H50Q MUTATION PROMOTING A-SYNUCLEIN BINDING TO VESICLE

ZHAN, T.

Hwa Chong Institution, Urban Vista 269734, Singapore
(e-mail: zhan.tyx0619@outlook.com)

(Received 22nd Apr 2024; accepted 2nd Sep 2024)

Abstract. The abnormal accumulation of α-synuclein (αSyn) is thought to be linked to the progression of synucleinopathies, such as Parkinson's disease, with lipids being identified as one of the primary triggers. Mutations associated with familial Parkinson's disease (FPD) are hypothesized to alter the characteristics of αSyn, potentially influencing its interaction with lipids. Through the utilization of various fluorescence techniques in our investigation, we discovered that the H50Q mutation enhances the binding of αSyn to synaptic-like vesicles. This finding may contribute to a better understanding of the pathology associated with the H50Q mutation in αSyn.
Keywords: *lipids, misfolded aggregates, Parkinson's disease, lipid-protein interaction, therapeutic target*

Introduction

In the past 24 years, a direct relationship has been discovered between the formation of neuropathological lesions and degenerative processes (Perl, 2010). Parkinson's disease (PD) has been found to be one of the most common neuropathological degenerative diseases, affecting 1%-2% of the general population above the age of 65 (Tanner and Goldman, 1996). PD is identified by the degeneration of nigrostriatal dopaminergic neurons, affecting not only the dopaminergic region in the substantia nigra but also impacting cells in various other areas of the nervous system. The most defining neuropathological characteristic of this disease is the misfolded aggregates of a type of protein, named α-synuclein (αSyn), in the form of Lewy Body or Lewy neurites (Kouli et al., 2018). αSyn is a type of 14 kDa intrinsically disordered protein which is highly conserved (Liberski and Ironside, 2014). The physiological function of αSyn is not very well understood, though evidence indicates that it probably plays a role in maintaining neuronal plasticity and regulation in synaptic vesicle recycling (Emanuele and Chieregatti, 2015). Moreover, the high proportion of αSyn found in the central nervous system, particularly in presynaptic terminals, suggests that αSyn may also act a role in synaptic vesicle trafficking (Stefanis, 2012). Additionally, αSyn promotes the formation of presynaptic soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes by directly interacting with the N-terminus of synaptobrevin-2/VAMP protein (Burré et al., 2010).

αSyn is intrinsically unstructured in solution but it undergoes a structural change upon its association with lipid membranes. A number of αSyn residue, up to the first 95th place, undergoes a conformational transformation from random coiling to α-helical structure, and the proportion of the involved sequence depends on the lipid-to-protein ratio (Bodner et al., 2009). When the N-terminal region begins binding with the membrane, the hydrophobic residues will present inside the membrane, residues with negative charge exposed to the solvent while positively charged residues bind with hydrophilic head of phospholipid membrane by electrostatic interactions. The arrangement of the formed a-helix structure is relevant to the membrane curvature (Jao et al., 2008). For example, the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_58635874

continuous α-helix that binds with 100 nm lipid vesicle can be split into two individual helices and form an extended helix, whereas α-helix will be present in a bent shape (Varkey et al., 2013). In general, the N-terminus of αSyn interacts with the membrane instead of the C-terminus, which maintains the high flexibility of αSyn. The binding of the protein with lipid is said to induce changes to the membrane's melting point, membrane thinning, membrane remodelling and membrane expansion (Shi et al., 2015). Chemical, physical and thermotropic properties of the membrane are the principal factors that affect the strength of the binding of αSyn. This lipid-protein interaction is said to not only play a pivotal role in αSyn native function, synaptic plasticity, but also in the pathology in the formation of PD. Studies have shown that the interactions of lipid vesicles with oligomeric or monomeric states of αSyn are likely to impact the initiation of both the reaction of fibrilisation formation and the amplification of toxic aggregates (Bell and Vendruscolo, 2021).

Different types of mutations are being widely researched in a-synuclein regarding the formation of familial forms of PD. The abnormal aggregation resulting from the mutation could cause mitochondrial dysfunction, which will result in many downstream effects. However, the relationship between the different types of mutation of a-synuclein protein and the phenotype of PD is still rather unclear. So far, studies show that most of the PD cases are sporadic while only 10% are familial disease. Six mutations including A30P (Lei et al., 2019), E46K (Boyer et al., 2020), A53T (Teravskis et al., 2018), A53E (Rutherford and Giasson, 2015), H50Q (*Fig. 1*) (Khalaf et al., 2014) and G51D (Lau et al., 2023) are identified on SNCA gene in familial PD. Interestingly, all of these mutations are found in the N-terminus of αSyn, indicating that they may have an effect on lipid binding. It has been shown that mutations such as E46K (Lei et al., 2019), and A53T (Boyer et al., 2020) can improve αSyn's lipid binding affinity and promote fibril formation, whereas A30P (Bell and Vendruscolo, 2021), G51D (Khalaf et al., 2014), and A53E (Teravskis et al., 2018) decrease lipid binding affinity and fibrilisation rate. The varied properties of the protein are caused by different mutation points. The effect of mutation, H50Q, in a-synuclein was also investigated, and results have shown that compared with wild-type αSyn, H50Q mutation increased the speed of fibrillization of a-synuclein in vitro (Khalaf et al., 2014). However, little research has been done on the relationship between H50Q mutation and αSyn-lipid aggregation.

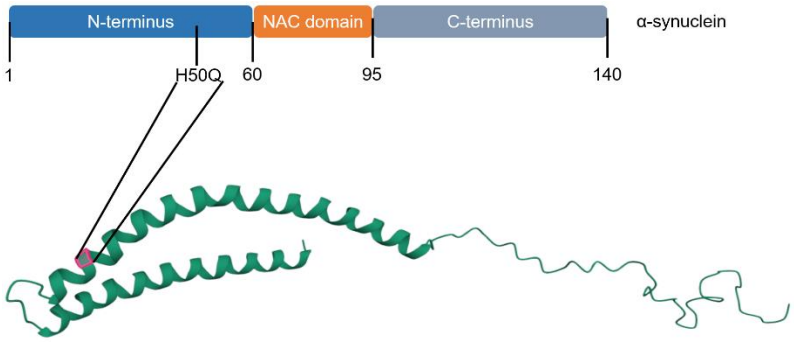

Figure 1. *The graphical representation of α-synuclein with the H50Q mutant*

Fluorescent methods are widely used to quantify and analyze the interactions of αSyn with lipid vesicles. Fluorescence correlation spectroscopy (FCS) is a powerful tool to quantify a-synuclein-lipid interactions, it provides insight into the biophysical interaction

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_58635874

mechanisms. For example, a research done by Rhoades et al. (2006) using FCS, has shown that the affinity of a synuclein with higher proportion of negatively charged lipids is higher than that with low proportions. Single molecule Förster resonance energy transfer (FRET) has also been used in several studies to probe the structure of αSyn bound on detergent micelles and lipid vesicles (Tosatto et al., 2015). FRET could potentially help to identify the amount of connections could be made after the binding of αSyn with lipid vesicles. It was used to analyze the binding affinity toward micelle and large unilamellar vesicles and the structure of protein chain after binding (Trexler and Rhoades, 2009). Fluorescence anisotropy is also used to analyze the binding affinity of αSyn and lipid membrane which is indicated by the free rotation of fluorophores (Gijsbers et al., 2016).

αSyn is believed to act as a crucial role in PD and Lewy body dementia yet the structure arrangement of αSyn in biomolecular interactions as well as the role in those diseases are still unclear. Our project will focus on the FPD mutation, H50Q, and its impact on the interactions with small unilamellar vesicles (SUV) using fluorescence methods, to have a deeper understanding of the conformation and pathological features of αSyn.

Methods

Lipid vesicle preparation

Lipid Coag Reagent I (DOPS: DOPC: DOPE = 3:2:5 w/w) was provided as lyophilized powders by Avanti Polar Lipids. In experiments, it was dissolved in methanol-chloroform (volume ratio: 1:2). The solution was then aliquoted into glass vials and subjected to drying under a nitrogen flow. The obtained lipid films were exposed to nitrogen overnight to completely eliminate any remaining solvent. To produce small unilamellar vesicles (SUV), the lipid samples were initially dissolved in required buffer with vortexing for an hour. The preparation was done by extrusion through membranes with a 50-nm pore diameter (Nuclepore, Whatman) using a glass extruder (Avanti Mini-Extruder). The produced SUV suspension may be kept at 4 degrees Celsius for two days.

αSyn purification and labelling

αSyn was first expressed though BL21(DE3) cell(Thermo Fisher Scientific Ltd) grown in LB at 37 ℃, which contains 100 μg/ml ampicillin. IPTG was added to 1 mM once the absorbance value at 600 nm (OD_{600}) came to 0.5, and LB was incubated for 3 h at 37 ℃ to induce the expression. The cells were subsequently collected by centrifugation in a Beckman J-26XP centrifuge (Beckman Coulter) at 8000 g for 30 minutes. 20 mM Tris-HCl buffer with a pH of 7.4, containing 5 mM EDTA and 1 mM protease inhibitor (cOmplete, Roche) was used to resuspend cell pellet, which was then lysed for two minutes using a tip sonicator. The generated suspension was then heated at 90 °C for 20 minutes before being centrifuged in a Micro Star 17 centrifuge (VWR) at 16000 g for 20 minutes. The collected supernatant was then filtered to eliminate any cell debris using a 0.2 m 25 mm diam. filter (Acrodisc, Pall Corporation). The supernatant was then treated with streptomycin sulfate at a concentration of 10 mg/ml to remove nucleic acid. The blend was agitated at 4 °C for 15 minutes. Following centrifugation at 16000 g for 20 minutes, the supernatant was collected, and ammonium sulfate was subsequently introduced to achieve 50% saturation. After stirring at 4 °C for 30 minutes, the mixture was centrifuged again at 16000 g. The pellet was recovered and resuspended in 20 mM

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_58635874

Tris-HCl and 50 mM NaCl (pH 7.4) before being dialysed overnight. The obtained protein concentration was measured using a Lambda 25 UV/Vis spectrometer with an extinction value of 5600 M^{-1} cm^{-1} (PerkinElmer, Massachusetts, USA).

Fluorescent labelling of protein was performed using a selective thiol-maleimide reaction. The first step was to generate a cysteine mutation at position 7 by a Phusion site-directed mutagenesis kit (Invitrogen). The dye will attach preferentially at position 7 since the altered residue 7 is the lone cysteine in the αSyn sequence. After that, the working buffer (20 mM Tris and 50 mM NaCl solution, pH 7.4) was bubbled with nitrogen flow for 1 hour to remove oxygen. To decrease disulfide linkages, the protein solution was always thawed on ice, then mixed with a 10-fold molar excess of TCEP. Next, to remove TCEP, the protein was eluted using the deoxygenated buffer through a disposable PD-10 desalting column (Cytiva). The concentration of obtained protein was calculated using an extinction value of 5600 M^{-1} cm^{-1} and the absorbance at 280 nm. Following that, a 3: 1 molar ratio (dye: protein) of Alexa Fluor 488 maleimide dye (Thermo Fisher Scientific, Massachusetts, USA) solution in DMSO was mixed with the eluted protein solution. The solution was stirred in the dark for 3 hours. The tagged protein solution was then desalted and concentrated for 5 minutes at 15000 g using a 10K MWCO protein concentrator (Pierce, Thermo Fisher Scientific). The protein samples were kept at -80 °C. The final concentration of labelled αSyn was calculated using an extinction value of 72000 M^{-1} cm^{-1} with Abs. at 495 nm. The total protein concentration was calculated by the following equation (1) (0.11 is a correction factor to dye's contribution to A_{280}).

$$Protein\ concentration\ (M) = \frac{A_{280} - 0.11(A_{495})}{molar\ \ of\ protein\ at\ 280\ nm} \qquad \text{(Eq.1)}$$

The labelling efficiency was around 95% based on the ratio of tagged to total protein content.

For FRET measurements, a dual labelled S9C-F94C Syn (WT and H50Q mutant) was generated. The donor fluorophore was Alexa 488 maleimide tag (Invitrogen), while the acceptor was Alexa 594 maleimide tag (Invitrogen). The labeling was done in the same way as single labeling, but with a different dye-protein mixing molar ratio (Alexa 594: Alexa 488: αSyn = 6: 2: 1).

Using a same genetic alteration technique, H50Q mutated αSyn was created. The sequencing result is shown in *Fig. 2*.

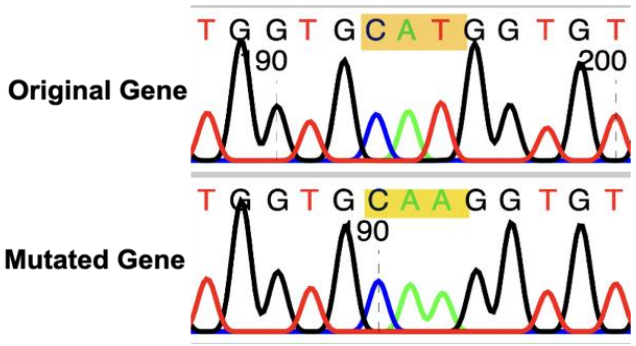

Figure 2. *H50Q mutation in vector. Sanger sequencing reveals a histidine at position 50 (CAT) to glutamine (CAA) codon change*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_58635874

CD measurements

CD experiments were carried out by a Chirascan V100 CD spectrometer (Chirascan V100, Applied Photophysics). A 110-QS quartz absorption cuvette (Hellma Analytics) was used in measurements. About 70 μM of αSyn diluted in 1× PBS buffer was prepared in the experiments.

FCS and smFRET measurements

FCS and smFRET experiments were conducted using a custom-built confocal microscope setup. The excitation light source utilized was a 35LAP321-230 argon ion laser (Melles Griot). This laser beam underwent several adjustments and optimizations before entering the microscope. Firstly, it passed through a neutral density filter to regulate its intensity. Subsequently, the beam was directed through a Dove prism to eliminate laser plasma lines. Following this, a beam expander focused the beam to a specific point, centered on a spatial filter to correct optical aberrations. A collimating lens then recollimated the beam, which was further refined in size and quality by passing through an aperture. Before entering the microscope, the laser power was measured using a S122 laser power meter (Thorlabs). The Eclipse TE2000-U inverted microscope (Nikon) utilized in these experiments was equipped with a dichroic mirror, a lens, and a CFI Apochromat TIRF 60×C Oil, NA 1.49 objective (Nikon). The incident beam was reflected by the dichroic mirror onto the objective, which was immersed in Type FF immersion oil (Cargille Labs). A chamber slide containing the sample was positioned on the objective, and the beam was focused into the sample solution 6 μm above the slide's bottom. The emission fluorescence from the sample passed through the dichroic and was focused by the lens onto a confocal pinhole. Subsequently, the emission beam was split by a beam splitter / dichroic mirror. The optical system employed a 50 ns dead time avalanche photodiode (APD) detectors. In FCS measurements, a beam splitter was used to divide the fluorescence beam, enabling independent detection and cross-correlation of the split beams to enhance temporal resolution by mitigating the dead time effect. While in smFRET experiments, the dichroic mirror separated donor and acceptor's fluorescence for detection. Emission filters were used to purify the beams from background before reaching the APDs (PerkinElmer). The APDs were connected to a 1.5625 ns sampling time digital hardware correlator for FCS experiments, and also linked to a multichannel scaler with a maximum input counting rate of 150 MHz for smFRET experiments.

Fluorescence anisotropy

Anisotropy investigations were carried out employing a spectrofluorometer (FluoroMax-4, Horiba). During measurements, samples were added into a quartz cuvette (105.250-QS, Hellma Analytics). This cuvette was then placed into a cell holder within the sample compartment for analysis. To measure fluorescence anisotropy, two polarizers situated at the optical entrance and exit of the sample compartment were engaged to select the desired polarized light. Anisotropy values were automatically computed based on the detected intensity parameters.

Data analysis

In this work, obtained FCS curves were fitted by the following equation:

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_58635874

$$G(\tau) = \frac{G(0)}{1+(\tau/\tau_D)}(1 - F + Fe^{-\frac{\tau}{\tau_m}}) + C \quad \text{(Eq.2)}$$

where $G(0)$ represents the obtained correlation curve amplitude, τ_D represents the average diffusion time for a fluorescent molecule diffusing through the observation volume, F represents the fraction of fluorescent molecules in the triplet state, and τ_m represents the average triplet lifetime.

The equation used to calculate binding affinity (K_d) from fluorescence anisotropy is a modified Hill-Langmuir equation:

$$\theta = \frac{A}{1+\frac{K_d}{[L]}} + C \quad \text{(Eq.3)}$$

where A represents the amplitude of curves, [L] represents the ligand concentration and C represents the value of the beginning point.

Results

Circular Dichroism (CD) measurement was conducted to study the binding of WT-αSyn and H50Q αSyn with synaptic-like SUV. For this experiment, 15 uM of αSyn samples were mixed with 1 mM of lipid. The results are shown in *Fig. 3*. It can be seen that with the presence of SUV, the spectra of both WT αSyn and H50Q αSyn show a signal decrease at 220 nm, which indicates that both WT and H50Q αSyn can bind to SUV and form a α-helix structure. While the H50Q αSyn induced signal decrease is a little larger than WT αSyn induced, which may suggest that H50Q mutation can promote the αSyn-SUV interaction.

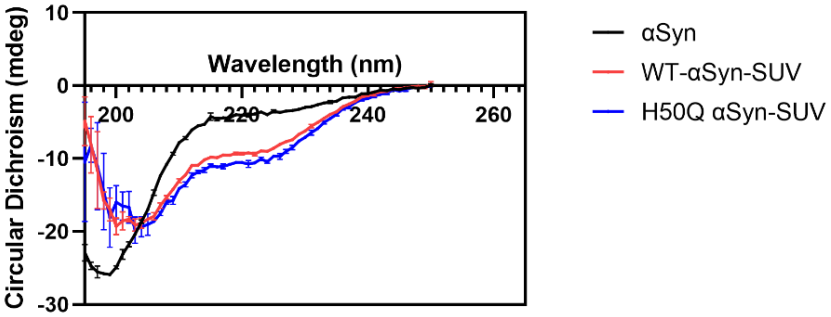

Figure 3. *CD spectra of αSyn with and without the addition of synaptic-like SUV*

To measure, 15 μM αSyn samples were added to a 1 mm cuvette. This experiment was carried out at 298 K in 1× PBS buffer (pH 7.5). Each spectrum is the average of three repetitions.

The binding of αSyn and SUV was then investigated using FCS technique. 10nM of labelled αSyn samples were mixed with 1 mM lipid (~40nM SUV). The molecular diffusion time was detected. The result is shown in *Fig. 4*. As can be seen, WT-αSyn and H50Q αSyn in the existence of SUV have longer diffusion times (τD = 5.09(6) ms and 5.14(3) ms, respectively) compared to free αSyn (τD = 0.34(3) ms), which further validates that both αSyn can bind to SUV.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_58635874

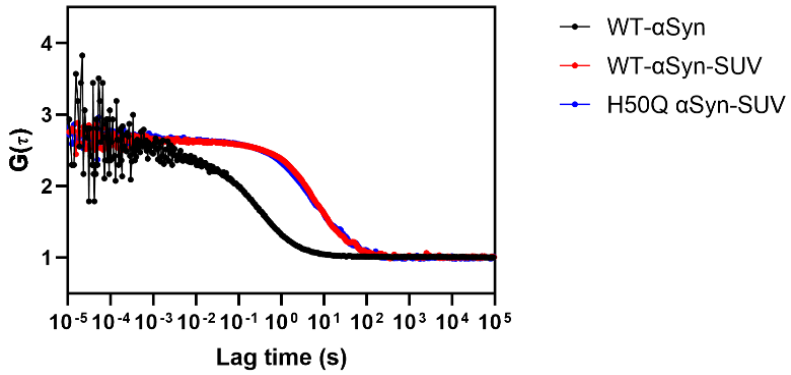

Figure 4. *FCS curves of αSyn with and without the addition of synaptic-like SUV*

Both WT and H50Q αSyn can efficiently bind with SUV according to the curves. The experiments were carried out at 298K in 50 mM HEPES, 100 mM NaCl (pH 7.5) buffer.

smFRET experiment was also performed to further study the binding between αSyn and lipid molecules. 5 nM doubly labeled αSyn was blended with 1 mM total lipid solution (around 40 nM SUV) in the experiment. Before measuring, the solution was incubated at room temperature for 10 minutes and diluted ten times. *Fig. 5* shows the FRET efficiency histograms that were produced. Comparing the frequency distribution plot of free αSyn in buffer solution (A) and the frequency distribution plot of αSyn with the addition of SUV (C), αSyn in SUV solution shows several peaks at various FRET efficiency, suggesting that αSyn monomer could bind with SUV via mixed stretched or bent helical configurations. In addition, the histogram shape of WT-αSyn bound on SUV is relatively similar to that of H50Q αSyn bound on SUV, which indicates that H50Q mutation does not influence the SUV binding configuration of αSyn.

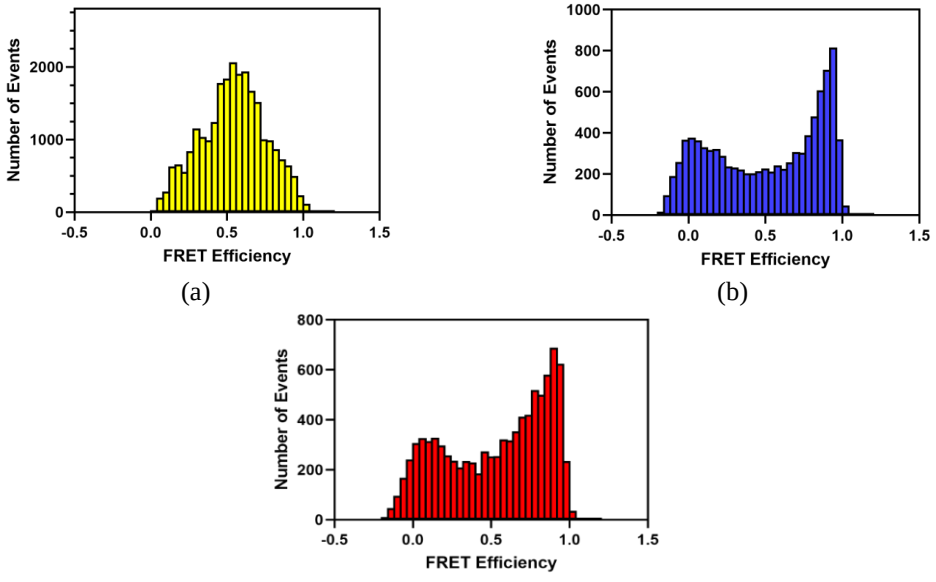

Figure 5. *smFRET histograms of αSyn with and without the addition of synaptic-like SUV. (A) αSyn in free buffer. (B) H50Q αSyn in the companionship of SUV. (C) WT-αSyn in the companionship of SUV. At 298 K, the experiments were carried out in a buffer solution of 50 mM HEPES, 100 mM NaCl (pH 7.5)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_58635874

Fluorescence anisotropy of αSyn was carried out to calculate the binding affinity between aSyn and SUV. 100nM αSyn sample was mixed with different lipid concentrations. The obtained anisotropy values were recorded by a spectrofluorometer and fitted by Hill-Langmuir equation (*Fig. 6*). This result suggests that H50Q mutation can actually increase the affinity of αSyn with lipid vesicle, as the Kd value of H50Q aSyn is 18 ± 1 μM, which is lower than WT-αSyn, which has a Kd value of 24 ± 2 μM. This result is consistent with the above CD result.

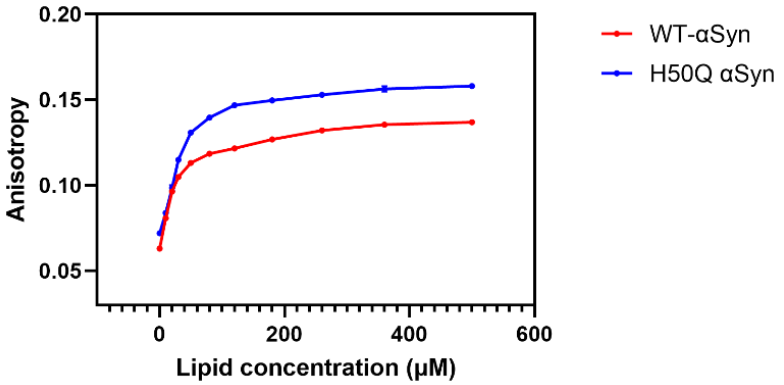

Figure 6. *Fluorescence anisotropy measurements of various αSyn isoforms binding to synaptic-like SUV. Each data point was produced by averaging ten repetitions. The experiments were conducted at 298 K in a buffer solution of 50 mM HEPES, 100 mM NaCl (pH 7.5)*

Discussion

The H50Q mutation in αSyn has emerged as a significant focus of research within the realm of neurodegenerative diseases, particularly PD (Kouli et al., 2018). αSyn, a presynaptic neuronal protein, plays a pivotal role in regulating neurotransmitter release (Kouli et al., 2018). Aberrant aggregation of αSyn, leading to the formation of insoluble fibrils known as Lewy bodies, is a hallmark of PD and other synucleinopathies. The identification of the H50Q mutation, characterized by the substitution of histidine (H) with glutamine (Q) at position 50 in the αSyn protein sequence, in individuals with familial PD has spurred investigations into the structural and functional consequences of this mutation (Rutherford and Giasson, 2015). Studies have shown that H50Q mutation can enhance the aggregation of αSyn by changing its structure and oligomeric state, showing higher toxicity to neuron and mitochondrion (Khalaf et al., 2014; Boyer et al., 2019). Lipid vesicles are crucial cellular components involved in various cellular processes, and αSyn is known to interact and regulate their trafficking function. One intriguing avenue of exploration is the interaction between the H50Q mutated αSyn and lipid vesicles, which are model systems commonly employed to mimic cellular membranes and study protein-lipid interactions (Jao et al., 2008; Bodner et al., 2009; Shi et al., 2015; Bell and Vendruscolo, 2021). Understanding the nuances of this interaction is crucial for unraveling the molecular mechanisms underpinning the pathogenicity associated with the H50Q mutation.

The interaction between αSyn and lipid vesicles is a dynamic and multifaceted process influenced by several factors, including lipid composition, vesicle size, and environmental conditions. In WT-αSyn, membrane binding is predominantly mediated by the N-terminal region, which encompasses several amphipathic alpha-helices (Jao et

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_58635874

al., 2008). This interaction not only contributes to membrane curvature sensing but also influences lipid clustering. The H50Q mutation's location within this critical N-terminal region underscores its potential significance in modulating the protein's interaction with lipid membranes.

Biophysical techniques, such as NMR, CD, and fluorescence spectroscopy, have been instrumental in probing the structural changes induced by the H50Q mutation in αSyn. These methods provide valuable insights into alterations in secondary structure, conformational dynamics, and thermodynamics associated with lipid vesicle binding. Initial studies suggest that the H50Q mutation may induce changes in the helical structure of αSyn, impacting its affinity for lipid membranes. This is confirmed by our CD and fluorescence anisotropy results, which demonstrate that H50Q mutated αSyn can induce more α-helical structure formation than WT-αSyn, and H50Q mutated αSyn has a smaller Kd binding with synaptic-like SUV. This could be a potential reason that H50Q mutation can cause PD development. As the stronger interaction with synaptic vesicle could dysfunction the detachment of αSyn molecule from synaptic vesicle, slowing down vesicle trafficking but promoting more fusion process, thus resulting in vesicle cycling dysregulation.

Lipid composition is a crucial determinant of the protein-lipid interaction, and the H50Q mutation may exhibit distinct preferences for specific lipid species. Anionic lipids, such as PS, have been shown to enhance the binding of αSyn to vesicles (Rhoades et al., 2006). Investigating the H50Q mutant's preference for particular lipid moieties can provide valuable insights into the selectivity of its interactions with cellular membranes (Jao et al., 2008; Shi et al., 2015; Gijsbers et al., 2016). Moreover, variations in lipid composition across different cellular compartments may influence the H50Q mutant's behavior, emphasizing the importance of studying its interaction with a diverse range of lipid environments (Jao et al., 2008; Gijsbers et al., 2016).

The size and curvature of lipid vesicles contribute to the complexity of the interaction between αSyn and lipid membranes (Rhoades et al., 2006; Tosatto et al., 2015; Bell and Vendruscolo, 2021). The ability of αSyn to sense and adapt to membrane curvature is a critical aspect of its function, particularly at synaptic terminals. The H50Q mutation may alter the protein's ability to interact with membranes of varying sizes and curvatures, potentially impacting its localization and function within the cell (Jao et al., 2008; Shi et al., 2015). Investigating the H50Q mutant's behavior across a spectrum of vesicle sizes can provide insights into its membrane-binding preferences and shed light on its potential role in cellular physiology.

The different lipid binding structure of αSyn may also contribute to the binding pattern, further impacting the normal function of lipid vesicle (Jao et al., 2008; Trexler and Rhoades, 2009; Bell and Vendruscolo, 2021). Here we used smFRET technique to study but found that H50Q mutated αSyn possess the similar lipid binding structure with WT-αSyn, which is a mixed stretched and bent structure. This indicates that H50Q mutation induced FPD may not be attributed to αSyn conformational change. In the future, research efforts could need to employ advanced imaging techniques, such as cryo-electron microscopy (cryo-EM), to visualize the structural details of αSyn-lipid interactions at the atomic level. These techniques enable researchers to observe the intricate interplay between αSyn and lipid vesicles, offering a three-dimensional perspective on how the H50Q mutation may alter these interactions. Cryo-EM studies can provide valuable information about the spatial organization of αSyn on lipid membranes, shedding light on potential structural changes induced by the H50Q mutation.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_58635874

Additionally, computational simulations, including molecular dynamics (MD) simulations, have become indispensable tools for studying protein-lipid interactions. MD simulations allow researchers to explore the dynamic behavior of the H50Q mutated αSyn in the presence of lipid vesicles over extended timescales. These simulations can provide insights into the stability of αSyn-lipid complexes, the dynamics of membrane binding, and the impact of the H50Q mutation on these processes. Integrating experimental and computational approaches offers a comprehensive understanding of the complex interplay between αSyn and lipid membranes.

The H50Q mutation's influence on αSyn-lipid interactions extends beyond its immediate structural effects. Exploring the potential downstream signaling events and cellular responses triggered by the H50Q mutant is crucial for connecting molecular changes to broader physiological consequences. The mutation may influence intracellular pathways, such as those involved in vesicle trafficking (Stefanis, 2006), endocytosis, and synaptic vesicle recycling (Emanuele and Chieregatti, 2015). Investigating these aspects can provide a holistic view of the H50Q mutation's impact on cellular homeostasis and its contribution to PD pathogenesis.

Moreover, the cellular context in which αSyn operates is complex, with interactions occurring in diverse subcellular compartments (Jao et al., 2008; Gijsbers et al., 2016). Studying the H50Q mutation's effects on αSyn-lipid interactions within different cellular membranes, including the plasma membrane and various organelles, is essential for capturing the full spectrum of its biological consequences. The mutation may exhibit distinct behaviors in different cellular environments, emphasizing the need for a nuanced and context-dependent understanding of its effects.

In conclusion, the H50Q mutation in αSyn represents a captivating focal point in PD research, particularly in the context of its interaction with lipid vesicles. The intricate interplay between the H50Q mutant and lipid membranes involves structural, thermodynamic, and dynamic aspects that collectively contribute to our understanding of PD pathogenesis. Integrating experimental techniques, advanced imaging methods, and computational simulations is essential for gaining a comprehensive view of the molecular events associated with the H50Q mutation. Unraveling the complexities of αSyn-lipid interactions in the presence of the H50Q mutation holds promise for uncovering novel therapeutic targets and advancing our understanding of neurodegenerative diseases. As research in this field progresses, a deeper comprehension of the H50Q mutant's impact on lipid vesicle interactions may pave the way for innovative strategies to intervene in PD and related disorders at the molecular level.

Summary

αSyn misfoldings and aggregations are closely associated with synucleinopathies, which also includes Parkinson's disease. Lipid is thought to be one of the main reasons for causing abnormal aggregations. Additionally, it is becoming clear that a growing number of SNCA genetic variants will cause familial Parkinson's disease, in which the H50Q mutation is the latest discovered one. The interactions between H50Q mutated αSyn and lipids is not well studied, thus it is consequently necessary to explore this area in order to understand the relationship between H50Q mutation and protein aggregation better.

In this work, we used a mimic synaptic vesicle system and found that H50Q mutated αSyn can also easily bind to synaptic-like SUV based on CD and FCS measurements.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_58635874

Moreover, by using an ultra-sensitive smFRET technique, H50Q mutated αSyn was found to be able to bind to synaptic-mimic SUV via mixed stretched and bent helical structures, which is actually very similar to that of WT-αSyn. Finally, we determined that H50Q mutated αSyn (Kd = 18 ± 1 μM) can bind synaptic-like SUV tighter than WT-αSyn (Kd = 24 ± 2 μM).

Overall, the pathophysiology of Parkinson disease and its function depend critically on the connection between αSyn and Lipid. Exploring relationships between αSyn and lipid can help to build a deeper understanding of its biomolecular function and designing effective cures against neurodegenerative disorders.

Acknowledgement. I would like to thank Dr. Xiangyu Teng from Imperial College London for his experimental support and help.

REFERENCES

[1] Bell, R., Vendruscolo, M. (2021): Modulation of the Interactions Between α-Synuclein and Lipid Membranes by Post-translational Modifications. – Front Neurol. 12: 661117. doi: 10.3389/fneur.2021.661117.

[2] Bodner, C. R., Dobson, C. M., Bax, A. (2009): Multiple tight phospholipid-binding modes of alpha-synuclein revealed by solution NMR spectroscopy. – J Mol Biol 390: 775-790.

[3] Boyer, D. R., Li, B., Sun, C., Fan, W., Sawaya, M. R., Jiang, L. (2019): Eisenberg DS. Structures of fibrils formed by α-synuclein hereditary disease mutant H50Q reveal new polymorphs. – Nat Struct Mol Biol 26: 1044-1052. https://doi.org/10.1038/s41594-019-0322-y.

[4] Boyer, D. R., Li, B., Sun, C., Fan, W., Zhou, K., Hughes, M. P., Sawaya, M. R., Jiang, L., Eisenberg, D. S. (2020): The α-synuclein hereditary mutation E46K unlocks a more stable, pathogenic fibril structure. – Proc Natl Acad Sci U S A. 117(7): 3592-3602. doi: 10.1073/pnas.1917914117.

[5] Burré, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M. R., Südhof, T. C. (2010): Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. – Science 329(5999): 1663-7. doi: 10.1126/science.1195227.

[6] Emanuele, M., Chieregatti, E. (2015): Mechanisms of alpha-synuclein action on neurotransmission: cell-autonomous and non-cell autonomous role. – Biomolecules 5(2): 865-92. doi: 10.3390/biom5020865. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4496700/.

[7] Gijsbers, A., Nishigaki, T., Sánchez-Puig, N. (2016): Fluorescence Anisotropy as a Tool to Study Protein-protein Interactions. – J Vis Exp. 116: 54640. doi: 10.3791/54640.

[8] Jao, C. C., Hedge, B. G., Chen, J., Harworth, I. S., Langen, R. (2008): Structure of membrane-bound α-synuclein from site-directed spin. – PNAS 105(50): 19666-19671. https://www.pnas.org/doi/full/10.1073/pnas.0807826105.

[9] Khalaf, O., Fauvet, B., Oueslati, A., Dikiy, I., Mahul-Mellier, A. L., Ruggeri, F. S., Mbefo, M. K., Vercruysse, F., Dietler, G., Lee, S. J., Eliezer, D., Lashuel, H. A. (2014): The H50Q mutation enhances α-synuclein aggregation, secretion, and toxicity. – J Biol Chem. 289(32): 21856-76. doi: 10.1074/jbc.M114.553297.

[10] Kouli, A., Torsney, K. M., Kuan, W. L. (2018): Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. – In: Stoker, T. B., Greenland, J. C. (eds.) Parkinson's Disease: Pathogenesis and Clinical Aspects [Internet]. Brisbane (AU): Codon Publications; Chapter 1. doi: 10.15586/codonpublications.parkinsonsdisease.2018.ch1.

[11] Lau, H. H. C., Martinez-Valbuena, I., So, R. W. L., Mehra, S., Silver, N. R. G., Mao, A., Stuart, E., Schmitt-Ulms, C., Hyman, B. T., Ingelsson, M., Kovacs, G. G., Watts, J. C.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_58635874

(2023): The G51D *SNCA* mutation generates a slowly progressive α-synuclein strain in early-onset Parkinson's disease. – Acta Neuropathologica Communications 11(72). https://doi.org/10.1186/s40478-023-01570-5.

[12] Lei, Z., Cao, G., Wei, G. (2019): A30P mutant α-synuclein impairs autophagic flux by inactivating JNK signaling to enhance ZKSCAN3 activity in midbrain dopaminergic neurons. – Cell Death Dis 10: 133. https://doi.org/10.1038/s41419-019-1364-0.

[13] Liberski, P. P., Ironside, J. W. (2014): Prion diseases. – ScienceDirect. https://www.sciencedirect.com/science/article/pii/B9780123982704000239.

[14] Perl, D. P. (2010): Neuropathology of Alzheimer's disease. – Mt Sinai J Med. 77(1): 32-42. doi: 10.1002/msj.20157. PMID: 20101720; PMCID: PMC2918894.

[15] Rhoades, E., Ramlall, T. F., Webb, W. W., Eliezer, D. (2006): Quantification of α-Synuclein Binding to Lipid Vesicles Using Fluorescence Correlation Spectroscopy. – Biophysical Journal 90(12): 4692-4700.
ISSN 0006-3495, https://doi.org/10.1529/biophysj.105.079251.

[16] Rutherford, N. J., Giasson, B. I. (2015): The A53E α-synuclein pathological mutation demonstrates reduced aggregation propensity in vitro and in cell culture. – Neurosci Lett. 597: 43-8. doi: 10.1016/j.neulet.2015.04.022.

[17] Shi, Z., Sachs, J. N., Rhoades, E., Baumgart, T. (2015): Biophysics of α-synuclein induced membrane remodelling. – Phys Chem Chem Phys. 17(24): 15561-8.
doi: 10.1039/c4cp05883f.

[18] Stefanis, L. (2012): α-Synuclein in Parkinson's disease. – Cold Spring Harb Perspect Med. 2(2): a009399. doi: 10.1101/cshperspect.a009399.

[19] Tanner, C. M., Goldman, S. M. (1996): Epidemiology of Parkinson's disease. – Neurologic clinics 14(2): 317-35. https://pubmed.ncbi.nlm.nih.gov/8827174/.

[20] Teravskis, P. J., Covelo, A., Miller, E. C., Singh, B., Martell-Martínez, H. A., Benneyworth, M. A., Gallardo, C., Oxnard, B. R., Araque, A., Lee, M. K., Liao, D. (2018): A53T Mutant Alpha-Synuclein Induces Tau-Dependent Postsynaptic Impairment Independently of Neurodegenerative Changes. – J Neurosci. 38(45): 9754-9767. doi: 10.1523/JNEUROSCI.0344-18.2018.

[21] Tosatto, L., Horrocks, M. H., Dear, A. J., Knowles, T. P., Dalla Serra, M., Cremades, N., Dobson, C. M., Klenerman, D. (2015): Single-molecule FRET studies on alpha-synuclein oligomerization of Parkinson's disease genetically related mutants. – Sci Rep. 5: 16696. doi: 10.1038/srep16696.

[22] Trexler, A. J., Rhoades, E. (2009): Alpha-synuclein binds large unilamellar vesicles as an extended helix. – Biochemistry 48(11): 2304-6. doi: 10.1021/bi900114z.

[23] Varkey, J., Mizuno, N., Hegde, B. G., Cheng, N., Steven, A. C., Langen, R. (2013): α-Synuclein Oligomers with Broken Helical Conformation Form Lipoprotein Nanoparticles. – Journal of Biological Chemistry 288(24): 17620-17630.
https://doi.org/10.1074/jbc.M113.476697.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_58635874