The Role of Alpha-Synuclein in Parkinson’s Disease

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Parkinson’s disease (PD) is a slowly progressing neurodegenerative disorder that affects the daily movements and coordination of a person. Currently, there are about 6.3 million people affected worldwide and 1 million people in the United States [1]. PD belongs to the motor system disorders that result from loss of dopamine-producing brain cells. Dopamine is a neurotransmitter which plays an important role in movement, coordination, sleep, behavior, mood, and pleasure. The most common feature of PD is the extreme slowness in the movements and reflexes known as the bradykinesia. In addition to motor symptoms such as resting tremor, inflexibility, and postural instability, there are other nonmotor symptoms experienced by a patient include depression, rapid eye movement sleep behavior disorder, and constipation [2]. Parkinson's disease patients are found to have high level of lewy bodies in the substantia nigra (a part of brain involved in movement, reward, and addiction). Lewy bodies are formed by abnormal aggregation of proteins in the nerve cells of substantia nigra. Alpha-synuclein has been found to be a major component of Lewy bodies in the PD patients. Thus, the focus of this paper is to understand the structure and role of the alpha-synuclein in the neuronal cells involved in Parkinson’s disease patient.

Parkinson’s disease has mostly been found in people older than 60 years of age. As the person ages, the chance of having PD increases. However, age is not the only factor involved in having PD symptoms. About 5-10% cases of PD are inherited from parents through gene alternations. PD can also be sporadic with no known history of PD in the family. It has been
really hard to pinpoint for specific causes for symptoms of PD in a person. PD is not limited to genetics and age, it is also influenced by the environment a person lives in. Parkinson's disease is a multifactorial disease which means it is the result of genetic and environmental factors interacting with each other. The primary cause for the motor symptoms has been found to be the decreasing number or absence of dopaminergic (DA) neurons within the substantia nigra (SN). One of the reasons for this loss of SN neurons has been linked to the overexpression of alpha-synuclein which further leads to oligomerization, fibrillation, and aggregation of this protein in the SN neurons. The aggregated alpha-synuclein interferes with the normal function of SN neurons and causes PD-linked symptoms [3].

Alpha-synuclein, a presynaptic neural protein, is a product of 140 amino acid sequence lacking both tryptophan and cysteine amino acids. Human alpha-synuclein of 140 amino acid sequence consists of three different regions. The amino acid sequence region from the first amino acid to 60th amino acid is called N-terminal amphipathic region. This region can form alpha chains and interact with the surface membranes of other cells, neurotransmitter vesicles, and micelles. The region from 61th amino acid sequence to 95th is known as central hydrophobic non-amyloid region. The last region from 96th amino acid to 140th amino acid is called C-terminal acidic part which is highly enriched in prolines and acidic residues such as serine and tryrosine and can be phosphorylated [3].

It had been understood in the past that alpha-synuclein only exists in monomer form; however, this is not an accepted fact anymore. Wang and Chittulure et.al determined that in the absence of other lipids, and micelles, the alpha-synuclein forms a tetramer structure. This tetramer structure made up of a four-helix bundle did not aggregate under normal conditions and was not toxic to the membranes of other cells. Only when alpha-synuclein was isolated by using
denaturing conditions, it lacked a stable structure and formed aggregation. In order to carry out the experiment, Wang et al constructed an expression vector in *E.coli* where N-terminal GST-tagged alpha-synuclein protein was expressed. To prevent any inclusion body formation, the expression of GST-tagged protein was carried out at 20°C. After the extraction and purification of protein by GST affinity chromatography, the N-terminal tag was removed by protease. The further purification was done by size-exclusion chromatography. The protein was used to find the normal structure of quaternary structure of alpha synuclein. Later the normal structure was compared to structure denatured by detergent, concentration, heat, and disease-associated mutations. To find the normal structure, first SDS/PAGE gel was run loaded with a chemical cross-linked sample of purified protein. There were four bands seen on the gel corresponding to the presence of tetramer structure of protein. MALDI-TOF mass spectrometry was used to identify the bands of tetramer of alpha-synuclein. Further analysis on the electron microscopy revealed the protein was homotetramer oligomer. By analyzing two negative bands at 222nm and 208nm and one positive band at 193nm on CD spectra charactized the presence of 65% alpha-helix, 17% turns, and 8% unfolded. To detect if there was any hydrophobic core present in this structure, a ThermoFluor assay was performed. It was concluded from both CD spectra and a ThermoFluor assay that hydrophobic interactions were used to hold the subunits together [4].

The protein sample was then boiled at 95°C and a precipitation formed. The precipitated sample did dissolved when mixed; however, the structure of the protein was disordered after boiling. This disordered structure began to aggregate on day 4. Then, the sample was tested against concentration and it was found that alpha-synuclein protein was more in the monomer form than oligomer when low concentration was present. However, below 0.5mg/ml concentration, the protein was most disordered on the CD spectra. The interesting point that was
seen in the concentration test was that in normal and high concentrations, the tetrameric form of protein was not toxic to neuronal tissue and did not cause any pores in the membranes. The results from both boiling sample test and concentration test concluded that in order to form aggregation, the protein has to go through shape changes that makes it more prone to fibrillation and aggregation. In disordered form, the protein was toxic at high levels and formed pores in the membranes. Mutated proteins were also analyzed and it was found out that mutated proteins were more disordered than tetrameric ordered form. Overall, the study showed that alpha-synuclein existed in ordered tetrameric form in the absence of lipids bilayer or micelles. It was concluded in vitro, and probably in vivo, there was equilibrium between monomer, tetramer oligomer, and fibrilled proteins. This equilibrium can be shifted to either side by changing conditions such as genetic mutations, age, environmental conditions, and intracellular processes. When a high concentration of alpha-synuclein is present, it appeared in the experiment that less helical structure was favored, which over time led to aggregated and amyloid fibrils product. Being able to extract ordered tetrameric form that was resistant to aggregation has provided hope for the possibility of using this protein structure in future to treat PD [4].

The normal function of alpha-synuclein is still under investigation. However, it has been found that alpha synuclein helps in recycling synaptic vesicles and releasing neurotransmitters such as dopamine. The N-terminal portion of the alpha-synuclein interacts with SNAREs complex and helps control the exocytosis of the dopamine. The exocytosis of dopamine is regulated by alpha-synuclein which helps in regulating SNARE's complex, vesicle pool and dopamine reserve. In a study done by Murphy et al, the researchers showed how alpha-synuclein regulated the size of the presynaptic vesicular pool of hippocampal neurons. First, hippocampal neuronal cell culture was prepared from rat brain. The culture was used to prepare coverslip for
electron microscopy and to make tissue wells for Western blot analysis. Then cells were incubated with different antibodies for immunocytochemistry analysis. The cells were incubated with Syn102 antibody which recognized alpha and beta synuclein. The second antibody that was used was SNL-1 which recognized alpha-synuclein only. The third antibody syn207 was specific for beta-synuclein protein. To image cells, the cells were first washed and incubated with secondary antibodies. A Laser-scanning microscope was used to image the cells. It was found that both alpha synuclein and beta synuclein were found to be expressed exclusively at the presynapse once the presynaptic neuronal terminals were mature. Thus, the alpha and beta synuclein are proteins associated with presynapse. The Western Blot analysis was also done on the cell culture. The western blot procedures revealed that alpha-synuclein level decreased over the 3 weeks where as the level of beta-synuclein was unchanged when the neuronal cells were maturing. The alpha synuclein was found to be 100% at first week, then 75% and about 60% on the week 3 when neurons presynaptic terminal was matured. However, alpha-synuclein was fully expressed only when the presynaptic terminals were fully matured. Thus, this late start expression in neurons even though the level of alpha-synuclein was found to higher at the start of the week 1 suggested that the alpha-synuclein protein might be important for later maintance of neurons not the initial formation [5].

The hippocampal cell culture was also treated with antisense oligonucleotides that were specific for alpha-synuclein region. The goal was to turn off the expression of synuclein by antisense oligonucleotides and to see what happened to the cell expression. Also, a control cell culture was set up which did not have antisense oligonucleotides rather had a sense nucleotide. Both cell cultures were then analyzed by electron microscopy. The researchers found that the expression of beta-synuclein stayed unchanged in cell culture incubated with antisense
oligonucleotides specific for alpha-synuclein. However, the expression of alpha-synuclein was decreased over time with antisense oligonucleotides. To see the effect of this suppression, the presynaptic terminals were analyzed especially the vesicles. It was found that the reserve pool of vesicles was decreased as the alpha-synuclein protein level decreased due to suppression of its expression by AS oligonucleotides. The docked pool of vesicles was unaffected. The control did not have any changes in both vesicle pools. Overall, the study showed that alpha and beta synuclein were presynaptic proteins found in neurons. However, as the neurons matured the alpha-synuclein level decreased whereas the level of beta synuclein was unchanged. When this already low level of alpha-synuclein in mature neurons was suppressed by antisense oligonucleotides, it caused the decrease in reserve pool of vesicles [Fig 1]. Thus, the low level of alpha-synuclein compared to normal alpha-synuclein in a neuron caused the only reserve pool of vesicles not the docked vesicle to decrease. This also showed that alpha-synuclein might have a role in vesicle maintenance after the neuronal presynaptic terminals were fully matured [5].

Figure 1: showing the decreased reserve pool and increased release of neurotransmitter (in this picture dopamine) as the concentration of alpha-synuclein decreases in the neurons.
It was now known that low level of alpha-synuclein causes decrease in the reserve pool; however, more researched was needed to see if the high level of alpha-synuclein had any effects. A study done by Nermani et al. showed that elevated level of alpha-synuclein decreased the size of the recycling pool of vesicles in neurons while interfering with the process of exocytosis and endocytosis. This overexpression of alpha-synuclein has been seen to cause considerable toxicity in the cells. This study used neuronal cells with vesicles transfected with vesicular glutamate transporter 1-pHluorin to directly observe the effects of overexpressed (more protein being formed) alpha-synuclein on the cells. VGLUT1-pHluorin fluoresces when exposed to alkaline solution. However, it fluoresced less when exposed to acidic solution. This change in pH was really important in observing exocytosis of neural vesicles. When the vesicles were released, VGLUT1-pHluorin came in contact with the extracellular alkaline environment and it fluoresced. However, when the neurons were in resting state with no exocytosis of vesicles, VGLUT1-pHluorin did not fluoresce. In order to relate this to human cells, the researchers used dopamine human cells, and transfected them with VGLUT1-pHluorin. The change in fluorescence was observed in normal concentration alpha-synuclein versus elevated concentration of alpha-synuclein in dopamine neurons. When alpha-synuclein was in high amount, the VGLUT1-pHluorin did not or little fluoresced. However, under normal concentration, the VGLUT1-pHluorin did fluoresce. This showed that over expression of alpha-synuclein interfered with the vesicle exocytosis and inhibited the dopamine releasing vesicles. Thus, the over-expression caused the reduced release of dopamine neurons by interfering with exocytosis. Another experiment was done to see what effect the over-expression had on the process of endocytosis. Following endocytosis, the reclustering of the vesicles was also slowed in the presence of over expressed alpha-synuclein protein. Since the vesicles could not be reclustered at a normal rate,
there was a decrease in the recycle pool of the vesicle. To observe this in lab, the researchers measure the uptake of styryl dye by the neurons expressing normal and elevated level of alpha synuclein. Styryl dyes are organic molecules with fluorescent properties and are used in the field of neurobiology as a means of labeling recycling vesicles. The cells were given stimulant and allowed to uptake the dye. The cells were allowed to remain in the dye solution to have a full endocytosis process. The neurons were given another stimulant to unload the dye. The cells were allowed to uptake the dye again. The second stimulant for unloading was used to measure the size of the recycling pool of the vesicles. The cells expressing elevated level of alpha-synuclein showed 50% decrease in the uptake of the dye compare to a normal cells. The rate which endocytosis happened was not changed in both cells containing elevated and normal level of alpha-synuclein. It was the vesicle size that was reduced and lower amount of dye was uptaken by the cells due to high level of alpha-synuclein [Fig 2]. Thus, the high level of alpha-synuclein not only interfered with the exocytosis process where the vesicles were not able to fuse at a normal rate and release the contents outside but also interfered with the endocytosis process. In endocytosis, the size of the recycle vesicle was reduces leading to lower amount of substance uptake [Fig 2]. Overall, the study showed that alpha synuclein plays a major role in the uptake and release of neurotransmitter [6].
Figure 2: showing the decreased recycling pool size and decreased release of neurotransmitter (in this picture dopamine) as the concentration of alpha-synuclein increases in the neurons.

The experiments had shown that elevated levels of alpha-synuclein interfered with the process of exocytosis by reducing the release of dopamine or endocytosis in which the recycling vesicle pool size was reduced. However, there was not any study done until 2012 at the molecular level to show how alpha-synuclein interacts with the membrane of the neurons and interferes with their normal functions. A study done by Bong-Choi et al showed that large alpha-synuclein oligomers interfered with SNARE complex during exocytosis and release of dopamine. In order to obtain large oligomers, alpha-synuclein was incubated with 10-fold molar excess of dopamine. After the incubation of alpha-synuclein, the solution was run on the SDS/PAGE to get different bands. One of the bands that was produced belonged to the high molecular weight of alpha synuclein oligomer. Oligomers were only produced in the presence of dopamine. In the absence of dopamine, there were no oligomers present in the solution. Thus, high amount of dopamine causes high molecular weight oligomers formation. The size exclusion chromatography was used to separate the high molecular weight oligomer from the other species. The weight of the oligomer was around 250kDa. In vitro lipid-mixing assay, the researchers tested whether the alpha-synuclein has any effect on the SNARE-mediated lipid mixing by using proteoliposomes. In order to carry out the lipid-mixing assay, two populations of proteoliposomes were created. The t-SAREs constituted the t-vesicle while the v-SNARE synaptobrevin-2 constituted the v-vesicle. Under normal conditions, when these two populations
of proteoliposomes were mixed, the vesicles fused together to form one vesicle. The fusion of vesicle was monitored by FRET signal. FRET also known as the fluorescence resonance energy transfer is used in the protein-protein interactions and in this study for vesicle-vesicle interactions. When there was fusion of the vesicles, the signal was increased. However, when these two proteoliposomes v-vesicle and t-vesicles were allowed to fuse in the presence of 30mM concentration of large alpha-synuclein oligomers, the fusion rate decreased to 75% with 25% of inhibition. The concentration of oligomers was given in monomer units. The same concentration 30nM but now in the monomer form of alpha-synuclein had no effect on the vesicle fusion. Thus, it was concluded that high molecular weight alpha-synuclein oligomers interfered with the SNARE-mediated lipid mixing and the fusion of vesicles was inhibited [7].

To understand the mechanism behind the inhibition of SNARE-mediated lipid mixing, more experiments were carried out by the researchers. The researchers tried to test three known modes of interactions between alpha-synuclein and SNARE proteoliposomes to see which interaction was common in large alpha-synuclein oligomers and SNARE proteoliposomes. The first mode of interaction could be large alpha-synuclein oligomers interacting with a negatively charged membrane of the vesicles. To test this hypothesis, the researchers incubated a large amount of alpha-synuclein oligomers with protein free liposome. They stated if the oligomers did interact with the membrane of vesicles, then there would be a decrease in the amount of free-floating large alpha-synuclein oligomers and there was a chance of inhibition of vesicle mixing. However, this inhibition could be overcome by addition of large amount of protein free liposome. Addition of protein free liposome had no effect on the 50% inhibition. Even when large amount of alpha-synuclein oligomers were added, the inhibition still stayed at 50%. The researchers concluded that alpha-synuclein oligomers did not bind to the protein free liposome or
phospholipids of vesicle membrane. The second mode of interaction where oligomers caused leakage of membranes was tested. In order to test this hypothesis, the researchers used the sulforhodamine B (SRB) dequenching method. The vesicles were prepared from addition of 20nM of SRB to lipid film. These vesicles with concentration 10μM were incubated with 170nM of large alpha-synuclein oligomers. To observe the dequenching of SRB (result of membrane disruption), SRB fluorescent emissions were monitored. There was not any noticeable increase in the fluorescent emissions leading to the conclusion that the large oligomers of alpha-synuclein did not cause leakage of membranes of vesicles. The last mode of interaction was that large oligomers of alpha-synuclein actually bind to the v-SNARE synaptobrevin-2 one of the SNARE proteins. To test this hypothesis, the researchers used coflotation assay. This assay helped to observe the interactions between large alpha-synuclein oligomers and v-vesicle synaptobrevin-2. The researchers obtained large alpha-oligomers bound to t-vesicles and v-vesicle. The western blot was used to quantify the amount of alpha-oligomers bound to each vesicle. The v-vesicle had higher amount of bounded oligomers than t-vesicles and protein free liposomes. The researchers concluded the large oligomers of alpha-synuclein had preferential binding for v-vesicle over other vesicles. Thus, in the exocytosis process SNAREs complex, the oligomers interacted with the v-vesicles and caused the inhibition of formation of SNAREs complex and release of dopamine vesicles [7].

The mode of interaction was determined; however, it was still unclear how the large oligomer of alpha-synuclein interacted with the v-SNARE synaptobrevin-2. To determine the mechanism behind this interaction, the researchers used mutant synpatobrevin-2 without any N-terminal region. Their hypothesis was that if the large oligomer used N-terminal region of synaptobrevin-2 to bind to it, then the large oligomer will no longer to bind to mutant
synaptobrevin-2 lacking N-terminal region. Their hypothesis was supported by the coflotation assay which showed that oligomers had lower binding affinity for mutant synaptobrevin-2 lacking N-terminal than v-SNARE synaptobrevin-2 with N-terminal region. It was now known that oligomers did not bind to mutant synaptobrevin-2 lacking N-terminal region, next step was to see if the low affinity for binding also lowered the inhibitory effect of oligomers on the lipid mixing. The paper does not give any specific method for this procedure and only stated that there was no inhibitory effect of oligomers seen in the presence of synpatobrevin-2 lacking N-terminal region. Thus, the researchers concluded that large oligomers of alpha-synuclein specifically bind to N-terminal region of v-SNAREs synaptobrevin-2 to inhibit the lipid mixing in a cell [7].

The next question put up by the researchers was exactly how oligomers' binding to N-terminal region of synaptobrevin-2 led to inhibition of lipid mixing. Their hypothesis was that oligomers inhibit the SNAREs complex which led to vesicle reduced docking. Docking in the molecular biology means the orientation of one substance to another which then leads to the product. In order to relate to this concept to this situation, the orientation of v-SNAREs with t-SNAREs was called docking and the lipid mixing was called the product. A single-vesicle assay known as ALEX used to observe the docking and lipid-mixing. ALEX used different color for docking, lipid-mixing steps, and unreacted t-and v-vesicles. The vesicles were incubated with the large oligomers of alpha-synuclein for 30 minutes at 37°C. To use a control, the vesicles were also incubated with alpha-synuclein monomer at the same conditions. It was seen that in the presence of oligomers, the lipid-mixed vesicles were reduced and the exocytosis was decreased. The vesicles were not able to mix with the lipid bilayer. If the lipid-mixed vesicles were low and it was due to lipid-mixing step at the end, then there should be increase in the vesicles of the docking step. However, the subpopulation of docking step vesicles did not change. This means
the oligomers inhibit the initial SNAREs complex formation of t- and v-SNAREs synaptobrevin-2 at the docking step where v-and t-vesicle come together which will later allow the lipid layers to mix. Overall, this study showed that the role of large oligomers of alpha-synuclein in exocytosis process which can be formed when there was high concentration of dopamine or mutated alpha-synuclein or other environment cause was present. The large oligomers interact with the N-terminal region of v-vesicle snyaptobrevin2 and inhibit the initial SNAREs complex formation with the t-vesicle. Since the SNAREs complex cannot be formed, the membranes cannot fuse to release the substance outside [Fig 3]. Similar results were seen in vivo, where large alpha-synuclein oligomers inhibited the exocytosis in PC12 cells. This study provided very important insights in understanding the role of alpha-synuclein in the cells [7].

Figure 3: showing the general process of monomer alpha-synuclein forming into large alpha-synuclein oligomers in the presence of high amount of dopamine. These large alpha-synuclein oligomers then go bind to v-SNAREs synaptobrevin-2. The v-SNAREs can no longer bind to t-SNAREs. Since the v-SNAREs and t-SNAREs cannot interact, the fusion of two membranes cannot be accomplished leading to inhibition of exocytosis.
The aggregation of alpha-synuclein in the neurons of substantia nigra in human leads to reduce exocytosis of dopamine release. When there is high level of dopamine as seen in the past experiments, the more alpha-synuclein oligomers will be formed leading to overstressed cell with no exocytosis process. The overstressed cells will eventually die leading to neuronal death. The reduced released amount of dopamine leads to movement disorders, depression, and other motor disorders that require the dopamine to function properly. There is still more research being done around the world to see other ways alpha-synuclein can cause the disease. It is a very elusive process because there is more than one factor contributing to neuronal cell death. Although alpha-synuclein has been found to be a major component of lewy bodies, there are still other proteins that might interact and cause the cell death. Thus, more research needs to be done to fully understand the cause behind the neuronal cell seen in PD. Due to these difficulties, there is still no cure for Parkinson's disease. However, there are some treatments available to reduce the aggregation of proteins or death of the neurons. These treatments are not effective to inhibit the aggregation of proteins; thus, patient still has Parkinson's disease and symptoms associated it. The current treatment involves medications such as substituting dopamine and therapy such as deep brain stimulation to suppress pathological neuronal oscillations.

There are numerous studies being done to find a cure for the Parkinson's disease. One focus of these studies is to slow or inhibit the fibrillation or aggregation of alpha-synuclein. Aggregated alpha-synuclein causes inhibition of exocytosis and leading to high level of dopamine in the presynaptic neuronal cells. A study done by Hyun Koo et al., the researchers tested whether the self-fibrillation-defective α-synuclein mutants were able to prevent the polymerization of wild-type and/or PD-linked α-synuclein variants. The cDNA that coded for mutant self-fibrillation defective alpha-synuclein proteins was cloned into E.coli plasmid. The
plasmid was introduced into the *E.coli* and was allowed to replicate the cDNA and produce mutant alpha-synuclein produce. The mutant protein was extracted from the cells by lysis of the cell and then purification of the supernatant that contained the protein. The purification of the mutant alpha-synuclein was done by anion exchange column chromatography and later on the SDS-polyacrylamide gel. After getting purified mutant alpha-synuclein, it was used to test the inhibition of fibrillation of the wild-type and PD-linked alpha-synuclein protein (A30P, A53T, and E46K). These all four alpha-synuclein proteins were incubated with or without the mutant self-fibrillation defective protein in PBS buffer. The absence of mutant alpha-synuclein was used as a control. At certain points, the 20ul of each of the samples was taken and mixed with thioflavin T solution to find the degree of fibril formation. Thioflavin T is a benzothiazole salt and used as a dye to visualize and quantify the presence of misfolded protein aggregates called amyloid. When it binds to amyloid aggregates, the dye displays enhanced fluorescence. Fluorescence emission was measured for each of the sample. As the solubility of the protein decreased, more fluorescence color would be seen [8]. Thus, more amyloid aggregates were present.

When the concentrations of both wild-type alpha-synuclein and PD-linked alpha-synuclein were decreased, the time for fibrillation of proteins was increased. Thus, in the absence of mutant self-fibrillation defective alpha-synuclein protein, the time for fibrillation is concentration dependent. The wild-type alpha-synuclein in the presence of mutant self-fibrillation defective alpha-synuclein protein, the fibrillation of the wild-type protein was inhibited and almost no fibrillation was recorded by fluorescence emission. Also, all PD-linked alpha-synuclein in the presence of mutant self-fibrillation defective alpha-synuclein protein had slow or no fibrillation seen. [8]. Thus, the method used in this study to slow or inhibit the
fibrillation can serve as a therapeutic method in the future to treat Parkinson’s disease or other
disease caused by aggregation of alpha-synuclein.

In conclusion, Parkinson’s disease is the second most common neurodegenerative disease
and affects many people worldwide. It is very important in today’s world to know the causes of
Parkinson’s disease and find cure for this disease. The most studied cause for PD is the
aggregation of alpha-synuclein which is found to be a major component of lewy bodies. This
protein is found most at the presynaptic terminals of brain cells. By understanding the
mechanism involved behind the aggregated alpha-synuclein in neurodegeneration can also
greatly help with other diseases such as neurodegenerative synucleinopathies. As of this moment,
alpha-synuclein protein is found to be involved in the maintance of neurons. However, when
expressed in high concentrations, it can greatly reduce the neurotransmitter release by inhibiting
the SNAREs complex involved in the exocytosis. It not only inhibits the SNAREs complex but
decrease the recycling vesicle pool size. This leads to lower uptake of neurotransmitter at the
synapse. There are many studies being done to find cure such as the inhibition of aggregation or
fibrillation of alpha-synuclein. With new growing technology and research methods, it might be
possible to find cure in the future and help all the people suffering from this disease.
Work Cited


