

Saposin C Protects Glucocerebrosidase against α -Synuclein Inhibition

Thai Leong Yap,[†] James M. Gruschus,[†] Arash Velayati,[‡] Ellen Sidransky,^{*,‡} and Jennifer C. Lee^{*,†}

[†]Laboratory of Molecular Biophysics, Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, and [‡]Section on Molecular Neurogenetics, Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892, United States

S Supporting Information

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 nuclear magnetic resonance 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 that 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 hallmark of PD.

A growing number of studies show a correlation between GCase deficiency and increased α -syn levels,⁴ leading some to speculate that the accumulation of GluCer 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 the question of whether reduced GCase activity alone leads to increased α -syn levels is currently unresolved.⁷ Because 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 cofactor saposin C (Sap C),⁸ a 9 kDa membrane-interacting protein (Figure 1a, bottom).⁹ Sap C has been proposed to function by altering lipid bilayer properties or through direct association with GCase.¹⁰ Although rare, Sap C deficiency alone can result in GD symptoms in patients,¹¹ demonstrating its

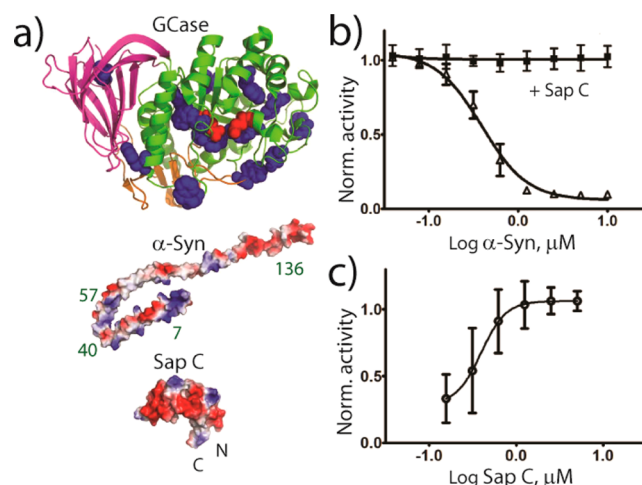


Figure 1. (a) Molecular structures of GCase, α -syn, and Sap C. GCase [Protein Data Bank (PDB) entry 2NSX] with its 12 Trp residues (used as Förster energy transfer donors) colored blue and active site residues (E235 and E340) colored red. α -Syn (PDB entry 1XQ8) and Sap C (PDB entry 1SN6) with positive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

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 cofactor *in vivo*, can affect GCase activity inhibition by α -syn *in vitro*.^{5b} Consistent with our previous study,^{5b} an IC_{50} of ~ 0.4 μ M is obtained for α -syn inhibition using a fluorogenic substrate and vesicle assay [Figure 1b, equimolar phosphatidylcholine (POPC) and phosphatidylserine (POPS) (pH 5.5)]. Remarkably, inhibition is completely abolished in the presence of Sap C (5 μ M) at all measured concentrations of α -syn (Figure 1b). At the highest α -syn concentration (10 μ M), Sap C protects GCase in a

Received: August 28, 2013

Revised: September 26, 2013

Published: September 26, 2013

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.

Because Sap C had been proposed to bind to GCase directly, we questioned whether Sap C displaces α -syn from GCase, 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 (three Asp and five Glu) residues was identified as the site of interaction with GCase.^{5a} Association with GCase ($\sim 60 \text{ kDa}$) 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 unchanged intensities (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. Because Sap C and α -syn do not interact in solution,^{5a} the displacement of α -syn is due to Sap C binding to GCase.

The association between Sap C and GCase 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 GCase. Because 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 GCase. Given that Sap C has regions that are rich in acidic residues (Figure 1a), some of which have been previously proposed to interact with GCase,¹³ Sap C and α -syn could have overlapping GCase binding sites (Figure S1 of the Supporting Information), though displacement via an allosteric effect cannot be ruled out.

To study the α -syn–GCase interaction both in solution and in the presence of vesicles, we turned to site-specific fluorescence spectroscopy. In the GCase-bound form, the 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 a reduced mobility and a more hydrophobic environment.⁵ Adding Sap C reverses these trends with a concomitant decrease in intensity and a spectral red shift (Figure 2b, inset). We confirmed that Sap C has a negligible effect on Dns136 fluorescence in the absence of GCase in solution^{5a} and on the membrane [Figure 2b (Δ)]. By comparison, the concentration of Sap C required to dissociate α -syn from GCase 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 GCase– α -syn and GCase–Sap C binding models were tested, and our data are best described by a model with a 1:1 GCase: α -syn ratio and a 2:1 GCase:Sap C ratio (Figure S2 of the Supporting Information). 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 the Dns136 mean wavelength (λ) and intensity can be fit to yield apparent K_D values for binding of Sap C to GCase: 0.26 ± 0.13 and $2.4 \pm 0.5 \mu\text{M}$ in solution and in the presence of vesicles, respectively.

To further characterize the dissociation of α -syn from GCase induced by Sap C, we performed Förster energy transfer experiments using intrinsic Trp residues in GCase as donors and Dns- α -syn as the acceptor. Sap C does not contain any native Trp residues. Unlike the case in solution, sites in both N- and C-terminal regions of α -syn interact with and are in the proximity (~ 11 – 33 \AA) of GCase on the lipid membrane.^{5b} Using Dns sites (7, 40, 57, and 136), the extent of transfer of energy between Trp

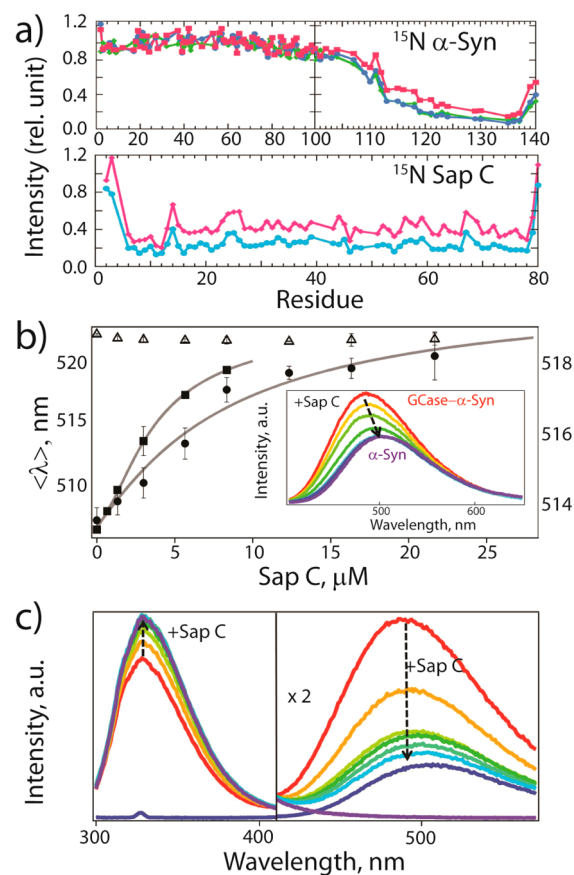


Figure 2. (a) NMR ^{15}N HSQC spectra backbone amide intensities plotted by residue of α -syn (top) and Sap C (bottom). The top panel shows data for α -syn with GCase (Taliglucerase alpha) alone (blue) and in the presence of Sap C (pink). Data for α -syn with GCase (Imiglucerase) are colored green. The 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 GCase is the same within error. They differ in the composition of glycans and amino acids at the N- and C-termini.¹⁵ Protein concentrations were $50 \mu\text{M}$ for GCase, α -syn, and Sap C. The bottom panel shows data for Sap C with GCase alone (blue) and in the presence of α -syn (pink). Protein concentrations for GCase, α -syn, and Sap C were 33, 76, and $38 \mu\text{M}$, respectively. (b) Spectral changes of the Dns136- α -syn–GCase complex as a function of increasing Sap C concentration. The mean wavelength, λ , is plotted with left and right axes for proteins in solution [(■) 50 mM MES (pH 5.5) and 25 mM NaCl] and vesicles [(●) 0.9 mM POPC/POPS], respectively. Dns136- α -syn ($2 \mu\text{M}$) and GCase ($5 \mu\text{M}$) were used. Data for α -syn alone in vesicles are also shown (Δ , left axis). In the absence of vesicles, $\lambda = 523 \text{ nm}$. Error bars indicate standard deviations from two independent measurements. The inset shows titration of the Dns136- α -syn–GCase complex with Sap C. (c) Förster energy transfer from GCase ($2 \mu\text{M}$) to Dns136- α -syn ($5 \mu\text{M}$, red curve) in the absence of vesicles followed by the addition of Sap C. Fluorescence spectra of GCase and Dns136- α -syn alone are colored purple and blue, respectively. The intensity scale for Dns wavelengths has been expanded for the sake of clarity.

and Dns was monitored with added Sap C (Figure 2c and Figure S3 of the Supporting Information). As expected, the addition of Sap C ($10 \mu\text{M}$) reduced the efficiency of energy transfer as a result of α -syn displacement, with dequenching and quenching of Trp and Dns fluorescence, respectively. We note that the Trp intensity from GCase in the presence of vesicles is slightly changed upon addition of Sap C, indicating that Sap C

interaction may influence the environment of one or more Trp residues in GCase (Figures S3 and S4 of the Supporting Information). Nevertheless, energy transfer is lost as measured via Dns fluorescence, confirming that Sap C impedes the interaction of GCase both on and off the membrane.

In summary, Sap C protects GCase from α -syn inhibition and competes with α -syn binding. Taken together with the reported inverse correlation between GCase 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 GCase 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–GCase interaction promotes PD pathology via the inhibition of activity,^{5b} then Sap C could play a protective role by removing α -syn from GCase. In this scenario, Sap C deficiency would be a risk factor for PD. Alternatively, if interaction of α -syn with GCase 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 among Sap C, α -syn, and GCase is involved in PD. Resolution of these different viewpoints will require quantification of the physiological concentrations of α -syn, Sap C, and GCase in lysosomes from brain samples of patients with *GBA1* mutations as well as PD, GD, and healthy individuals.

■ 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>.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: sidranse@mail.nih.gov. Telephone: (301) 402-6438. Fax: (301) 402-6438.

*E-mail: leej4@mail.nih.gov. Telephone: (301) 496-3741. Fax: (301) 402-3404.

Author Contributions

T.L.Y. and J.M.G. contributed equally to this work.

Funding

Supported by the Intramural Research Program at the National Institutes of Health, the National Heart, Lung, and Blood Institute, and the National Human Genome Research Institute.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Recombinant GCase was a gift from Protalix Biotherapeutics (Carmiel, Israel). The Sap C plasmid was provided by Gilbert Privé (University of Toronto, Toronto, ON). We thank Nico Tjandra [National Heart, Lung, and Blood Institute (NHLBI)] for the use of NMR spectrometer, Duck-Yeon Lee (NHLBI Biochemistry Core Facility) for technical assistance with mass spectrometry, and Zhiping Jiang (NHLBI) for the expression of isotopically labeled Sap C.

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