News and Views – Talaga et al.

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The causes of conditions such as Alzheimer's and Parkinson's diseases are becoming better understood. The misfolding of proteins from their native structure is implicated in these and other so-called amyloid diseases, in which proteins form aggregates of β-sheet fibrils termed amyloid.[1, 2] Understanding fibril formation is therefore critical and so *in vitro* studies are often conducted to examine the conditions, mechanism and kinetics of the fibrillisation process. It has been suggested that fibrillisation kinetics can be influenced during mixing by mass transfer effects. In addition, mixing leads to shear forces when the protein or peptide is dispersed in solution. This can influence the growth of fibrils by perturbing the equilibrium between isolated protein molecules and proteins aggregated into fibrils, since fibrils can fragment and create new nuclei.[3]

Writing in JACS,[4] Talaga and coworkers highlight an additional factor - the presence of hydrophobic interfaces - that can influence the fibrillisation of amyloid-forming proteins. They study the fibrillisation of α-synuclein which is implicated in Parkinson's disease using a standard fluorescence dye technique used to assay amyloid formation. The dye Thioflavin T binds to amyloid fibrils, but not to isolated protein. The kinetics of fibrillisation in dilute aqueous solutions of the protein were monitored as a function of incubation time, the samples being subjected to agitation in the presence of different types of ball of 1-2 mm size. Balls were made of borosilicate glass which is chemically inert, polymethylmethacrylate (PMMA) which is slightly hydrophilic or polytetrafluoroethylene (PTFE) which is hydrophobic. Some samples were also agitated in the presence of controlled volumes of air (which is

hydrophobic). The fibrillisation kinetics, which showed a typical sigmoidal growth curve, were found to depend strongly on the number of PTFE balls, i.e. to the hydrophobic surface area. The initial slope of the fluorescence increase, and the asymptotic fluorescence level reached, were proportional to the number of PTFE balls, according to power law behaviour. The inverse lag time also increased in a non-linear fashion with the number of PTFE balls. Further nucleation and growth of fibrils was induced by addition of PTFE balls to a sample containing fibrils that had already developed upon agitation in the presence of PTFE balls. Several control experiments were also performed. An increase in dye fluorescence was observed in the presence of air, although fibrils were not observed using atomic force microscopy. In the case of glass balls, no fibril formation was observed. Fibrillisation was observed using PMMA balls, but to a much lower extent than with PTFE balls. As a further control, quiescent samples were examined and these showed no increase in ThT fluorescence in the absence of agitation.

This report relates to earlier work[5] which examined amyloid fibrillisation in the presence of nanoparticles with controlled surface hydrophilicity/hydrophobicity. Polymeric nanoparticles with varying surface chemistry can either increase or decrease the fibrillisation of amyloid proteins, depending on the nanoparticle hydrophobicity and also the unfolding behaviour of the protein and the hydrogen bonding capacity of subunits within it.[5] Talaga and coworkers interpreted their results in the context of several proposed models for fibrillisation. First, simple mass transfer effects on mixing were considered. These eliminate local concentration gradients. Since one PTFE ball can mix the sample on a timescale shorter than the rate of reaction, this model would predict no dependence on the number of PTFE balls. This

model is evidently in conflict with the experimental results. Second, fragmentation of fibrils was considered. Fibril break-up occurs due to forces experienced during flow under agitation. PTFE balls moved more rapidly but less turbulently than PMMA balls, while glass balls moved similarly to PTFE. This does not suggest a simple relationship between flow force and the observed fibrillisation kinetics. Furthermore, the fibril length from atomic force microscopy images did not depend on the number of PTFE balls. The asymptotic fluorescence would also be expected to be lower if shorter fibrils were produced by a fragmentation mechanism. Since the asymptotic fluorescence actually increased with number of PTFE balls, this is also inconsistent with just a simple fragmentation model. A final factor - the influence of hydrophobic interfacial area - was therefore considered. The fibrillisation kinetics were evidently proportional to the PTFE surface area, but not to the surface area of glass or PMMA. The contact angle of PTFE decreases in the dramatically in the presence of protein, showing that the protein coats the PTFE surface progressively reducing the amount of available catalytically active interface. Moreover, addition of more PTFE balls lead to re-initiation of growth indicates that saturation of adsorption had not occurred since fibril-capable protein was still present in solution. Accelerated fibrillisation was also observed in the presence of air, although the morphology of fibrils was different (globular aggregates were observed).

These results clearly show the importance of hydrophobic interfaces in accelerating the fibrillisation of the amyloid-forming protein α-synuclein. At a molecular level, the results suggest that conformational changes may accompany fibrillisation, specifically that hydrophobic residues are selectively adsorbed at hydrophobic interfaces. The interface may also nucleate contacts between hydrophobic regions, leading to

accelerated fibrillisation. It is known that α-synuclein contains hydrophobic sequences, in particular the NAC domain (non-Aβ component of amyloid plaques) is mainly hydrophobic. Other amyloid peptides including Aβ are known to be amphiphilic.[6] These findings provide an important insight to understand issues of sample-to-sample reproducibility that plague *in vitro* studies of amyloid fibrillisation. It is already known that very careful protocols have to be followed in studying fibrillisation of Aβ(42) for example, for example starting from a well-defined state of unaggregated peptide (achieved by initial dissolution in a hydrophobic solvent) and then carefully controlling the addition of water or buffer to a dried film.[7] The results of Talaga and coworkers reveal an additional complicating influence. Heterogeneous interfaces, specifically hydrophobic interfaces including air, are demonstrated to exert a strong effect on fibrillisation kinetics. The role of amyloid peptide amphiphilicity, and interfacial hydrophobicity *in vivo* is not clear, although aggregation of hydrophobic sequences is already known to enhance amyloid fibril-formation.[8] Variability in morphology resulting from mixing in the presence of hydrophobic interface may also be important since fibril polymorphism, resulting for instance from sonication, has a profound effect on toxicity.[9] Agitation in the presence of air bubbles, or using PTFE stirrers or vials is expected to lead to variability in fibril formation kinetics, unless the hydrophobic interfacial area can be carefully controlled.

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