

Design and synthesis of pseudopeptide inhibitors for aggregation and fibril formation of Amyloid β protein

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Introduction

Amyloid β peptide ($A\beta_{42}$) consisting of 42 amino acids has been regarded as the main protein component of amyloid plaques (Fig.1). Many efforts were made to investigate the mechanism of aggregation of $A\beta_{42}$ however, the mechanism of the aggregation and fibril formation of this protein is not fully understood. Thus, research to elucidate the mechanism of fibril formation of $A\beta_{42}$ and the intermediate structure of aggregation was necessary to develop novel inhibitors for aggregation of $A\beta_{42}$. The core region (16-22) of $A\beta_{42}$ is considered as key part for fibrillogenesis as well as aggregation. Recently, many research groups have reported that pseudopeptides containing N-methyl amino acids corresponding to the core region of $A\beta_{42}$ are effective inhibitors for fibrillogenesis. Similarly, the pseudopeptides containing ester bonds instead of amide bonds have been used to investigate the main chain hydrogen bonding and its effect on fibrillogenesis.

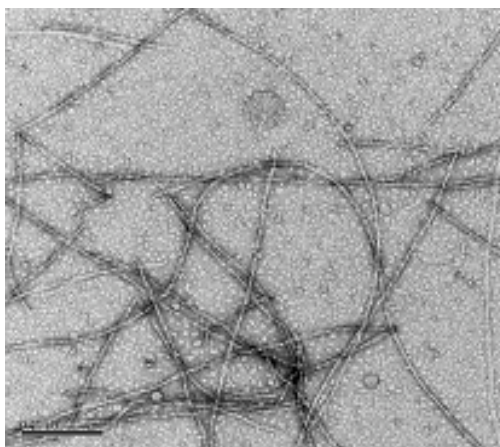
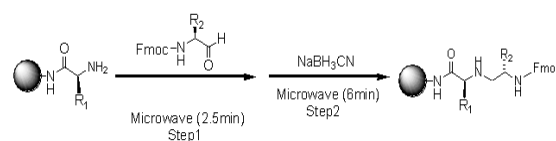


Fig. 1. TEM image of amyloid β protein fibril

In this communication, we studied the inhibitory activity of the pseudopeptides containing reduced amide bond for the aggregation and fibril formation of $A\beta_{42}$. The inhibition activity of the pseudopeptides was investigated by using

dynamic light scattering method, Thioflavin T assay and Congo red assay, respectively.



Scheme 1. Preparation of Pseudopeptide assisted by microwave system

Results and Discussion:

All peptides containing reduced amide bonds were synthesized using solid phase synthesis assembling the peptide chain on Rink amide MBHA resin. The reduced amide bond was incorporated using the method as shown in Scheme 1.

Table 1: Peptide sequence and measured mass.

Peptide	Sequence	Calculated mass/ Measured mass
$A\beta_{16-22}$	KLVFFAE-NH ₂	853.48/852.62
R1	Ac-K Ψ [CH ₂ (CH ₃ CON)]LVFFAE-NH ₂	923.11/922.00
R2	Ac-KL Ψ [CH ₂ NH]VFFAE-NH ₂	881.07/ 880.10
R3	Ac-KLV Ψ [CH ₂ NH]FFAE-NH ₂	881.07/880.10

All peptides were characterized by reverse phase HPLC using Waters C18 column and further analysed by ESI mass. The peptides were purified by semi-prep column and purities were > 95 %. The peptide sequence, and measured mass were shown in Table 1.

The size of aggregation of $A\beta_{42}$, $A\beta_{16-22}$, and the pseudopeptides were investigated using dynamic light scattering. The size of the core peptide ($A\beta_{16-22}$), R3 pseudopeptide and $A\beta_{1-42}$ together with R3 were shown in Figure 2 and Figure 3.

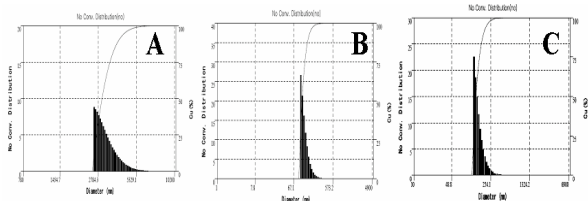


Fig. 2. Size analysis distribution by DLS of (A) Aβ16-22 (30μM, 0-1 hr Avg. 3188.7nm±615.4nm), (B) R3 (28 μM at 0 hr Avg. 117.6nm±29.0nm), and (C) R3 (28μM, 18 hr avg. 152.4nm± 36.7nm)

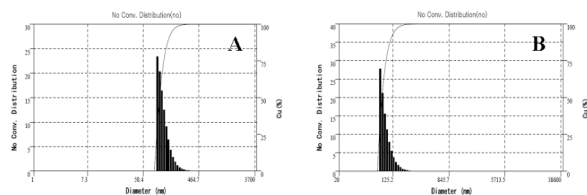


Fig. 3. Size analysis distribution by DLS of Aβ42 in the presence of R3. (A) 0 hr Avg. 120.9nm±29.3nm, (B) 16 hr Avg. 96.0nm±21.3nm

The size analysis study by DLS indicated that the core peptide aggregated instantly in this condition (Figure 2a) whereas the inhibitor peptide did not show aggregation property even after incubation for 18 hours in the same condition. (Figure 2b, c) Similarly, Aβ42 along with R3 did not show aggregation. (Figure 3) Further, the inhibition activity of R3 on the aggregation of Aβ42 were investigated in the different temperature and concentration. The size of aggregation of Aβ42 in the presence of R3 was depicted in Figure 4.

To investigate the fibrillogenesis, Thioflavin T-assay was performed in a time dependent manner. As shown in Figure 5 (a), the fibril formation of core peptide was

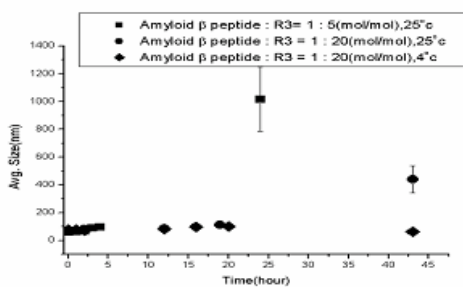


Fig. 4. Size analysis by DLS of mixture (R3+ Aβ1-42) by DLS at different temperature and concentration.

completed within 45 min, whereas Aβ1-42 required 4 hour. This result was consistent with the result of size analysis.

Furthermore, the fibril formation study was conducted using congo red assay based on the absorbance of congo red (Figure 6). The change in the absorbance caused by inhibition of R3 with Aβ1-42 at different time interval was measured.

In conclusion, overall study indicated that the core peptide Aβ16- 22 easily aggregated whereas the inhibitor

peptide (R3) containing reduced amide did not show the aggregation even after incubation for long time. Thus, reduced amide bond containing peptide might be potent inhibitor for the study of aggregation and fibrillogenesis of amyloid beta protein.

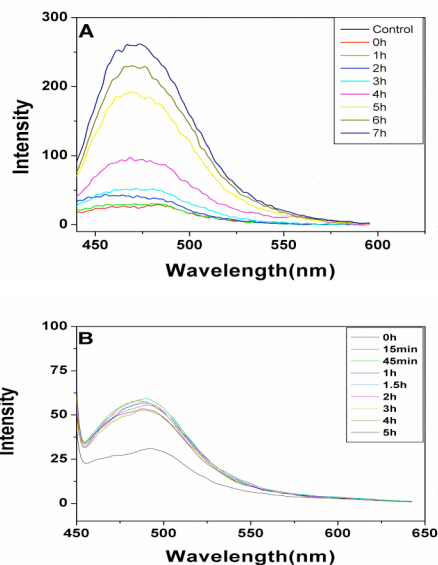


Fig 5. Fluorescence response of Thioflavin T at different time interval. (Excitation at 430nm) (A) 5.5μM Aβ 42 in 10 mM phosphate buffer (pH 7.4, 150 mM NaCl) (B) 5.5 μM Aβ16-22 in 10 mM phosphate buffer (pH 7.4, 150 mM NaCl)

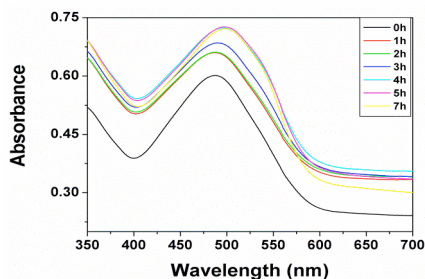


Fig 6. Uv-Vis Spectra of a 5 μM Congo red with Aβ42 (5.5 μM) and R3 inhibitor 20 eq at different time interval

Acknowledgments

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References

- Zurdo, J.; Guijarro, J. I.; and Dobson, C. M. (2001) *J. Am. Chem. Soc.*, **123**, 8141-8142.
- Iwata, K., Eyles, S. J. and Lee, J. P. (2001) *J. Am. Chem. Soc.*, **123**, 6728-6729.
- Bothner, B.; Aubin, Y. and Kriwacki, R. W. (2003) *J. Am. Chem. Soc.*, **125**, 3200-3201.
- Morgan, D. M.; Dong, J.; Jacob, J.; Lu, K.; Apkarian, R. P.; Thiyagarajan, P.; Lynn, D. G. (2002) *J. Am. Chem. Soc.*, **124**, 12644-12645.
- Lu, K.; Jacob, J.; Thiyagarajan, P.; Conticello, V. P.; Lynn, D. G. (2003) *J. Am. Chem. Soc.*, **125**, 6391-6393.