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Saposin C Protects Glucocerebrosidase against α -Synuclein Inhibition

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Abstract

Mutations in *GBA1*, the gene for glucocerebrosidase (GCase), are genetic risk factors for Parkinson disease (PD). α -Synuclein (α -syn), a protein implicated in PD, interacts with GCase and efficiently inhibits enzyme activity. GCase deficiency causes the lysosomal storage disorder Gaucher disease (GD). We show that saposin C (Sap C), a protein vital for GCase activity *in vivo*, protects GCase against α -syn inhibition. Using NMR spectroscopy, site-specific fluorescence, and Förster energy transfer probes, Sap C was observed to displace α -syn from GCase in solution and on lipid vesicles. Our results suggest that Sap C might play a crucial role in GD-related PD.

Mutations in the *GBA1* gene are the most common of the known risk factors for Parkinson disease (PD).¹ Despite a wealth of studies linking *GBA1* mutations and PD, the underlying mechanisms have yet to be fully defined.² *GBA1* encodes glucocerebrosidase (GCase, Figure 1a top), a lysosomal enzyme which hydrolyzes glucosylceramide (GluCer) into glucose and ceramide and is deficient in Gaucher disease (GD). Both GCase and α -synuclein (α -syn, Figure 1a middle), a membrane binding protein associated with PD, have been observed in Lewy bodies,³ a classic PD hallmark.

A growing number of studies show a correlation between GCase deficiency and increased α -syn levels,⁴ leading some to speculate that GluCer accumulation affects normal α -syn turnover.^{4b} Intriguingly, we discovered that α -syn physically interacts with GCase under acidic conditions found in lysosomes,⁵ a site of α -syn degradation.⁶ In further substantiating this relationship, we found that α -syn inhibits GCase activity on the membrane;^{5b} although, it is currently unresolved whether reduced GCase activity alone leads to increased α -syn levels.⁷ Since only a minority of GD patients and carriers develop PD, other factors are also expected to play a role in promoting pathogenesis. Obvious molecules of interest include those that modulate GCase activity and α -syn-GCase interaction.

In vivo degradation of GluCer by GCase is facilitated by the co-factor saposin C (Sap C),⁸ a 9 kDa membrane-interacting protein (Figure 1a bottom).⁹ Sap C has been proposed to

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ASSOCIATED CONTENT

Supporting Information. Experimental details and Figures S1–S4. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Notes

T.L.Y. and J.M.G. contributed equally. The authors declare no competing financial interest.

function by altering lipid bilayer properties or through direct association with GCCase.¹⁰ Although rare, Sap C deficiency alone can result in GD symptoms in patients,¹¹ demonstrating its essential role in GluCer metabolism. Sap C deficiency was shown to cause severe GD phenotypes and enhanced storage of GluCer in a GD-mouse model.¹²

Here, we investigated whether Sap C, a vital co-factor *in vivo*, can affect GCCase activity inhibition by α -syn *in vitro*.^{5b} Consistent with our previous study,^{5b} an $IC_{50} \sim 0.4 \mu\text{M}$ is obtained for α -syn inhibition using a fluorogenic substrate and vesicle assay (Figure 1b, equal molar phosphatidylcholine and phosphatidylserine, POPC:POPS, pH 5.5). Remarkably, inhibition is completely abolished in the presence of Sap C ($5 \mu\text{M}$) at all measured concentrations of α -syn (Figure 1b). At the highest α -syn concentration ($10 \mu\text{M}$), Sap C protects GCCase in a concentration dependent manner, yielding an AC_{50} of $0.4 \mu\text{M}$ (Figure 1c). Under the high POPS condition used here, activity enhancement by Sap C is negligible, making this rescue even more remarkable.

Since Sap C had been proposed to bind to GCCase directly, we questioned whether Sap C displaces α -syn from GCCase, thus affording rescue of its activity. In prior work, using nuclear magnetic resonance (NMR) spectroscopy with isotopically labeled α -syn, a C-terminal region, residues 118–137, rich in acidic (3 Asp and 5 Glu) residues was identified as the site of interaction with GCCase.^{5a} Association with GCCase ($\sim 60 \text{ kD}$) slows the molecular tumbling of these C-terminal residues, reducing their NMR signal intensities while N-terminal residues remain in the highly disordered unbound state with intensities unchanged (Figure 2a, top panel). Upon addition of Sap C, partial recovery of intensity is seen for the C-terminal α -syn residues, indicating an increase in the fraction of unbound α -syn. Since Sap C and α -syn do not interact in solution,^{5a} the displacement of α -syn is due to Sap C binding to GCCase.

The association between Sap C and GCCase was detected by NMR spectroscopy using isotopically labeled Sap C (Figure 2A, bottom panel). Intensities for all residues of Sap C were reduced in the presence of GCCase. Since Sap C is a folded protein, all of its residues undergo slower tumbling upon interaction. Adding α -syn results in partial recovery for all residues, indicating that α -syn also can cause Sap C to dissociate from GCCase. Given that Sap C has regions that are rich in acidic residues (Figure 1a), some of which have been previously proposed to interact with GCCase,¹³ Sap C and α -syn could have overlapping GCCase binding sites (Figure S1), though displacement via an allosteric effect cannot be ruled out.

To study the α -syn-GCCase interaction both in solution and in the presence of vesicles, we turned to site-specific fluorescence spectroscopy. In the GCCase-bound form, fluorescence of dansyl (Dns)-labeled α -syn at residue 136 exhibits an intensity increase and a spectral blue-shift upon enzyme interaction both in solution and on lipid vesicles reflecting reduced mobility and increased hydrophobic environment.⁵ Adding Sap C reverses these trends with a concomitant decrease in intensity and spectral red shift (Figure 2b inset). We confirmed that Sap C has negligible effect on Dns136 fluorescence in the absence of GCCase in solution^{5a} and on the membrane (Figure 2b, triangles). By comparison, the concentration of Sap C required to dissociate α -syn from GCCase on the membrane is higher than that in solution. This observation may result from differences in membrane binding affinities between α -syn and Sap C.

Several GCCase: α -syn and GCCase:Sap C binding models were tested, and our data are best described by a 1:1 and 2:1 GCCase to α -syn and Sap C model, respectively (Figure S2). Using the previously determined α -syn dissociation constants ($K_D = 1$ and $4 \mu\text{M}$ in the absence and presence of vesicles, respectively), the changes in Dns136 mean wavelength

(< >) and intensity can be fit to yield apparent K_D values for Sap C binding to GCCase: 0.26 ± 0.13 and 2.4 ± 0.5 μM in solution and in the presence of vesicles, respectively.

To further characterize the dissociation of α -syn from GCCase induced by Sap C, we performed Förster energy transfer experiments using intrinsic Trp residues in GCCase as donors and Dns- α -syn as the acceptor. Sap C does not contain any native Trp residues. Unlike in solution, sites in both N- and C-terminal regions of α -syn interact with and are in close proximity (~11–33 Å) to GCCase on the lipid membrane.^{5b} Using Dns-sites (7, 40, 57, and 136), the extent of energy transfer between Trp and Dns was monitored with added Sap C (Figure 2c and Figure S3). As expected the addition of Sap C (10 μM) reduced the efficiency of energy transfer as a result of α -syn displacement, with de-quenching and quenching of Trp and Dns fluorescence, respectively. We note that Trp intensity from GCCase in the presence of vesicles is slightly changed upon the addition of Sap C, indicating that Sap C interaction may influence the environment of one or more Trp residues in GCCase (Figure S3 and S4). Nevertheless, energy transfer is lost as measured from Dns fluorescence, confirming that Sap C impedes the interaction of GCCase both on and off the membrane.

In summary, Sap C protects GCCase from α -syn inhibition and competes with α -syn binding. Taken together with the reported inverse correlation between GCCase and α -syn levels derived from tissue culture, animal models, and patients,⁴ Sap C could act as a modifier in the homeostasis of α -syn. Our results might suggest two possible disease implications. First, the high levels of α -syn in neurons could lead to greater attenuation of neuronal GCCase activity in patients deficient in Sap C, and this association could potentially explain why *GBA1* mutations cause neuronopathic GD in some patients, but not in others. Second, if α -syn-GCCase interaction promotes PD pathology *via* activity inhibition,^{5b} then Sap C could play a protective role by removing α -syn from GCCase. In this scenario, Sap C deficiency would be a risk factor for PD. Alternatively, if interaction of α -syn with GCCase is involved in its normal lysosomal degradation as previously hypothesized,^{5a} then increased Sap C levels displacing α -syn could potentially be harmful. In fact, high levels of Sap C have been observed in the spleen and blood of GD patients,¹⁴ though this has not been evaluated in the brain. Further investigation is clearly needed to determine if and to what extent Sap C and/or the interplay between Sap C, α -syn, and GCCase is involved in PD. Resolution of these different viewpoints will require quantification of the physiological concentrations of α -syn, Sap C, and GCCase in lysosomes from brain samples of patients with *GBA1* mutations as well as PD, GD, and healthy individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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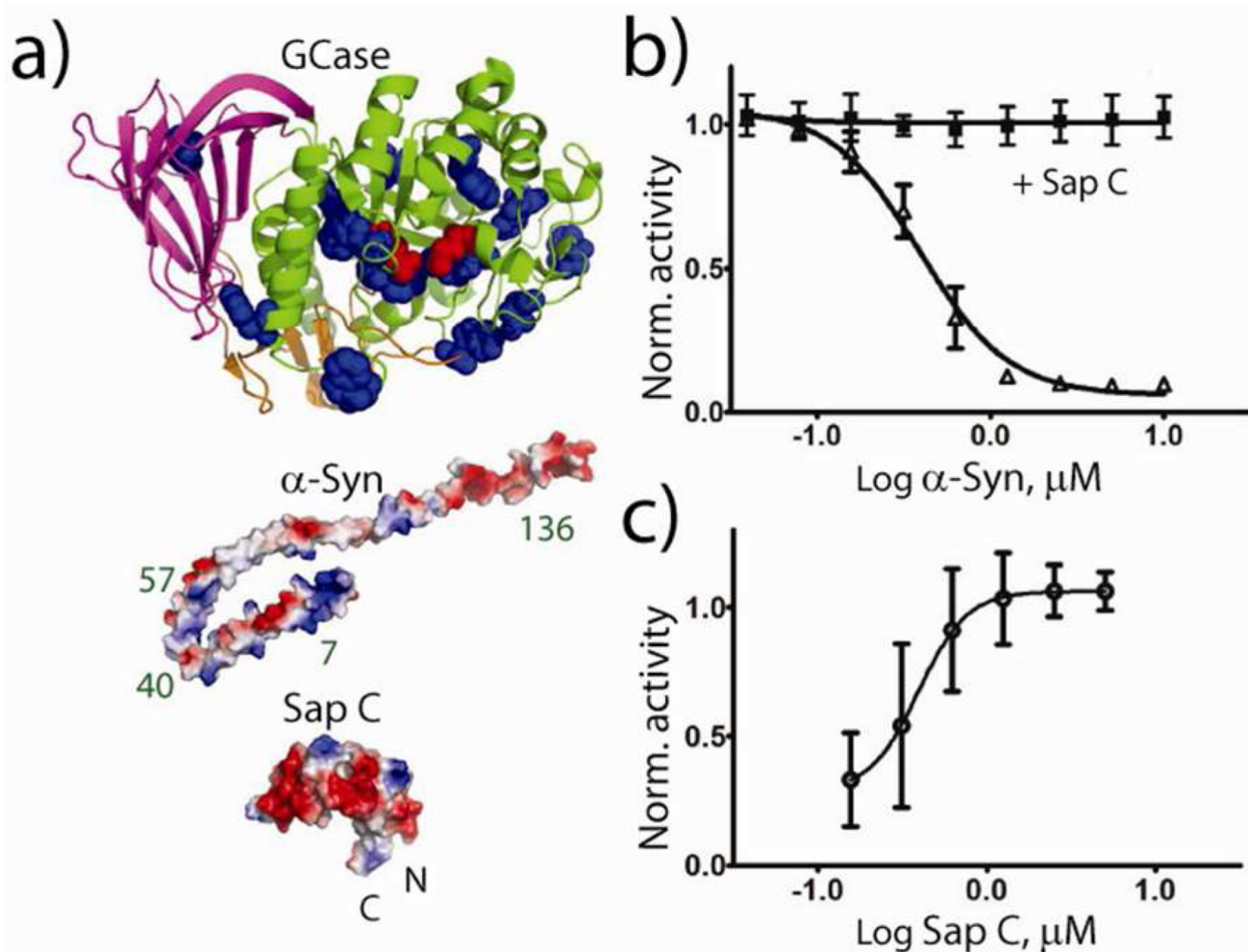


Figure 1.

(a) Molecular structures of GCCase, α -syn, and Sap C. GCCase (PDB code 2NSX) with its 12 Trp residues (used as Förster energy transfer donors) shown in blue and active site residues (E235 and E340) in red. α -Syn (PDB code 1XQ8) and Sap C (PDB code 1SN6) with positive (blue) and negative (red) electrostatic potentials shown. Cys-mutation sites of α -syn used for Dns labeling are noted. (b) GCCase activity (50 nM GCCase, 1 mM 4-methylumbelliferyl β -D-glucopyranoside, 350 μ M POPC:POPS vesicles, pH 5.5) with increasing α -syn concentration in the absence (triangles) and presence of 5 μ M Sap C (squares). (c) GCCase activity titrated by increasing concentrations of Sap C in the presence of 10 μ M α -syn. Activity levels are normalized to GCCase alone and error bars indicate standard deviations from two independent measurements.

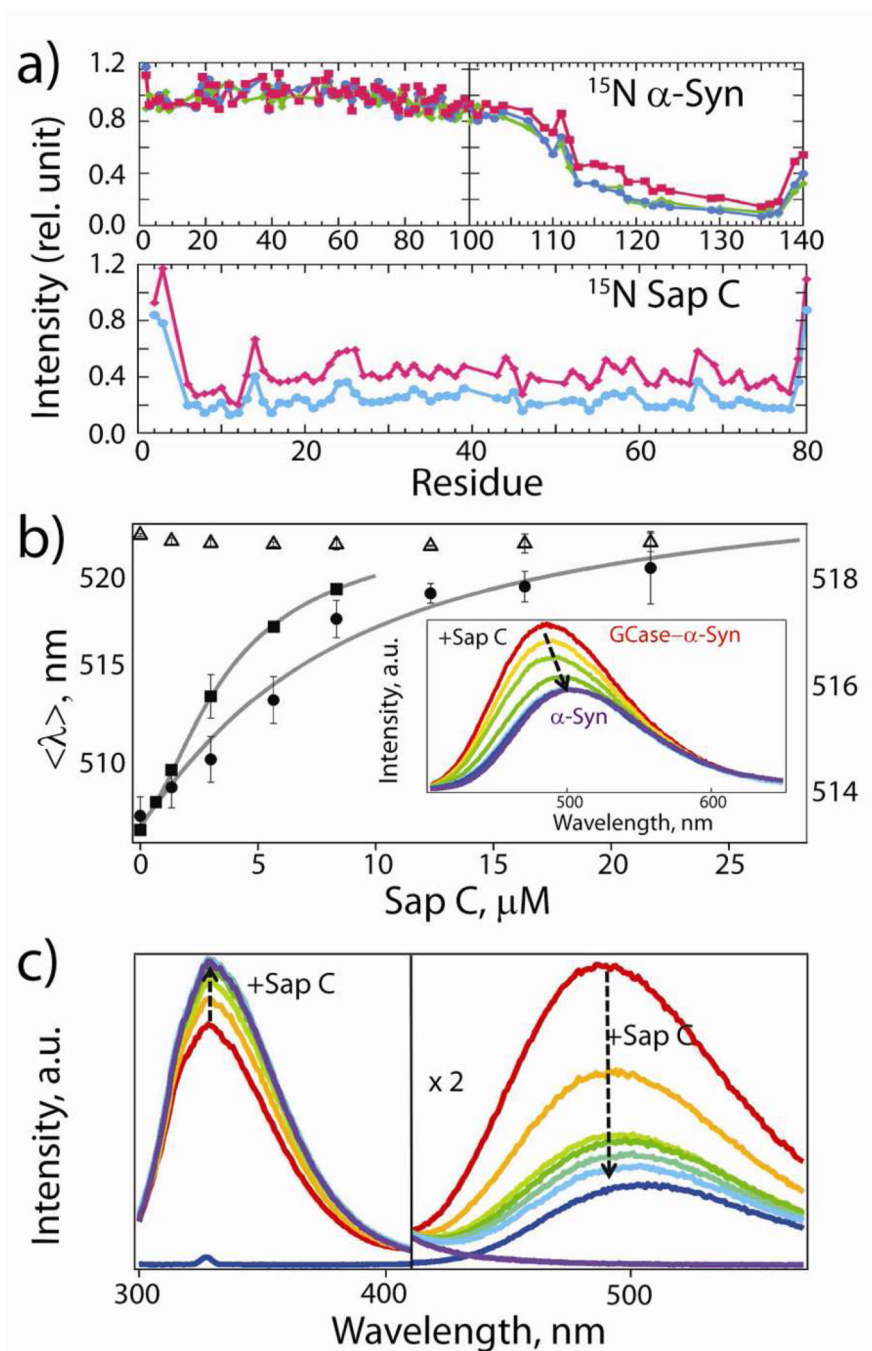


Figure 2. (a) NMR ^{15}N HSQC spectra backbone amide intensities plotted by residue of α -syn (top) and Sap C (bottom). (Top) Data for α -syn with GCCase (Taliglucerase α) alone and in the presence of Sap C are shown in blue and pink, respectively. Data for α -syn with GCCase (Imiglucerase) are shown in green. The portion of the acidic C-terminal region of α -syn (residues 101–140) that experiences the greatest intensity quenching is expanded. The α -syn intensity quenching due to interaction with both FDA approved versions of recombinant GCCase is the same within error. They differ in the composition of glycans and amino acids at the N- and C-termini. ^{15}N Protein concentrations were $50 \mu\text{M}$ for GCCase, α -syn and Sap C.

(Bottom) Data for Sap C with GCase alone and in the presence of α -syn are shown in blue and pink, respectively. Protein concentrations for GCase, α -syn, and Sap C were 33, 76, and 38 μ M, respectively. **(b)** Spectral changes of Dns136- α -syn-GCase as a function of increasing Sap C. Mean wavelength, $\langle \lambda \rangle$, is plotted with left and right axes for proteins in solution (squares, 50 mM MES pH 5.5, and 25 mM NaCl) and vesicles (circles, 0.9 mM POPC:POPS), respectively. Dns136- α -syn (2 μ M) and GCase (5 μ M) were used. Data for α -syn alone in vesicles are also shown (triangles, left axis). In the absence of vesicles, $\langle \lambda \rangle = 523$ nm. Error bars indicate standard deviations from two independent measurements. (Inset) Dns136- α -syn-GCase titration with Sap C. **(c)** Förster energy transfer from GCase (2 μ M) to Dns136- α -syn (5 μ M, red curve) in the absence of vesicles followed by the addition of Sap C. Fluorescence spectra of GCase and Dns136- α -syn alone are shown in purple and blue, respectively. Intensity scale for Dns wavelengths have been expanded for clarity.