

The Mechanism of β -Amyloid Aggregation under Different Surface Conditions

Introduction

Dual Polarisation Interferometry (DPI) is an important tool for the study of the structural events that underpin biomolecular function and interaction ⁽¹⁾. This application note describes the use of DPI to investigate the molecular basis for the action of a small molecule inhibitor of β -amyloid aggregation.

A key feature of Alzheimer's disease (AD) is the accumulation of fibrillar deposits on the surface of neuronal cells in the brain. The formation of these plaques is implicated in the pathogenesis of AD. The plaques are thought to result from aggregation processes involving β -amyloid peptides. Many researchers now consider the early oligomeric forms of β -amyloid to be the most neurotoxic; hence their detection is of critical importance (*Farfield Application Note 004*). The molecular events occurring during the formation of β -amyloid fibrils and the factors that influence the onset of aggregation are areas of intensive investigation in the search for AD therapies. As such there is intense interest in identifying and understanding molecular processes that make β -amyloid aggregation more likely. However, the lack of sensitivity provided by many analytical techniques has made the study of inhibition of early stage aggregation events particularly challenging to date ⁽²⁾.

We were interested in using DPI to gain a real time understanding of the molecular basis for the onset of the aggregation process through the study of the interfacial behaviour of β -amyloid and comparison of its subsequent aggregation on surfaces of differing hydrophobicity.

Experimental

The DPI experiments were performed on a Farfield **AnaLight**[®] instrument. Two surfaces were used in these studies, a C-18 functionalised silicon oxynitride **AnaChip**[™] and a trimethyl silane functionalised silicon oxynitride **AnaChip**[™]. The temperature of the samples was controlled throughout to 37°C. Water used in buffer and reagent preparation was deionised and free from organic impurities. All buffers and reagents were analytical grade or higher, and solutions were degassed prior to use.

β -Amyloid Aggregation Studies: The synthetic peptide corresponding to the residues 1 to 40 of β -amyloid **Ab1-40** was used in these studies. A β 1-40 was introduced in solution (50 μ M in 10mM phosphate buffer, 150mM NaCl, pH7.4) to the **AnaChip**[™] surface via the **AnaLight**[®] instrument fluidics. The flow was stopped when the peptide solution covered the **AnaChip**[™] surface and the accumulation of peptide on the **AnaChip**[™] surface was measured. Identical conditions were used for the C18 and subsequently the trimethyl silane **AnaChip**[™] surfaces. In each case the process was monitored for a total of 72 hours after which the **AnaChip**[™] was removed from the instrument and SEM images were obtained in order to confirm the presence or absence of mature β -amyloid fibrils.

Results and Discussion

β -Amyloid Aggregation Studies: The results of the comparative surface study are shown in **Figure 1**. The data consist of the core DPI measurements of real-time thickness and mass on the **AnaChip**[™] surface. Both the trimethyl silane and the C18 surfaces offer the peptide hydrophobic alkyl (hydrocarbon) chain lengths. The trimethyl silane surface is effectively a 'shallow' hydrophobic surface with a hydrocarbon chain length of 1 carbon atom, whilst the C18 surface offers a 'deep' hydrophobic surface with a hydrocarbon chain length of 18 carbon atoms. This means that the experiment offers a comparison of β -amyloid aggregation on a deep and a shallow hydrophobic surface.

It is thought that providing support for the hydrophobic tail region of the β -amyloid molecule aids the aggregation process. In the case of trimethyl silane, the surface is hydrophobic but there is no depth to the layer to allow insertion. When the amyloid is introduced to the trimethyl silane surface, the mass and the thickness of the resulting layer increase concurrently (**Figure 1**). This is consistent with the peptide molecules physisorbing onto the shallow hydrophobic surface but not being able to penetrate into it. The process tails off very quickly and it is found to be very difficult to reliably obtain aggregates on such a surface.

Application Note 016

In the case of the C18 surface, a very different process takes place. Initially a sharp increase in the mass of the layer is observed but with very little increase in its thickness (**Figure 1**). This means that during the initial stages after introduction of the peptide the molecules are inserting into the C18 layer, leading to a sharp increase in the deposited mass of the layer. Given the hydrophobic nature of the C18 functionalised surface, it can be assumed that the hydrophobic tail region of the β -amyloid molecule is inserting into the 'deep' hydrophobic layer, an orientation believed by many to be conducive to aggregation. After this initial insertion phase, the layer then proceeds to increase in mass and thickness monotonically as the β -amyloid aggregates. C18 surfaces have been observed to routinely and reliably support β -amyloid aggregation processes (*Farfield Application Note 015*).

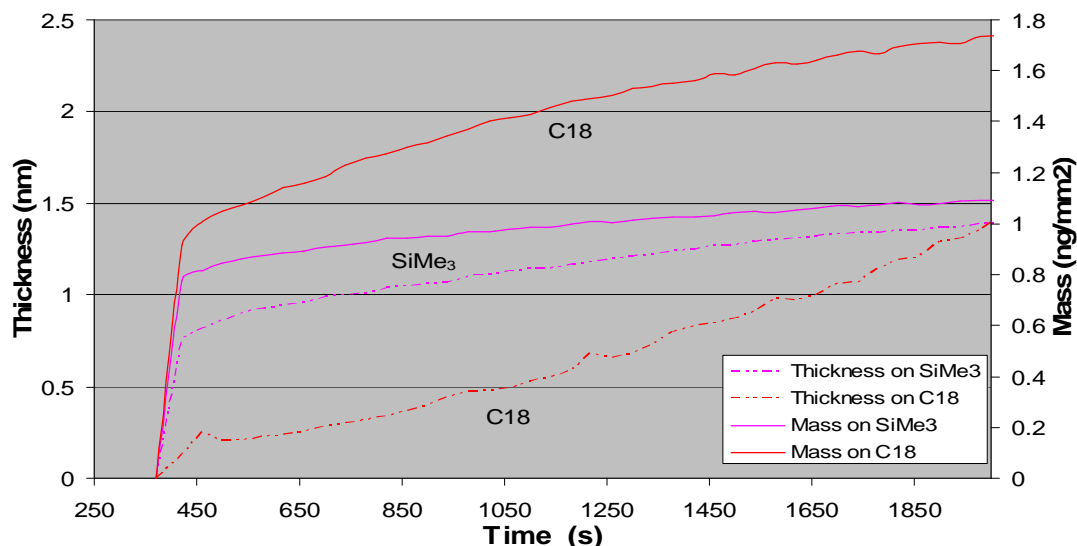


Figure 1: Early stage β -amyloid aggregation on two hydrophobic surfaces with different carbon chain lengths

Conclusions and Benefits

This study demonstrates DPI as an enabling technique to study interfacial aggregation processes from the previously difficult to measure early stages. Simple fibrillar aggregates of β -amyloid can be formed on an **AnaChip™** surface (see 004). Details of the molecular processes can be studied at C18 and trimethyl silane surfaces, from which details of the mechanisms by which aggregation occurs can be elucidated in real time. DPI also displays unsurpassed instrument stability to allow these processes to be measured over a time period of several days

The **AnaLight®** instrument range and associated experimental protocols give the researcher a unique combination of high-resolution data in real time on thickness, refractive index (density) and surface coverage from a bench top technique, providing an important enabling tool to

- Clearly understand the molecular processes involved in early stage aggregation
- Postulate the likely surface conditions and molecular mechanisms through which aggregation is enhanced
- Obtain real time data on the growth characteristics of amyloid aggregation
- Compare the analytical data obtained with complimentary techniques
- Utilize unsurpassed instrument stability to allow processes to be measured over several days

Farfield gratefully acknowledges that these experiments were carried out according to protocols and using samples provided by Professor David Allsop from the Department of Biological Sciences, Lancaster University, Lancaster, UK.

For further applications information contact: applications@farfield-scientific.com or Telephone the applications team on +44 (0) 870 950 9717

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 (2) D. Walsh, A. Lomakin, G. Benedek, M. Condron, & D. Teplow. *J. Biol. Chem.* **272** (1997) 22364-22372