Circular Dichroism Spectroscopic Study of β-Amyloid Aggregation and the Effects of Inhibitors

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INTRODUCTION

Neurodegenerative disorders, including Alzheimer's disease (AD) and Parkinson's disease (PD), are characterized by progressive cognitive decline, and global efforts are underway to develop effective treatments. Key proteins such as β-amyloid, [1] α-synuclein, and tau are implicated in the pathogenesis of these diseases. the aggregation of β-amyloid plays a central role in neurodegenerative diseases such as AD, and research on this phenomenon is crucial for understanding the pathology and developing therapeutic strategies. β-amyloid exists extracellularly and undergoes an aggregation process, in which monomeric peptides form micellar intermediates under certain stimuli, which then further grow into fibrils (fibrous structures) or form amyloid plaques. These amyloid plaques accumulate between neurons, and ultimately lead to neuronal death. Understanding this aggregation process is essential for discovering new approaches to prevent or treat the disease. Circular dichroism (CD) spectroscopy is well known as a convenient and sensitive method for investigating the higher-order structure (HOS) of proteins in solution. This technique can monitor the changes in HOS and the formation of aggregates by measuring CD signals of β-amyloid peptide,[2-4] making it valuable for elucidating disease mechanisms and advancing the development of effective therapeutics and preventive agents. In this presentation, we report the results of the evaluation of the performance of ionic liquids as inhibitors of amyloid aggregate formation and as solubilizers of β-amyloid peptide aggregates using CD spectroscopy and the BeStSel program, which allows accurate and detailed evaluation of secondary structure of proteins.

EXPERIMENTAL

Materials

Amyloid β-peptide (Human, 1-42) (from Peptide institute. Inc.)

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala(Trifluoroacetate Form)

(M.W. **4514.0**) $C_{203}H_{311}N_{55}O_{60}S$

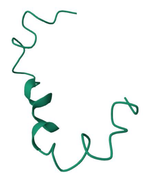
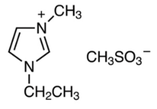


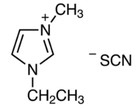
Fig. 1 Image of β-amyloid peptide (PDB 6SZF)[3]

Ionic liquid (IL) (from Tokyo Chemical Industry Co., Ltd.)

[EMI] [Ms] : 1-Ethyl-3-methylimidazolium Methanesulfonate



[EMI] [Tc] : 1-Ethyl-3-methylimidazolium Thiocyanate



[EMIM][Cl] : 1-(2-Hydroxyethyl)-3-methylimidazolium Chloride

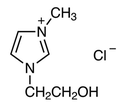


Fig. 2 Structures of ILs

Measurement method

- Sample conditions: 25 μM amyloid β-peptide dissolved in 67 mM phosphate buffer (pH 7.0) in the absence of IL or in the presence of 0.005% of three different ILs.
- Data acquisition: 400 μL sample was loaded into a 1 mm optical path length cell, and spectra were collected at 20 min intervals for 400 min.
- Measurement conditions: bandwidth 1 nm, scanning speed 50 nm / min, response 2 sec.

Instrument and analysis program

J-1500 CD Spectrometer

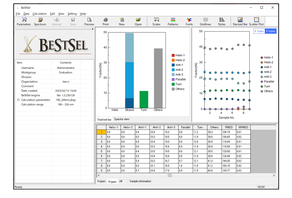
- Simultaneous CD and absorbance measurements with high accuracy
- Measurement of protein solutions at a wide range of concentrations

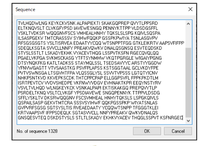
PTC-510 Peltier Thermostatted Cell Holder

- Temperature setting range -40 to 130 degrees

Fig. 3 Image of CD spectrometer and cell holder

Spectra Manager Ver.2.5/CFR BeStSel





- Detailed and accurate secondary structure analysis of a wide range of proteins, including β-sheet-rich proteins
- Batch analysis of 3D data or multiple data sets
- Concentration calculations using amino acid sequences

Fig. 4 Images of [BeStSel] program for Spectra Manager

RESULTS

Inhibition effect of ionic liquids

The CD signal at 217 nm derived from the β-sheet was decreased over time for amyloid β-peptide (Human, 1-42) due to aggregation (Fig. 5 A). In the presence of [EMI][Ms], the small change in the CD spectrum as well as the smaller initial amount of change in the CD signal just after the addition of IL than in the absence of IL (Fig. 5 B) indicate that IL inhibits the aggregation of peptides. On the other hand, for [EMI][Tc] and [EMIM][Cl], the initial change in CD signal just after the addition of IL was larger than that without IL, suggesting that IL promotes peptide aggregation in the first-step aggregate formation.

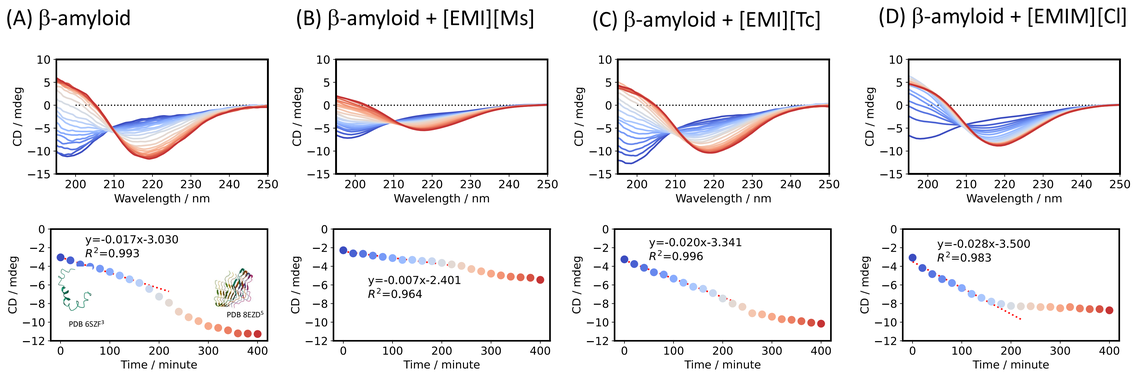


Fig. 5 Far-UV CD spectra (upper) and $CD_{217\ nm}$ vs. time (bottom) of amyloid β-peptide in the absence or presence of ILs . (A) without IL, (B) with [EMI][Ms], (C) with [EMI][Tc], and (D) with [EMIM][Cl].

Secondary structure analysis using BeStSel

Secondary structure analysis using BeStSel allows us to evaluate the time dependence of the secondary structure fractions (Fig. 6 A-D). Overall, β-strands showed more or less an increasing trend, while others showed a decreasing trend. It is also possible to classify β-sheet into four types: parallel β-strand and three types of antiparallel β-strands. Fig. 7 shows the time dependence of the parallel β-strand fraction, which is especially important during aggregate formation. This result indicates that parallel β-strand is not formed when [EMI][Ms] is present. The intermediate time for the first stabilization of parallel β-strand formation and the average value of parallel β- strand fraction after this stabilization were as follows. Intermediate times were fastest in the presence of [EMIM][Cl], followed by [EMI][Tc], then β-amyloid only. The average was largest in the presence of β-amyloid only, followed by [EMI][Tc], then in the presence of [EMIM][Cl].

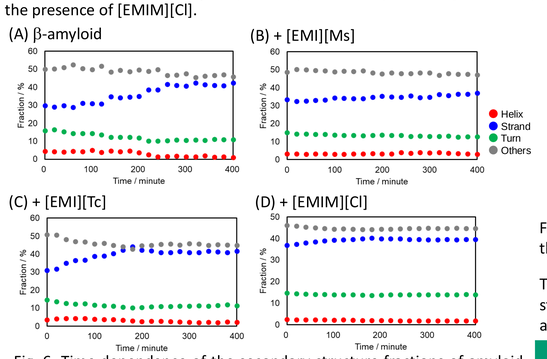


Fig. 6 Time dependence of the secondary structure fractions of amyloid β-peptide in the absence or presence of the three ILs. Time vs. secondary structure fractions of (A) without IL, (B) with [EMI][Ms], (C) with [EMIM][Cl], and (D) with [EMI][Tc].

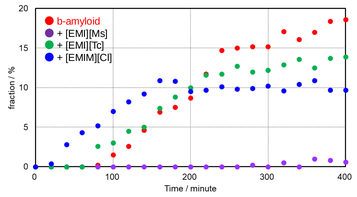


Fig. 7 Time dependence of parallel β-strand fractions in the absence or presence of the three ILs.

Table 1 Intermediate time and averages of fraction after stabilization of parallel β- strand formation in the absence or presence of the three ILs.

	β-amyloid	+ [EMI][Ms]	+ [EMI][Tc]	+ [EMIM][Cl]
Middle point / min	154	---	143	78
Average	16	---	13	10

SEM analysis

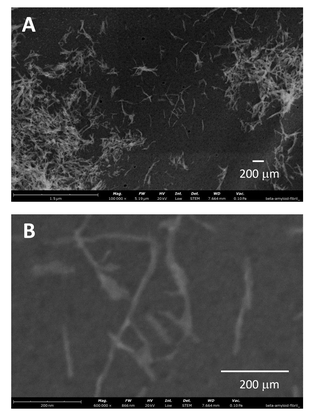


Fig. 7 STEM micrograph of aggregates of Amyloid β peptide.
(A) after 8hrs incubation at 37 °C,
(B) scale-up. Each scale bar shows in each micrograph.

CONCLUSIONS

- ✓ Only [EMI][Ms] ionic liquid inhibited the first-step aggregation of amyloid β-peptide (1-42).
- ✓ The results demonstrate that the combination of CD spectroscopy and the BestSel program is effective for the evaluation of the secondary structure fractions of amyloid β-peptides in detail.

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