Electric field assisted inhibition of aggregation in amyloidogenic proteins

Gaurav Pandey, Vibin Ramakrishnan

Indian Institute of Technology Guwahati, Assam, India

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Introduction

Tauopathies represent a group of progressive neurodegenerative disorders, associated with pathological aggregation of tau protein into neurofibrillary tangles. These neurodegenerative disorders are a significant unmet medical need because of their prevalence, severity, the cost associated with them, and lack of mechanism-based treatments. The failure of invasive molecular therapies has resulted in a shift towards developing relatively inexpensive and non-invasive approaches to impede the progression of tauopathies. The present study describes the application of the electric field for retarding the aggregation of core peptide segment of tau protein (Acetylated-VQIVYK-amide/PhF6) (Figure 1 (a),(b)). VQIVYK has been reported to be crucial for aggregation tau protein and is often used as model peptide system for developing aggregation inhibitor specific to tau protein. Our results utilising electric field as an aggregation inhibitor are in good agreement with the previously reported experimental [1-3] and theoretical [4,5] investigations on amyloid forming systems.

Method

Peptide synthesis using Fmoc chemistry → Peptide → Characterization by HPLC & MALDI → Aggregation assay under ambient as well as electric field conditions → Qualitative estimation of extent of aggregation using: Thioflavin T fluorescence (ThT) assay, static light scattering assay transmission; morphology examined by electron microscopy (TEM).

Results and Discussion

The model peptide PhF6 was synthesized by solid phase peptide synthesis and characterized for purity using HPLC and MALDI-TOF (Figure 1 (c), (d)). For testing our hypothesis, the PhF6 peptide sample was allowed to aggregate under the presence and absence of AC as well DC electric fields of varying strengths (Figure 1 (b)), for 16 hours. After 16 hours, the electric field was turned off. Both treated and untreated samples were analyzed for the extent of aggregation using ThT fluorescence and static light scattering assay. The degree of aggregation is said to be directly proportional to fluorescence and scattering intensities. We observed that samples allowed under AC/DC electric field showed reduced ThT fluorescence (Figure 1 (e)) and scattering intensity (Figure 1 (f)) compared to the untreated (0 Vcm⁻¹) sample. These observations indicate that electric mediated stress arrested the aggregation of the PhF6 peptide. The samples were examined under TEM microscopy, to investigate the effect of electric field induced modulation on the morphology of PhF6 aggregates. We observed that the untreated sample showed fibrillar structures in contrast the electric field treated samples displayed sphere-like structures.

Conclusions

We infer that electric field interceded stress can retard the aggregation and self-assembly of the core peptide segment of tau protein. Electric field mediated perturbation can also modulate the morphology of aggregated structures and prevent the formation of toxic fibrillar assemblies. Initial results from this study showing the effect of electric field on the model peptide system are promising. Rigorous toxicity and side-effects related assessments are necessary to employ it as a cost-effective and non-invasive therapeutic against tauopathies.

Figure 1: Schematic illustration (a) of our approach, (b) experimental set-up, characterization of purity of synthesized peptide by (c) HPLC and (d) MALDI-TOF, quantification of extent of aggregation of peptide samples allowed to aggregate under electric field and ambient conditions by (e) ThT fluorescence assay and (f) static light scattering assay, (g) electron micrographs of electric field treated and untreated samples

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