Extrapolating the reaction curve to time zero yields a non-zero product (Figure 2) that corresponds to a burst phase where 4.8% of the N-succinimidyl acetate reacted in the 50 μM αS sample.