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The impact of ionic liquids on amyloid fibrilization of Aβ16-22: tuning the rate of fibrilization using a reverse Hofmeister strategy†

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We have shown that the amyloid fibrilization of Aβ16-22 follows a reverse Hofmeister trend in pILs. Fast fibrilization rates of seconds can be achieved.

The self assembly and subsequent amyloid fibril formation of proteins has implications in many human diseases, including Alzheimer’s disease.1,2 The pathogenesis of Alzheimer’s disease involves the self assembly of Aβ monomers into dimers, multimers and oligomers.3,4 The oligomers self assemble into higher order aggregates termed protofilaments which continue to grow into fibrils.4 The processes of Aβ peptide assembly from monomers to oligomers and into fibrils, and their associated neurotoxicity is still an active area of research.5–8 Currently the neurotoxic species in the amyloid fibril assembling process is thought to be some form of soluble oligomer, most likely those that form during the early stages of Aβ aggregation.9,10 Recent trends have been directed at finding strategies to control the Aβ assembly process to allow characterization of the early state oligomers.9,11,12 In addition to developing methods to trap the early state oligomers, the development of drugs specifically targeted at early stage Aβ aggregation are currently under investigation.12

Solvents can play a key role in controlling the fibrilization of Aβ,13,14 as such, various solvents and additives have been used to study to Aβ amyloid fibrilization. Ionic liquids are designer solvents comprised entirely of ions. The advantages of ionic liquids as solvents include low volatility, recyclability, excellent solvating properties, variable polarity and a tremendous selection of cation and anion combinations.15,16 Since the formation of amyloid fibrils proceeds via the formation of intramolecular hydrogen bonds we sort to use protic ionic liquids to manipulate the hydrogen bond nature of the solvent to better control amyloid fibril formation. The use of ionic liquids as solvents for biological applications is currently receiving considerable attention, and ionic liquids have been used successfully as refolding additives for protein renaturing studies.17,18

The kinetics of amyloid fibrilization can be measured using the ThT binding assay. ThT fluorescence intensity is sensitive to beta sheet structure, with an increase in the fluorescence intensity being measured when aggregation have proceeded to the organized amyloid stage. Fig. 1 shows the ThT intensity kinetics of the Aβ16-22, in a series of different pILs with the common cation triethylammonium, (Tea). pILs investigated in increased protein shelf life,19 enhanced thermal stability20,21 and as additives which increase enzymatic reaction rates.22 Recently pILs where shown to both promote and inhibit amyloid fibrilization of hen egg white lyszyme.23,24

Hwang et al. also reported the ability of 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide to accelerate the fibrilization of α-synuclein.25 Therefore, the characterization of ionic liquid solvents and the impact they have on amyloid fibrilization is important, especially since ionic liquids potentially have an important role to play in furthering our knowledge of the amyloid fibrilization process and in drug design. In this context, our aim was to study the influence pILs have on the amyloid fibrilization of the Aβ16-22 peptide. This fragment has been previously identified as a core fragment for amyloid fibril formation in full length Aβ,26,27 making this fragment an appropriate peptide sequence to further our understanding of the role ionic liquids can play in amyloid fibrilization.

We have used a combination of ThT fluorescence intensity, circular dichroism, and transmission electron microcopy to analyze and characterize the amyloid fibrilization of the Aβ16-22 peptide using protic ionic liquid solvents.

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Fig. 1 ThT intensity as a function of time for Aβ16-22 in 90% TeaHSO4 (red curve), 90% TeaH2PO4 (blue line), 90% TeaTFac (green line), 90% TeaLa (purple line), 90% TeaTf (light blue line), 90% TeaMs (orange line) and phosphate buffer (black line).
This study include, triethylammonium phosphate (TeaH$_2$PO$_4$), triethylammonium hydrogen sulfate (TeaHSO$_4$), triethylammonium trifluoroacetate, (TeaTfac), triethylammonium lactate, (TeaLa), triethylammonium triflate, (Tea Tf), and triethylammonium mesylate, (TeaMs). Since both TeaH$_2$PO$_4$ and TeaHSO$_4$ have melting points above room temperature, we measured the ThT intensity of the A$\beta$16-22 peptide from 97%TeaH$_2$PO$_4$:3%water (v/v) through to 5%TeaH$_2$PO$_4$:95%water (v/v) and found that 90% TeaH$_2$PO$_4$ showed fast kinetics (ESI Fig. 1f). Since we are interested in high pIL content solvents we therefore decided to compare the kinetics of fibrilization in the above mentioned pILs at a fixed concentration of 90%pIL:10%water (v/v). The ThT intensity of the A$\beta$16-22 peptide in different pILs is shown in Fig. 1. Both TeaH$_2$PO$_4$ and TeaHSO$_4$, showed remarkably fast fibrilization with the maximum ThT signal observed after 1 min. The kinetics of both TeaTfac and TeaLa show a lag phase with fibrilization proceeding to a measured maximum after several hours for TeaTfac and 2 days for TeaLa. TeaTf showed a longer lag phase with a maximum ThT signal observed after 2 weeks. In the cases of TeaTfac, TeaLa and TeaTf, the maximum ThT signal is less than 1, this may indicate either the presence of aggregated states or a different amyloid structure. Interestingly, no fibrils formed in the 90%TeaMs solution even after 4 months, and more importantly the solution remained clear indicating the A$\beta$16-22 was still in solution. No change in secondary structure, as measured using CD, was observed over this time period. Included in Fig. 1 is the ThT response for A$\beta$16-22 in phosphate buffer, which reached a maximum on day 9.

A reverse Hofmeister trend is observed when the pILs are listed in order of fibrilization rate. That is, TeaHSO$_4$, TeaH$_2$PO$_4$, TeaTfac, TeaLa, TeaTf, and TeaMs. Traditionally, both phosphate and sulphate are considered to be kosmotropic anions. These anions are considered to be water structuring and generally enhance protein stability. The reverse Hofmeister trend observed here in the 90% pIL solutions suggests that competitive hydrogen bonding between the anion of the pIL and water is driving the self assembly of the A$\beta$ peptide into amyloid fibrils. In this context both TeaH$_2$PO$_4$ and TeaHSO$_4$ are “salting out” the A$\beta$ peptide and resulting in amyloid fibrils. It also suggests that in high pIL concentration solutions such as the ones studied here (90%pIL;10%water (v/v)), does not completely dissociate into individual ions, and probably ion pairs or higher order aggregates exist or the presence of microsegregated IL phase. This would explain the deviation from the Hofmeister series, which is often used to explain the salting in and salting out effects of salts with respect to protein stability and solubility.

The fast kinetics of fibrilization in 90%TeaH$_2$PO$_4$ confirmed by the ThT binding experiment supports our initial observation of white precipitates forming in the 90%TeaH$_2$PO$_4$ seconds after the monomeric peptide is added. (ESI shows movie clip of fibrilization). We then decided to examine the fibrils using TEM, long mature fibrils were present in the solution after one minute as shown in Fig. 2a. Fig. 2b shows an overview of the amyloid fibrils that can be achieved after one minute in 90%TeaH$_2$PO$_4$. A dense highly branched network is observed. This extremely fast fibrilization rate holds considerable promise in the application of amyloid fibrils for biomaterials.

**Fig. 2** TEM images shows amyloid fibrils of the A$\beta$-22 in 90%TeaH$_2$PO$_4$ at 1 min and (b) overview of (a) showing a dense amyloid network after 1 min. Scale bar 200 nm.

Fig. 3a shows the micrograph of the A$\beta$16-22 peptide, after 1 min, in 90%TeaHSO$_4$. This pIL also registered a maximum ThT intensity after one minute. However, as can be seen from the micrograph, in this case fibrils are not present, rather spherical objects can be seen in the micrograph. The spherical objects, are termed annular oligomers. The formation and stabilization of annular oligomers is an important finding as it has been suggested that annular oligomers may be wholly or partly responsible for the cytotoxicity associated with the formation of amyloid fibrils. The presence of the annular oligomers and amyloid fibrils in the same solution supports the complexity of amyloid fibrilization process and why pinning down the toxic conformation is difficult.

Fig. 4 shows the CD spectrum of A$\beta$16-22 for 90%TeaHSO$_4$ and 90%TeaMs. Given the difference in fibrilization kinetics observed in the pIL solutions we measured the CD spectrum of Abeta in 90%TeaHSO4 and 90%TeaMs. Fibrilization in 90%TeaHSO4 occurs on the time scale of minutes whereas no fibrilization is observed in 90%TeaMs. Interestingly the conformation of the peptide in both these solutions is very similar. The similarity of the CD spectrum, which represents an extended 310 helix, suggests that the
initial conformations, for the Aβ16-22 peptide in both these solutions is similar. This further suggests that the anion–water interactions of the pIL is the driving force for the differences observed in the fibrilization kinetics.

We have shown that the kinetics of fibrilization for Aβ16-22 can be tuned by following a reverse Hofmeister strategy in pIL solutions using the triethylammonium cation. Fibrilization can be both accelerated or completely suppressed depending on pIL choice. This has potential impact in understanding the underlying process of amyloid fibrilization. Additional, the ability to create fibrils on the time scale of seconds is important from a biomaterials aspect. The use of amyloid fibrils to create nanostructures is a promising avenue of research and the ability to accelerate fibrilization is beneficial in this context. The structure of water involves ice-like structure in a “salting out” scenario, while pILs containing mesylate anions stabilize the annular oligomers, a key intermediate state.

The stabilization of annular oligomers is an important finding and future work is aimed at investigating the neurotoxicity of the ionic liquid stabilized states and the ability for ionic liquids to reverse the aggregation process of amyloid fibrils. The self assembly and superstructure organization of the amyloid fibrilization process was also found to be influenced by pIL composition, resulting in the stabilization of annular oligomers, a key intermediate state.

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Notes and references

Fig. 4 CD spectrum of Aβ16-22 in 90%TeaMs (black) and 90%TeaHSO₄ (red).

Conclusions
We reported the fibrilization kinetics and superstructure organization of the Aβ16-22 peptide in a series of protic ionic liquids (pIL). The kinetics of Aβ16-22 fibrilization follows a reverse Hofmeister trend. We find that pILs containing kosmotropic anions like phosphate or sulphate promote fibrilization with mature fibrils forming within seconds whilst pILs containing the mesylate anion completely suppresses amyloid fibrilization. The self assembly and superstructure organization of the amyloid fibrilization process was also found to be influenced by pIL composition, resulting in the stabilization of annular oligomers, a key intermediate state.