High Resolution Structural Studies of Isoform-Specific Apolipoprotein E Interactions with Tissue Plasminogen Activator

Introduction

Dual Polarisation Interferometry (DPI) is an important enabling tool for the study of protein-protein interactions. DPI provides a simple and unambiguous technology for the study of isoform-specific protein-protein interactions, and the differentiation between macromolecular complexes resulting from specific and non-specific protein-protein interactions.

DPI embodies a quantitative analytical technique, rather than a simple 'mass sensor' response, and provides absolute density measurements and structural dimensions of immobilised proteins (1). Changes in these physical parameters can be directly related to the structure and function of biomolecules at the measurement surface, which are in turn related to protein-protein interactions due to specific or non-specific binding events. With the continued focus on the study of protein-protein, and other biological molecule interactions in the drug discovery process, DPI provides an unequivocal approach for the analysis of these interactions through the provision of a unique combination of data.

Apolipoprotein E (Apo E), a member of a family of lipid-associated proteins, has three common isoforms – E2, E3, and E4. The isoforms differ by substitution between cysteine and arginine at residues 112 and 158 (2). Apo E is recognised as an important genetic risk factor for multiple neurological, vascular and cardiovascular diseases, and has recently been reported to show isoprotein-specific modulation of tissue plasminogen activator (tPA), a therapeutic clot-lysis protein. The efficacy of intravenous tPA (tPA/Activase®) in patients with acute ischemic stroke was shown to be enhanced in patients who have an Apo E2 genotype relative to the Apo E3 and Apo E4 genotypes. This raised questions as to the function or biological activity of isoforms of Apo E (3).

In this application note, DPI has been used to investigate isoprotein and state-specific intermolecular interactions between the Apo E isoforms and tPA, in order to provide insights into the molecular mechanisms through which Apo E may mediate its action on tPA-induced clot lysis.

Experimental

The DPI experiments were performed on a Farfield AnaLight® instrument, with all studies carried out on an amine-functionalised silicon oxynitride chip. The temperature of all samples was controlled throughout to 20°C. Water used in buffer preparation was deionised and free from organic impurities. All reagents were analytical grade or higher, and solutions were degassed prior to use.

Im mobilisation of tPA protein: The chip was calibrated by injecting an 80% (w/w) ethanol/water solution into a stream of PBS running buffer (10mM, 150mM NaCl, pH7.4) at a flow rate of 50μl/min (Farfield Technical Note 001). The amine-amine linker BS3 (bis[sulfosuccinimidyl]suberate, 2mg/ml in PBS) was added to both channels (experimental and reference) for 30 seconds at 10μl/min. Tissue plasminogen activator (tPA) solution (1mg/ml in PBS) was then added to the experimental channel only for 30 seconds at 10μl/min to block unreacted BS3 (Farfield Technical Note 003).

Isoform-Specific Protein-Protein Interactions: Following establishment of a stable baseline, Apo E2 (9.8µg/ml in PBS) was injected into the flow over the immobilised tPA for 3 minutes, then incubated for 40 minutes before elution to a stable signal. The same procedure was repeated for the Apo E3 and Apo E4 isoforms.

Results and Discussion

Isoform-Specific Protein-Protein Interactions: Figure 1 shows the thickness, density (RI) and mass changes occurring when the immobilised tPA was challenged with each of the Apo E isoforms. The mass (blue in Figure 1) of Apo E binding to the tPA is similar regardless of the isoform being studied. However, the thickness (green in Figure 1) of the resulting protein-protein complex layer is significantly greater in the case of Apo E3 than Apo E2 or Apo E4. This indicates that Apo E3 shows no specific affinity for tPA. This is in sharp contrast to the complex formed between tPA and Apo E2. The density changes which occur when Apo E isoforms interact with immobilised tPA are also shown (red in Figure 1). The density increases dramatically for Apo E2, while it decreases for the other two isoforms, indicating that the interaction between Apo E2 and tPA causes a specific and functionally relevant conformational tightening which does not occur with the other two isoforms. The DPI technique can be used to demonstrate that
Specific binding events typically involve a change in density as binding takes place. Comparison of the above changes in the immobilised protein layer upon addition of the Apo E isoforms yields useful information about the difference in nature of the binding events taking place. A decrease in density upon addition of protein is indicative of non-specific association, as appears to be the case for Apo E3 and E4. However, an increase in density upon interaction with another protein indicates a specific and direct protein-protein interaction, as for Apo E2.

Figure 1: Comparison of mass, thickness and density changes of the protein layer after interaction of tPA with Apo E isoforms

Figure 2: Visual schematic of the different protein-protein complexes formed upon binding of each Apo E isoform to immobilised tPA
The complex formed between tPA and Apo E2 is considerably thinner and more dense than in the diffuse layers of Apo E3 or E4. This suggests significant differences in the structures of the complexes, and that there is a more specific and intimate interaction between tPA and Apo E2 which forms a compacted quaternary structure. The complex formed between tPA-E4 is more diffuse than that of tPA-E2, and the thickness/density values suggest a different interaction from that of tPA-E3, with E4 binding to tPA in a specific fashion to form a more open complex than tPA-E2. Figure 2 shows that these interactions are clearly distinct from the non-specific nature of the tPA-E3 interaction.

These results provide important information about the mechanism through which Apo E displays isoprotein-specific effects on many of the biological processes and diseases involving blood clotting and flow. Using the Apo E proteins, or fragments thereof, for modifying or treating thromboembolic events could be very important in cerebral vascular, cardiovascular and peripheral vascular disease.

Conclusions and Benefits

This study clearly demonstrates that the interaction between Apo E2 and tPA leads to significant structural changes that are not observed during the corresponding interaction between Apo E3 or E4 with tPA. Apo E2, Apo E3 and Apo E4 interact directly with tPA in an isoprotein-specific manner, potentially explaining the difference in their effects on tPA-induced clot lysis and other biological processes and diseases involving blood clotting.

AnaLight® DPI systems offer next-generation technology, providing valuable insights into the relationship between structure and function during protein-protein interactions. ‘Biosensor’ techniques such as SPR and QCM cannot reveal this level of quantitative structural information. AnaLight® gives 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. AnaLight® is an important enabling tool for biophysicists, giving them the ability to:

- Understand the structural implications of interactions to a level beyond that provided by measuring mass changes alone
- Differentiate between specific and non-specific binding events
- Connect structural and functional events directly and in real time through a single set of high-content measurements
- Distinguish the binding mechanisms of different protein isoforms, which may have therapeutic implications

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