Investigating the Interactions Between Serum Albumin : Fatty Acid Complexes and Fibrinogen to explain abnormal blood clot formation.

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Introduction:

A quarter of all deaths in America are due to cardiovascular disease and strokes, and these diseases are connected to high fatty acid (FA) in the blood. The excess amount of FA affects the complex coagulation processes, leading to abnormal blood clot formation. Blood clots are composed of red blood cells, platelets, and fibrin, wherein fibrin is converted from fibrinogen (FBGN) by thrombin. It is well known that serum albumin serves as a fatty acid (FA) vehicle due to the low solubility of FA in the bloodstream. Recent studies have confirmed that human serum albumin (HSA) binds to fibrin. However, whether HSA binds to FBNG is not clear. We hypothesize that prior to clot formation, the albumin-FA complex binds to FBGN.

Materials and Methods:

FBNG, fatty-acid free bovine serum albumin (BSA), and oleic acid (c18:1, OA) were used in this research. We used BSA in place of HSA due to the well-known high degree of homology. FBGN and BSA at a 1:1 ratio were dissolved in HEPES buffer (150 mM NaCl and 20 mM HEPES). FAs were dissolved in ethanol (EtOH) to prepare high concentration stock solutions. We incubated a pre-warmed FA stock with BSA solution at 40 °C overnight; the FA-BSA solution was then incubated with the FBNG solution at 37 °C overnight. To probe the interaction of FA-BSA-FBGN complexes, we employed coherent anti-Stokes Raman scattering (CARS) and fluorescence lifetime (FLIM) spectroscopy. Moreover, we used the ClusPro docking server to obtain insights to the possible binding modes.

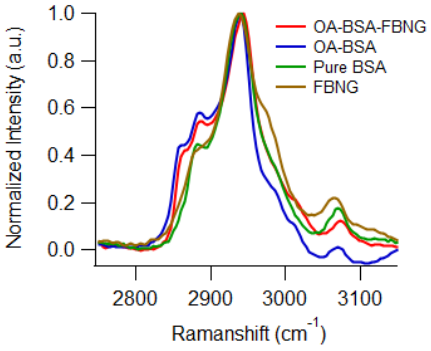

Fig 1. CH stretching region of Raman Spectra of OA, BSA and FBNG. All the spectra are normalized by the maximum intensity.

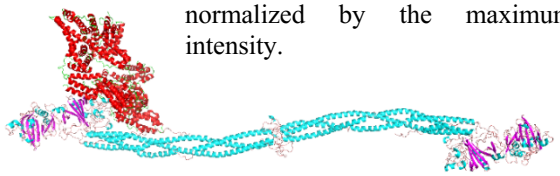

Fig 2. BSA+FBNG top preliminary docking result. BSA is in red. α helices of FBNG is in cyan, β sheets in purple and random coils in orange.

Results and Discussion: We acquired CARS spectra of pure substances and products of different mixing steps, from FA, EtOH, FA-EtOH, BSA and FA-EtOH-BSA. The OA-BSA-FBGN Raman spectra are shown in Fig. 1. From literature, 2800 - 3000 cm^{-1} is the CH stretching region, where FAs have a strong CH vibration at 2845 cm^{-1}. The FA-BSA spectrum shows an extra shoulder on the 2845 cm^{-1}, and FAs alone are not solube in aqueous solution, showing that FAs bind to BSA. The FA-BSA-FBNG spectra show a shoulder from FBGN. FLIM measurements showed BSA-FA-FBNG and BSA-FBNG interaction. The top ClusPro result for docking BSA (PDB ID 4F5S) with fibrinogen (PDB ID 3GHG) is shown in Fig. 2. The docking results include several probable structures; however, further analysis of these results and review of the existing literature will allow us to narrow down which possible binding modes are most likely. Bulk mechanical measurements showed that, the FA-BSA affects the fracture point in fibrin networks, showing that FA-BSA interaction with FBGN affects clot stability applied.

Conclusion: From the spectra and docking results, we confirm that high FA-BSA complexes bind fibrinogen. That leads to the explanation that FBGN is modified before the clotting, which results in abnormal clots. Computational docking methods provide additional mechanistic insights. Abnormal clots trouble us with cardiovascular disease and strokes.

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