공학석사 학위논문

# Gel-Sol transition of peptides derived from α-synuclein

## 알파-시뉴클레인으로부터 유도한 펩타이드의 젤-졸 전이현상

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Abstract

# Gel-Sol transition of peptides derived from α-synuclein.

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α-synuclein is a 140 residue protein, expressed in neurons. Through the specific self-assembly process, α-synuclein make insoluble protein aggregates such as Lewy bodies, the common characteristic of Parkinson's disease. The self-assembly of α-synuclein becomes popular because of its pathological reason and also non disease functional role. Using TANGO program to predict amyloidogenic propensity, we found a specific sequence, based on α-synuclein sequence (35-40) , EGVLYV. Although this sequence is not located in NAC region (61-95), core region of the fibrillation process of α-synuclein, when this sequence becomes tandem repeat, EGVLYV-EGVLYV, the modified peptide shows Gel-Sol transition. The self-assembly of peptide from α-synuclein is

studied in DMSO. The self-assembled structure (gel state) is transformed into sol state by applying proper force. When force is removed, the Sol state structure becomes Gel state. The assembled of peptide shows honey comb structure, confirmed by scanning electron microscopy. The characterization of Gel-Sol transition is proved by Advanced Rheometric Expansion System (ARES). The structure differences between Sol state and Gel state are proved by circular dichroism, and FT-IR. Comparing with other peptide gel, the peptide structure has a unique property such as Gel-Sol transition. Mechanical force sensitive self-assembled peptide is made by using peptide derived from α-synuclein. This novel peptide will be used to make mechanical sensitive materials.

Keywords : α-synuclein, Amyloid, Tandem Repeat Sequence, Self-assembly, Gel-Sol transition.

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# 1. Introduction

## 1.1 α-synuclein and Parkinson's Disease

Parkinson's Disease (PD) is a neurodegenerative disorder which is classified as motor disorder.[1] Abnormal protein aggregates, called Lewy bodies, were frequently found in PD patient's brain. Recent studies showed that α-synuclein is a main component of Lewy bodies [2,3,4] α-synuclein consists of 140-residue amino acid and it commonly found in neuron.

α-synuclein can separate in three region: Amphiphathic N-terminal region (residue 1-60), called membrane interaction region [5,6] , NAC region (residue 61-95) , and acidic C-terminal region (residue 96-140). The NAC region is called non-Aβ component of Alzheimer's disease amyloid, hydrophobic region which play critical role in protein aggregation. [7] C-terminal region represent highly acidic and soluble region, and recent studies reported C-terminal region behave like chaperone. [8,9]

Trough specific self-assembly process, α-synuclein form amyloid fibril. These amyloid fibrils have cross β-sheet conformation [10] and β-sheet conformation make fibrils stronger even comparing to spider silk. [11] Amyloid fibrils create new route to make supramolecular structures by applying the self-assembly process. [12].

## 1.2 The Self-assembly process application.

The self-assembly process of molecules such as peptides, amino acids and so on. [12-14] With rational design of molecules, supramolecular structure can be 2-D structure or 3-D structure such as films, hydrogels, nanotube, films. [12-21] These structures are becoming popular in drug delivery, tissue engineering part because of its biocompatibility, and degradability. In this study, we use a specific α-synuclein sequence to make modified tandem peptide which shows SOL-GEL transition.

## 1.3 TANGO program

There are some programs that help to predict amyloidogenic properties. Among these programs, TANGO can predict protein aggregation. TANGO program consider four different energy terms such as hydrophobicity, solvation energy, electrostatic interaction, and hydrogen bonding, to find β-sheet, β-aggregates, α-helix, β-turn. Through their own algorithm, these data show score of propensity. [22]

# 2. Materials and methods

## *2.1 Materials*

EGVLYV was synthesized by solid phase methods using standard Fast Moc (9-fluorenylmethyloxycarbonyl) protecting group and activation by HBTU (2-(1*H*benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) and HOBt (1-Hydroxybenzotriazole). Amino acids Fmoc-tyrosine(tbu)-OH, Fmoc-glu(OtBu)-OH, Fmoc-leucine-OH, Fmoc-valine-OH, Fmoc-phenylalanine-OH, H-valine-chlorotrityl resin, HBTU, and HOBt were purchased from beadtech. Diethyl ether were purchased from Merck. Methanol, DMF (dimethylformamide) were purchased from Fisher. MC (methylene chloride) was purchased from Daejung. Piperidine, trifluoroacetic acid (TFA), triisopropylsilane, DODT (2,2′-(Ethylenedioxy)diethanethiol), DIEA (diisopropylethylamine) and all other reagents were purchased from Sigma-Aldrich and were of the highest purity.

## *2.2 Peptide synthesis*

The peptide was synthesized on a 0.51 mmol scale using solid phase peptide synthesis method. The resin used for EGVLYV peptide synthesis was H-valine-chlorotrityl resin with 0.51 mmol $g^{-1}$ substitution. The peptide was assembled from the C-terminus toward the N-terminus. The first step of the reaction was to remove the Fmoc protecting group from the amino acid using a solution of 20% piperidine in DMF. The next step was activation of the carbonyl group of the new amino acid (dissolved in DMF) using HBTU, HOBt (dissolved in DMF) with adding DIEA. The activated amino acid was transferred from the activation vessel to the reaction vessel

containing the previously deprotected amino terminal group of the peptide chain, and coupling was performed. To obtain the highest coupling efficiency, three times excess of each amino acid was used in 0.51 mmol cycles. In the cleavage step, a mixture of 95% TFA, 2.5% triisopropylsilane, and 2.5% water was used. The sample was occasionally shaken at room temperature for approximately 2 h and the insoluble resin was washed with TFA. The peptide solution was precipitated in ice cold diethyl ether. Sample was separated by centrifugation and the solvent was decanted. The solid peptide was washed with diethyl ether and dried. During the cleavage the side chain protecting groups (Boc) were removed by TFA.

## *2.3 Field-Emission Scanning Electron Microscope ( FE-SEM )*

The modified tandem repeat peptide prepared in DMSO became GEL state. The modified peptide (GEL state) was put into liquid nitrogen and freeze-dried for two days. The freeze-dried peptide (GEL state) was coated with Gold by using Sputter Coater (BAL-TEC/SCD 005). Then the sample was examined by field-Emission Scanning Electron Microscope (SUPRA 55VP, Carl Zeiss)

## *2.4 Circular Dichroism (CD) spectroscopy*

Using Circular Dichrosim ( Chirascan plus ), the difference between SOL state and Gel state observed. The sample were dissolved in DMSO ( 30 mg/ml ) and loaded in 1 mm-pathlength quarts cell. Spectra scanning were performed from 250nm to 320nm with a 1 nm step resolution. The

scanning was performed at 25℃ and 80℃.

## *2.5 Advanced Rheometric Expansion System ( ARES )*

Advanced Rheometric Expansion System (Rheometric Scientific, UK) was performed to measure mechanical strength of modified peptide. The modified peptide was dissolved in DMSO with proper concentration, 30 mg/ml. The storage modulus, G', shows elasticity of the sample, and the loss modulus, G'', shows viscosity of the sample. The sample was loaded as circular cylinder shape, 25 mm diameter and 1 mm height.

## *2.6 Fourier Transform Infrared Spectroscopy (FTIR)*

FTIR spectrometer carried out to analyze secondary structure of tandem peptide. To observe EGVLYV-EGVLYV tandem repeat peptide (gel state) , dissolved in DMSO (30 mg/ml), the peptide was put into liquid nitrogen and freeze-dried for two days. Spectra were scanned 32 times over the range of 4000-650 $cm^{-1}$.

# 3. Results and Discussion

## 3.1 Gel-Sol transition of the peptide sequence, based on α-synuclein (35-40).

Scanning α-synuclein by using TANGO program with hexamer unit, there is a specific sequence (35-40), high amyloidogenic propensities. (figure 1.) When this sequence became tandem repeat, the score of amyloidogenic propensities was dramatically increased. The tandem peptide, prepared by solid phase peptide synthesis, was dissolved in 100 % DMSO. When the peptide completely dissolved in solvent, it became Gel state through self-assembly process after 30 min. However, with vortexing the peptide during 1 min, the sample became sol state. (Figure 2). When the force (vortex) was removed, the modified peptide returned gel state. This Gel-Sol transition phenomenon was observed over 100 cycles. This phenomenon showed that proper shear force is a critical factor to make Gel-Sol transition. Without a proper shear force, the peptide re-assembled to make the gel state.

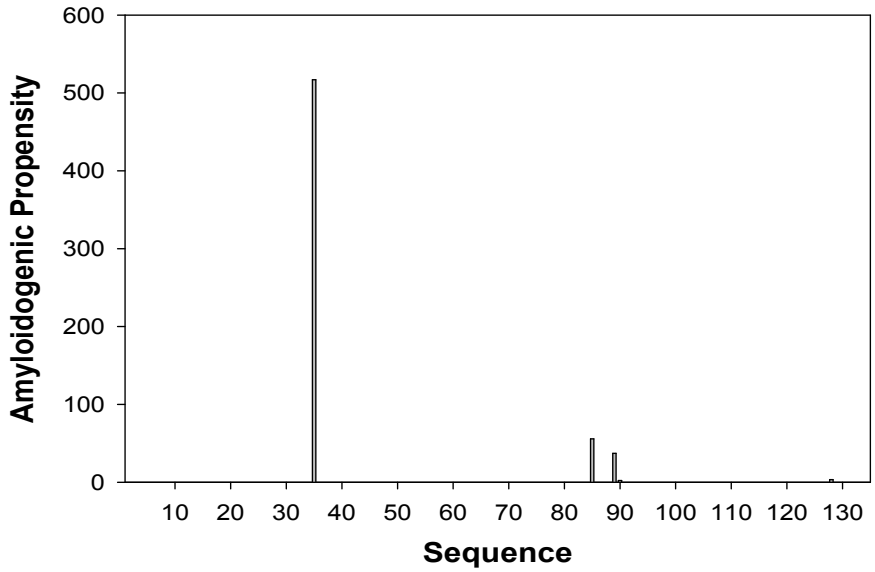


Figure 1. The graph of amyloidogenic propensity using TANGO program.

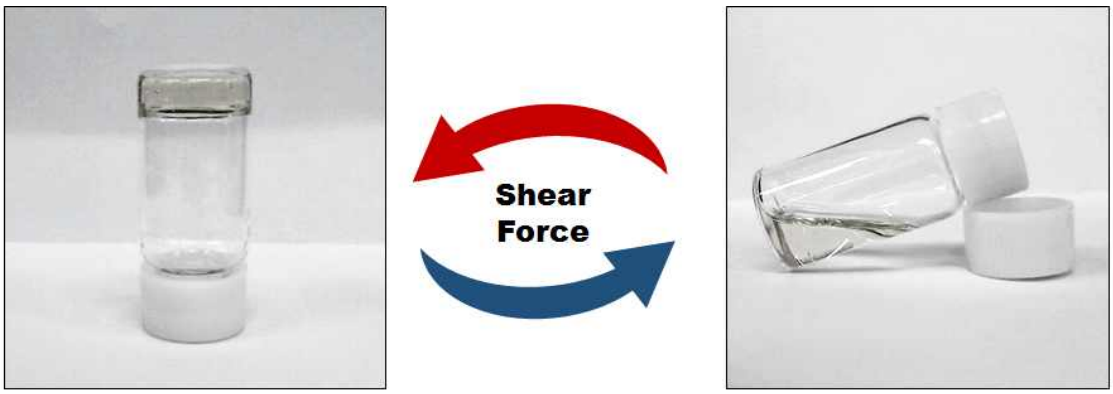


Figure 2. Image of Gel-Sol transition of tandem repeat sequence peptide, EGVLYV-EGVLYV. 30 mg/ml peptide dissolve in 100 % DMSO.

## 3.2 Conditions of Gel-Sol transition.

### *3.2.1 Sequence or tandem repeat effect of Gel-Sol transition.*

To figure out whether this phenomenon appears because of a specific sequence or tandem repeat reason, pruning strategy is used. General pruning strategy is removing one amino acid residue. However, we removed two amino acid residues due to tandem repeat. Therefore, GVLYV-GVLYV, VLYV-VLYV, LYV-LYV was synthesized to confirm. Although GVLYV-GVLYV, VLYV-VLYV, LYV-LYV, dissolve in same amount of concentration in DMSO, they did not show Gel-Sol transition. (Figure 3 b,c,d). In addition, when hexamer (EGVLYV) dissolved same concentration in DMSO, it also did not show Gel-Sol transition. (Figure 3). Another pruning sequence was chosen by picking important reside in EGVLYV-EGVLYV. Glutame and Tyrosie might play a critical role in Gel-Sol transition. Therefore, ELYV-ELYV was synthesized to confirm my assumption. However, this tandem sequence did not show Gel-Sol transition (figure 3e). The results show that Gel-Sol transition phenomenon depends on both a specific sequence reason and tandem repeat reason.

### *3.2.2 Peptide concentration effect of Gel-Sol transition.*

In order to observe minimum concentration of tandem repeat peptide, gelation

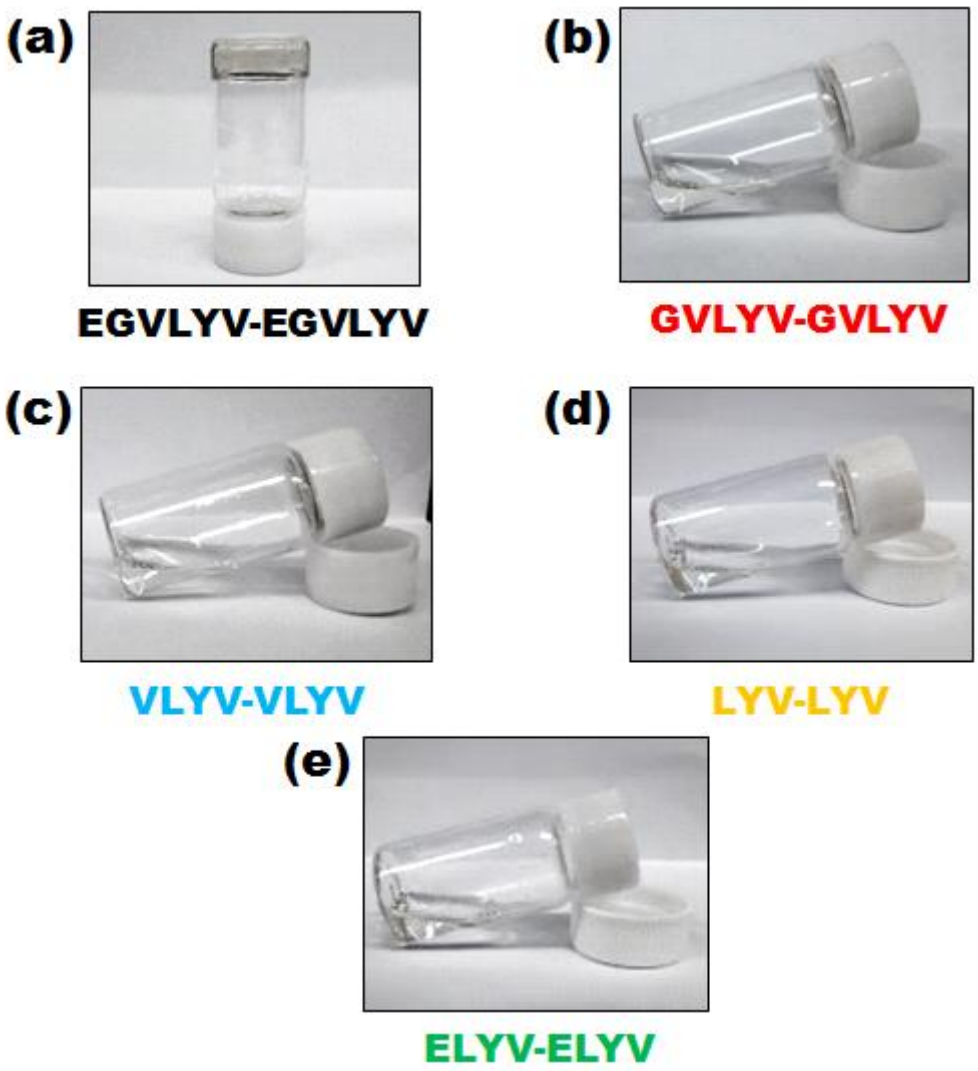


**Figure 3 : Image of Pruning tandem repeat sequence peptide.** 30 mg/ml peptide dissolve in 100 % DMSO. (a) EGVLYV-EGVLYV (b) GVLYV-GVLYV (c) VLYV-VLYV (d) LYV-LYV (e) ELYV-ELYV

concentration experiment was performed. Under 20 mg/ml concentration in DMSO, tandem repeat peptide did not become gel state. When the concentration of the tandem repeat peptide gel was 25 mg/ml, it became within 24 hours. However, when its concentration was more than 30 mg/ml, it became gel less than 2 hours. The results showed that Gel-Sol transition was dependent on peptide concentration. (Figure 4).

### *3.2.3 Solvent exchange effect of Gel-Sol transition.*

To determine whether DMSO is a proper solvent for the modified peptide, buffer was exchanged by water dialysis method. Before exchanging buffer, the sample prepared as a gel state. Then the sample immersed in water, and change water after 8 hours. Repeating this steps as three times tried to make less than 0.5% DMSO. When the sample was exchanged buffer as water, it lost Gel-Sol transition. The results showed that molarcular assembly was changed when buffer was exchanged (Figure 5). The SEM image proved that solvent change affected the molecular self-assembly process.

### *3.2.4 Temperature effect of Gel-Sol transition.*

To determine optimal temperature of Gel-Sol transition, the sample was incubated in 80℃ and room temperature for 1 hour. When peptide was incubated in 80℃, it completely lost the reversibility of Gel-Sol transition and it remained sol state. After few days, the tandem repeat peptide, incubated in 80℃, did not transform into gel state. The results represented the proper temperature of Gel-Sol transition is room temperature, 25℃ (Figure 6). In addition, when sample was incubated at 80℃, the structure of sample was completely changed.

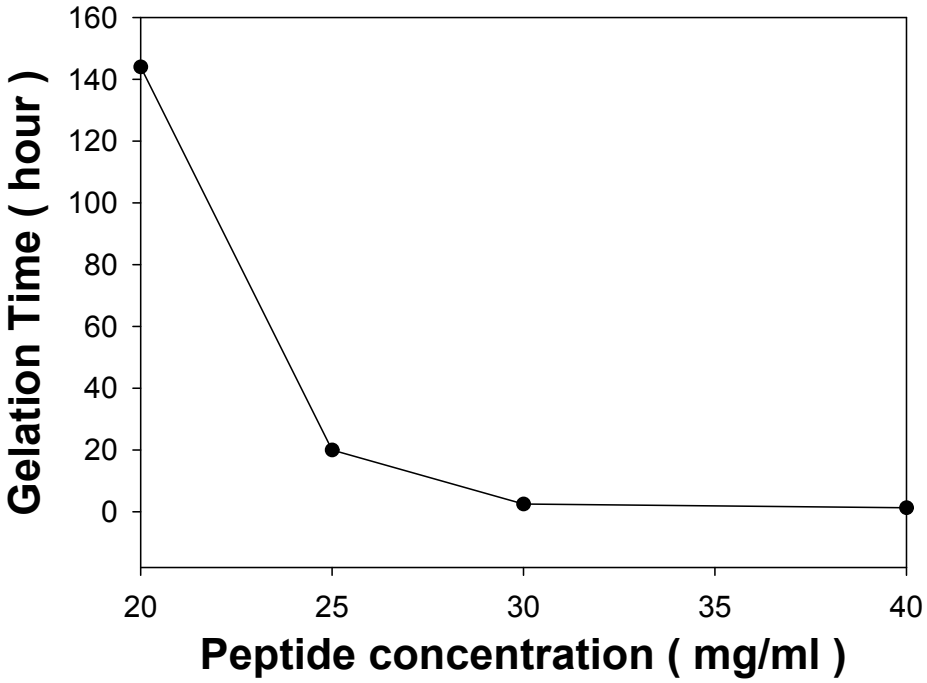


Figure 4 : Critical gelation concentration of tandem repeat peptide. Each peptide dissolve in 100% DMSO at 25℃

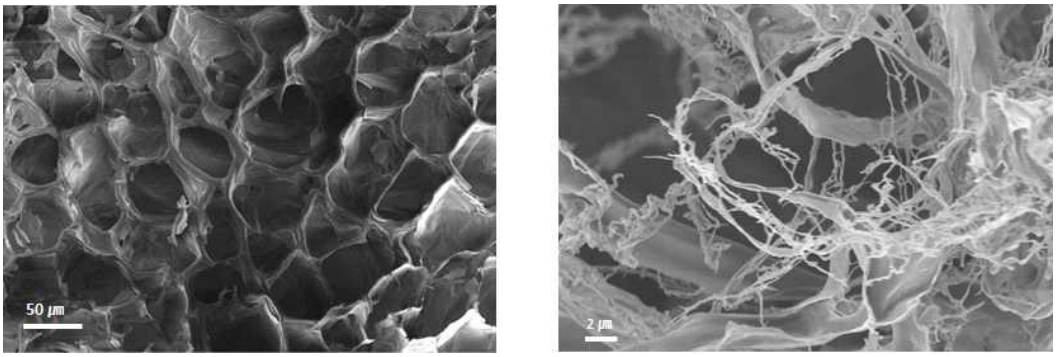


**Figure 5 : Field-Emission Scanning Electron Microscope images of GEL state and solvent exchanged GEL state.** The peptide dissolved in DMSO (30 mg/ml). The sample was put into liquid nitrogen and freeze-dried for two days.

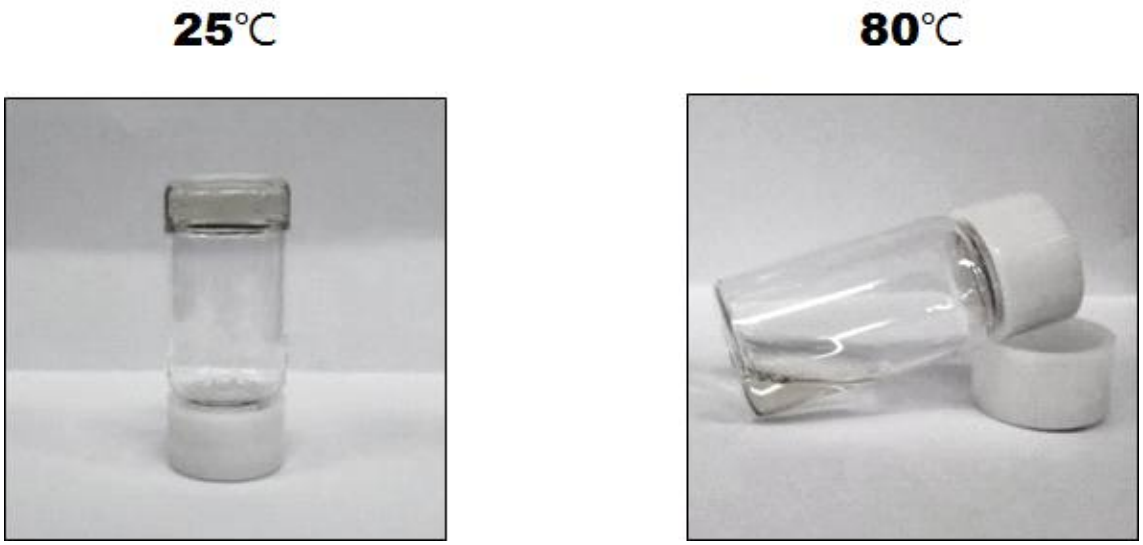


**Figure 6 : Image of 25℃ tandem repeat peptide and 80℃ tandem repeat peptide.** The samples were incubated for 1 hour at 25℃ and 80℃.

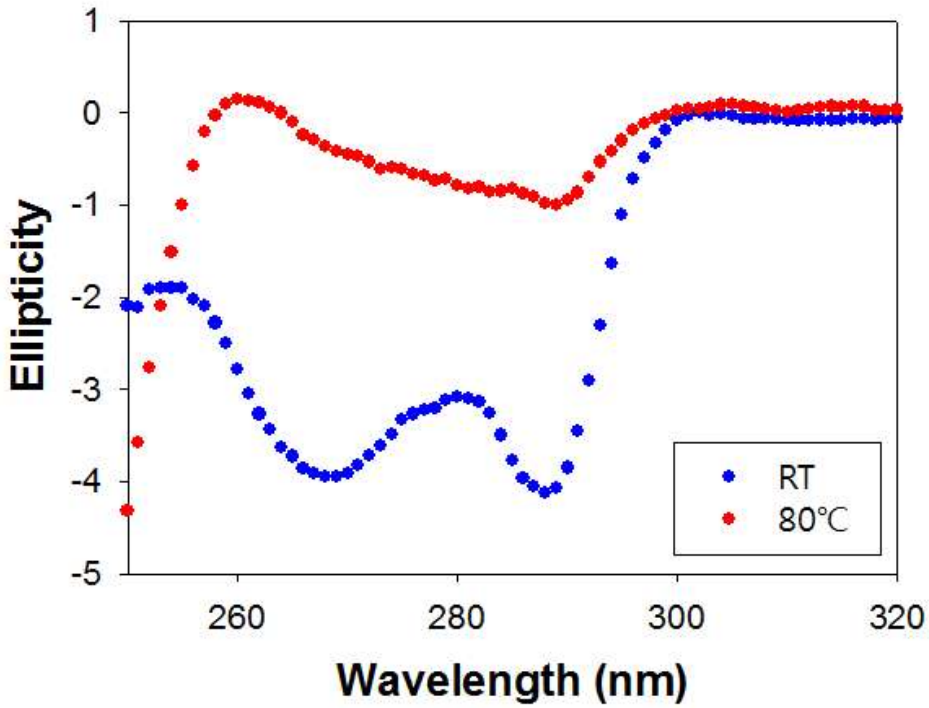


Figure 7 : Circular dichroism spectra of temperature effect of SOL state. The sample was dissolved in 100 % DMSO (30 mg/ml). Samples were incubated for 1 hour at different temperature. Red dot showed 80℃ and blue dot represented room temperature.

To figure out the structural difference between 80℃ incubated sample and 25℃ sample, Circular dichroism (CD) spectrum was performed. CD spectra showed structure difference following temperature change. Blue dot indicated room temperature CD spectra. When the sample was incubated in 80℃, CD data was dramatically changed (Figure 7).

## 3.3 Characterization of Gel-Sol transition

### *3.3.1 Internal structure of Gel state peptide.*

To analyze the internal structure of modified tandem repeat peptide (EGVLYV - EGVLYV), scanning electron microscope (SEM) was performed. SEM images of the peptide (gel state) showed the morphology of structure. The internal structure of the peptide (gel state) showed honey comb like structure with 30 ㎛ ~ 40 ㎛ pore size (Figure 8).

### *3.3.2 Structural difference between sol state and gel state*

Structural difference between gel state and sol state of tandem repeat peptide were examined by using Circular dichroism (CD), ATR-FTIR, and ARES (Figure 9, Figure 10, Figure 11). CD spectrum data showed that the structural difference was existed between sol state and gel state. (Figure 9) Before scanning CD, sol state of tandem repeat peptide was prepared. After scanning sol state, the sample, loaded in quarts cell, was given proper time to become gel state. After waiting 30 min, CD spectra of gel state were scanned. (Figure 9) However, scanning region from 250 nm

to 320 nm was not proper region to analyze secondary structure of peptide. Due to DMSO solvent, scanning less than 250 nm region was not afforded.

### *3.3.3 Reversibility of Gel-Sol transition.*

FTIR experiment was performed to find conformation structure. The focus region of FTIR data was on the amide I band. The amide I band was sensitive region to secondary structure. [23,24] The peak at 1628 and 1690 $cm^{-1}$ was observed in gel state. These peak showed that there are β-sheet conformation in the structure [23,24]. However, the peak tendency was different in sol state. There was a 1648-1650 peak range in the sol state of tandem repeat peptide. Through FTIR experiments, the secondary structure of sol state and gel state was completely different. gel state was composed of β-sheet conformation, but when it becomes sol state, there was not existed β-sheet conformation. (Figure 10)

ARES rheology test was performed to show mechanical sensitive Gel-Sol transition. Full red dot measured stress-strain curve, when force was proportionally increased. Empty red dot showed stress-strain curve, when force was proportionally decreased. Before testing ARES experiment, the sample was loaded then waiting 1 hour to make gel state. After making gel state, force was applied. When force was increased, the gel state showed elastic behavior until 90 % strain. Over 90 % strain, the graph did not show proportional slope. When strain became 150 %, the structure of tandem repeat peptide lost the property of gel state. This point indicated that the gel state transformed into sol state. Over 150 % region, there was a large change in strain but small increase in stress. However, when the force was decreased, the tandem repeat peptide showed similar graph tendency with gel state. (Figure 11) This result proved that the tandem

repeat peptide showed mechanical sensitive gel-sol transition. This Gel-Sol transition cycle will be repeated.

To observe how sol state is transformed into gel state, CD spectra was performed. Before loading sample in quarts cells, the sample was made sol state by applying mechanical strength. After scanning sol state, samples was located in the cell without adding any force. Then, the sol state sample became gel state (Figure 12). The CD spectra also proved that reversibility of Gel-Sol transition.

In summary, FTIR experiments showed a β-sheet secondary structure, when the tandem repeat peptide became gel state. CD, ARES data proved reversible Gel-Sol transition of tandem repeat peptide.

### *3.3.4 Strain sweep test of SOL-GEL transition*

To observe viscoelasticity, mechanical stability, and reversibility of tandem repeat peptide, strain sweep test was performed by ARES. In general, when storage modulus G', elasticity, is higher than loss modulus G'', viscosity, it gives that the sample is in the solid phase. The sample was prepared gel state. The strain was proportionally increased. Before increasing strain property, G' modulus is higher than G''' modulus. This data represented that the sample was in GEL state. When strain reached 150% strain, storage modulus G', elasticity, and loss modulus G'', viscosity cross the line. This point showed that the sample was deformed (Figure 13). When the sample became sol state, reverse sweep test was performed. The strain was proportionally decreased (Figure14). When the strain was decreased, the sample was lost sol property and gained gel property (Figure 14). However, comparing between strain sweep test and reverse strain sweet test cross point of G' and G'' was different. The reason was that

when the strain was increased in gel state, the structure tried to maintain its shape. In reverse sweep test, there were accumulated force was existed in the sample. Therefore, the cross point of G' and G'' was different.
Through ARES test, the sample recover reversible Gel-Sol transition even though the sample was completely deformed.

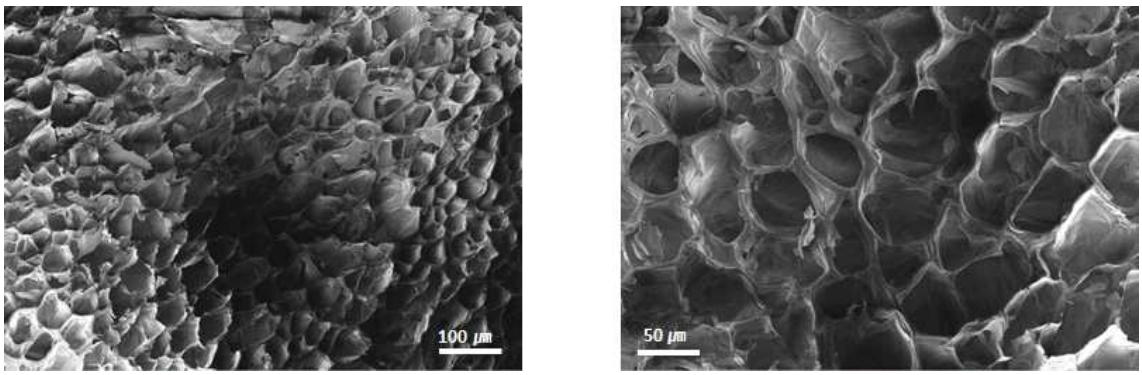


**Figure 8 : Field-Emission Scanning Electron Microscope images of gel state.** 30 mg/ml of the tandem repeat peptide (gel state) dissolved in 100 % DMSO was put into liquid nitrogen and freeze-dried for two days.

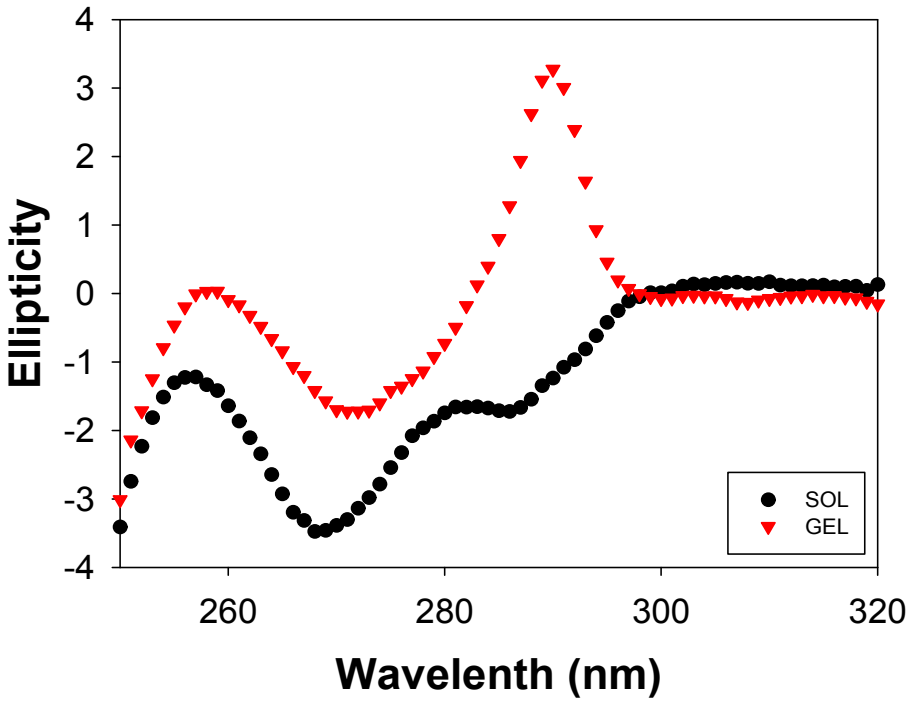


**Figure 9 : Circular dichroism image of Gel-Sol transition.** The sample were dissolved in DMSO (30 mg/ml) and loaded in 1 mm-pathlength quarts cell. Spectra scanning were performed from 250nm to 320nm with a 1 nm step resolution.

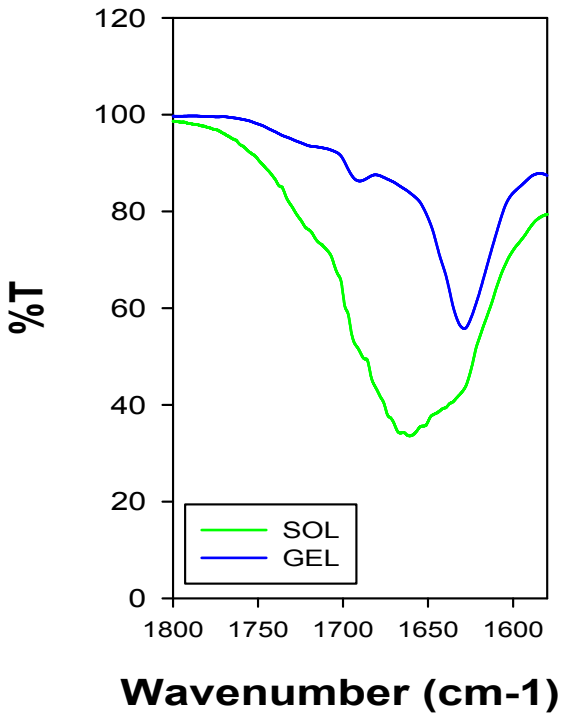


**Figure 10. ATR-FTIR spectra of the amide I band of sol state, gel state.** FTIR spectrometer carried out to analyze secondary structure of tandem peptide. To observe EGVLYV-EGVLYV tandem repeat peptide (gel state), dissolved in DMSO (30 mg/ml), the tandem peptide was put into liquid nitrogen and freeze-dried for two days.

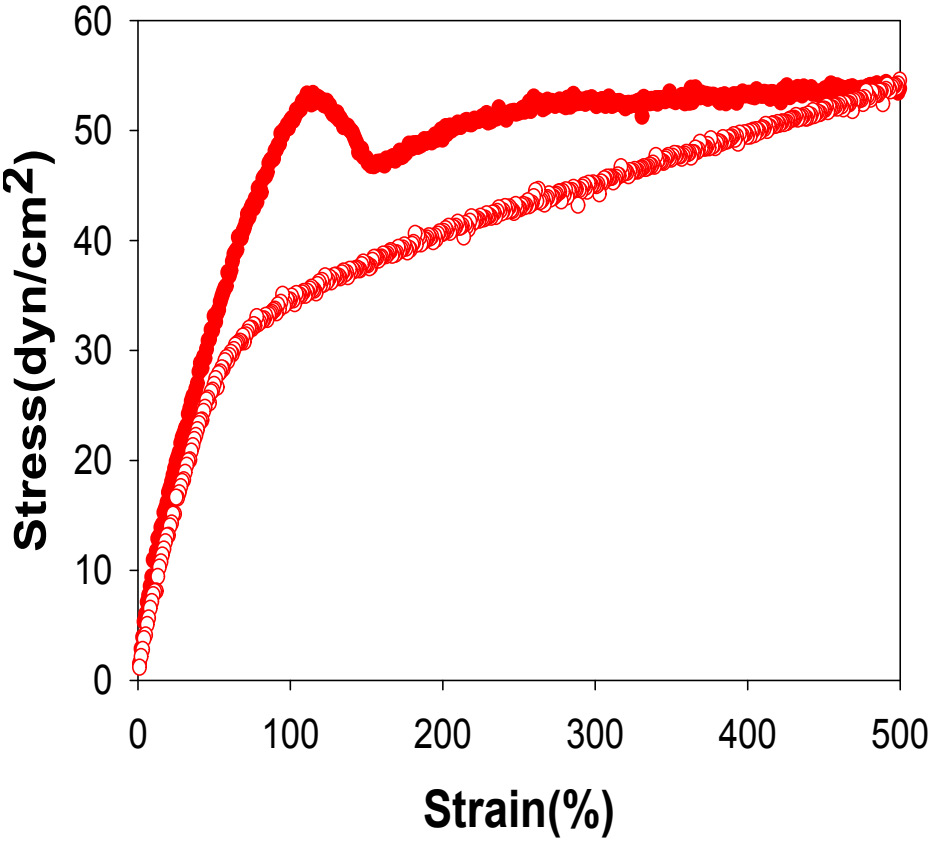


**Figure 11 : ARES stress-strain curve of Gel-Sol transition.** The peptide was dissolved in DMSO with proper concentration, 30 mg/ml. The sample was loaded as circular cylinder shape, 25 mm diameter and 1 mm height.

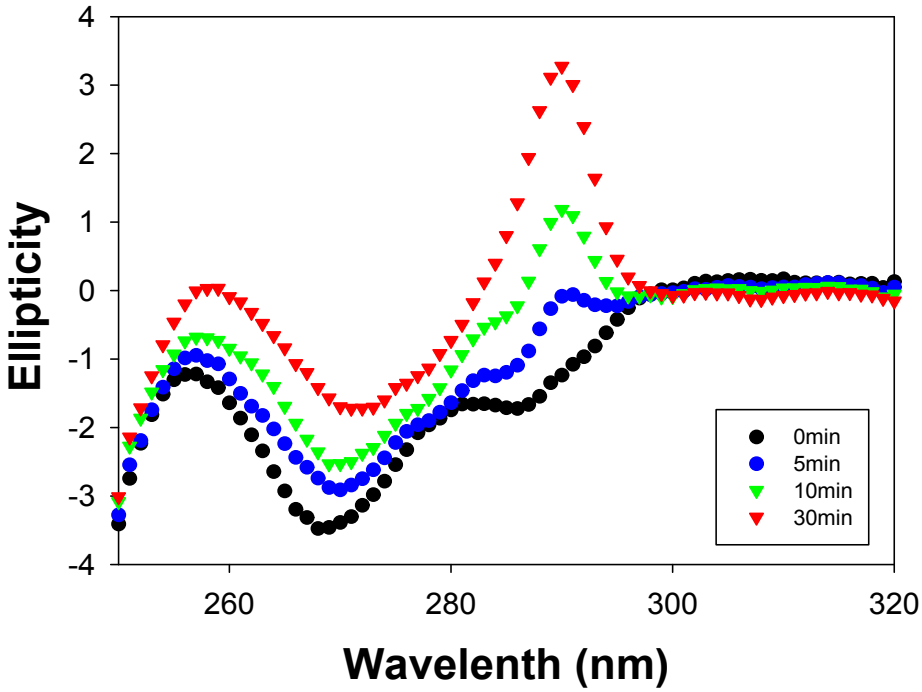


**Figure 12 : Circular dichroism spectra of Gel-Sol transition.** The sample were dissolved in DMSO ( 30 mg/ml ) and loaded in 1 mm-pathlength quarts cell. Spectra scanning were performed from 250nm to 320nm with a 1 nm step resolution.

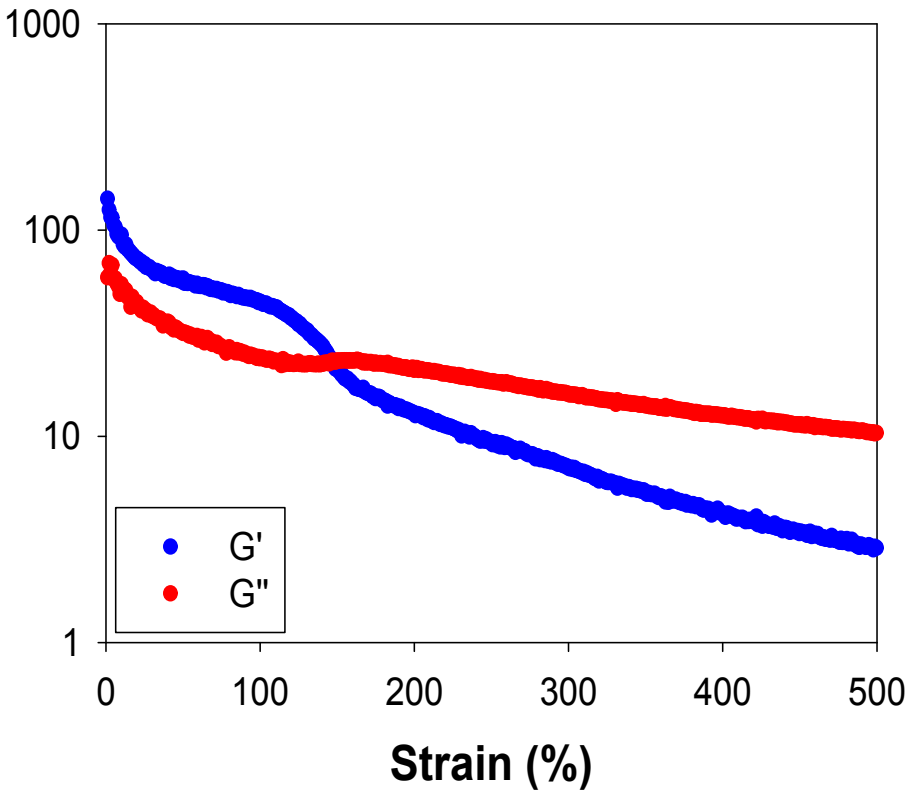


**Figure 13 : ARES strain sweep test of Gel-Sol transition.** The tandem repeat peptide was dissolved in DMSO with proper concentration, 30 mg/ml. The storage modulus, G', shows elasticity of the sample, and the loss modulus, G'', shows viscosity of the sample. The % of strain was increased.

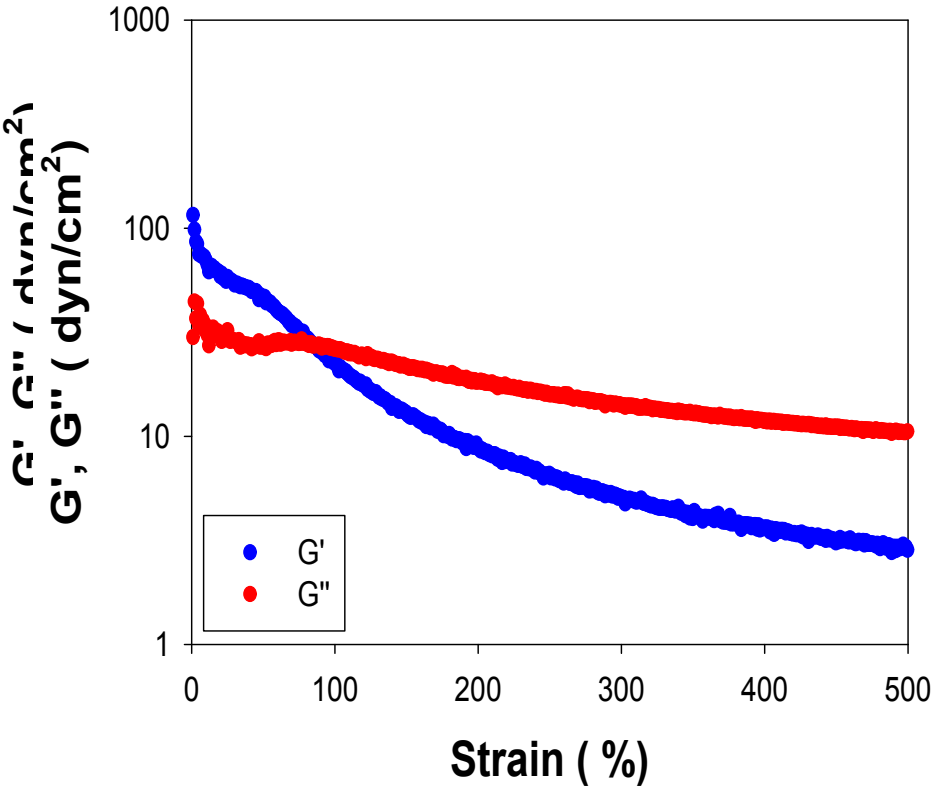


**Figure 14 : ARES reverse strain sweep test of Gel-Sol transition.** The tandem repeat peptide was dissolved in DMSO with proper concentration, 30 mg/ml. The storage modulus, G', shows elasticity of the sample, and the loss modulus, G'', shows viscosity of the sample. The % of strain was decreased.

# 4. Conclusion

Using TANGO program, there is a specific sequence, EGVLYV which has high amyloidogenic propensity. This sequence did not make any structure. However, when we made this sequence as tandem, EGVLYV-EGVLYV, it showed Gel-Sol transition. To figure out, this Gel-Sol transition was whether tandem repeat specific or sequence specific reason, pruning experiments performed. The results showed that Gel-Sol transition was both tandem repeat reason and sequence specific reason.

In order to proper condition of Gel-Sol transition, temperature, peptide concentration, water dialysis experiments were performed. Trough these experiments, Gel-Sol transition was well formed when tandem repeat peptide concentration was 30 mg/ml in DMSO solvent at 25 ℃. Changing solvent or temperature affected Gel-Sol transition. The reason was that molecular self assembly was changed.

Through previous experiments, there were specific conditions that was successfully formed Gel-Sol transition. Under this conditions, Gel-Sol transition continuously observed. To prove this reversibility of Gel-Sol transition, ARES, CD experiments were performed. ARES showed reversibility of Gel-Sol transition. When property of strain was increased, the sample became sol state. When strain was decreased, it transform into gel state. Through CD spectrometer, sol state was successfully changed to gel state when force was eliminated.

Through FTIR and SEM, internal structure and secondary structure of gel state was observed. SEM image showed honey comb structure with equal pore size. FTIR data represented that gel state was consist of a β-sheet structure..

This molecular structure, made by tandem repeat peptide from α-synuclein, could be used in various way such as sensor, affording

matrix for entrapping particles, and tissue engineering.

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# 국문 초록

파킨슨병은 신경퇴행성 질환인데 파킨슨병을 가지는 환자들의 공통적인 특징은 루이소체가 발견된다는 점이다. 이러한 루이소체를 형성하는 중요 단백질은 알파-시뉴클레인이라고 많은 연구에서 보고가 되었다. 알파-시뉴클레인은 140개의 아미노산으로 이루어져 있고 주로 뇌에 많이 분포하고 있는 단백질이다. 이 단백질은 특정한 자가 조립화현상을 통하여 응집체를 형성한다. 이러한 알파-시뉴클레인의 자가조립화 현상은 병리학적 이유 뿐 만 아니라 재료학 적으로도 크게 각광받고 있다. 단백질의 응집체 형성을 예측할 수 있는 TANGO 라는 프로크램을 이용하여 알파-시뉴클레인으로부터 특이한 서열 (EGVLYV) 을 발견하였다. 이 서열은 알파-시뉴클레인에 (35-40)에 위치하고 있고 N-terminal 영역에 있다. 비록 EGVLYV는 많은 연구에서 보고되는 베타-쉬트 형성에 주요한 영역인 NAC 영역에 포함되지는 않지만, 이 서열을 tandem repeat (EGVLYV-EGVLYV) 하게 만들었을 때 졸 젤 전이현상을 보였다. 이러한 졸 젤 현상은 펩티드를 DMSO에 녹였을 때 나타나는 현상이고 젤 상태에서 일정이상의 힘을 가하면 졸로 형태변화를 하였다가 다시 가하였던 힘이 사라지면 젤로 변화하는 현상을 보였다. 이렇게 졸 젤 전이현상을 보이는 물질은 젤 상태가 되었을 때 honey comb구조로 이루어져있고 증류석 투석이나 온도변화를 통하여 졸 젤 현상이 잘 일어나는 조건을 찾았다. 이 조건에서는 졸 젤현상이 끊임없이 나타는 것을 확인하였다. 이렇게 알파-시뉴클레인에서 유도된 펩티드의 졸 젤 전이현상은 알파-시뉴클레인의 자가 조립화현상이 N-term 영역에서도 중요한 역할을 할 수 있는 가능성을 제시하였고, 이렇게 특이한 졸 젤 전이현상은 나노테크놀로지재로로서 활용을 할 수 있다.

**주요단어 : 알파-시뉴클레인, Tandem repeat 서열, 자가조립현상, 졸 젤 전이현상**

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