SANS and USANS Investigation of the Structure of Coarse Fibrin Clots

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Abstract

Fibrin is the major protein component of blood clots, forming a cross-linked network of fibers and is important in the blood coagulation process. The relationship of the structure of fibrin networks to their function is crucial to understanding the processes of haemostasis (the halting of bleeding) and fibrinolysis (the breakdown of clots once damage has been repaired). However, studying the structure of these materials has been difficult due to the small size and wide range of size of the structures and to the high turbidity of the materials. Small- and Ultra-small angle neutron scattering (SANS and USANS) will be used to examine the structure of fibrin clots as a function of concentration over a size range of nanometers to micrometers.

Introduction

Biopolymer networks have mechanical properties that are remarkably different from those of most synthetic materials[1]. These properties are directly related to the internal structure of the polymer fibers and of the network. Fibrin, the major protein component of blood clots, has drawn particular interest due to its important role in blood coagulation.[2] It has also been recognized that the relationship between the structure and function of fibrin networks is critical to our understanding of important biological processes including haemostasis and fibrinolysis. Despite its paramount importance, many structural parameters of fibrin have been difficult to measure in unperturbed samples. This is due to the high turbidity of the materials and the small size of these structural features. Thus, previous research has been unable to probe the structure of these materials over a wide range of length scales and protein concentrations. In this experiment, small angle neutron scattering (SANS) will be used to characterize the structure of coarse fibrin clots formed in saline solutions over length scales that extend over five orders of magnitude (1 nm – 20 μm). Furthermore, SANS and Ultra SANS experiments allow us to analyze bulk solvated samples spanning a wide range of concentrations (1 to 15 mg/mL).

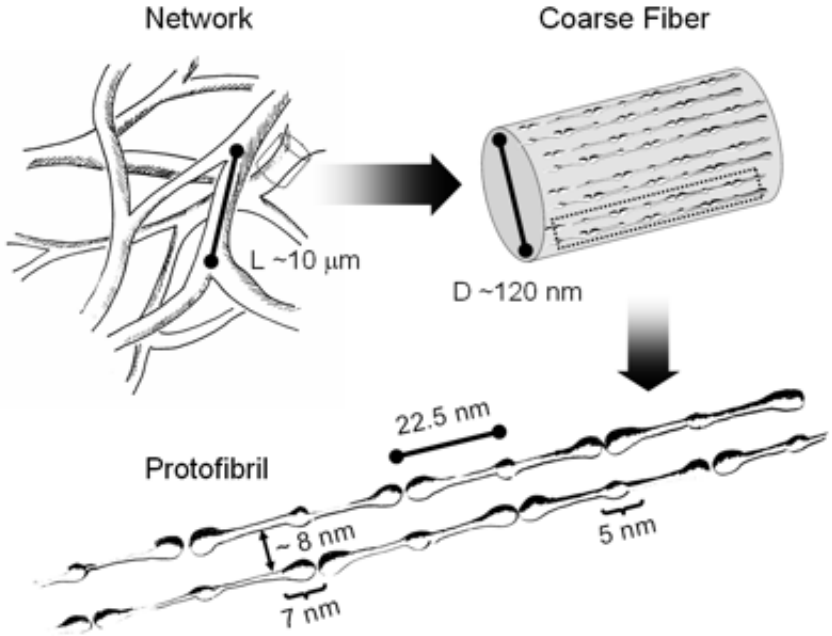

Figure 1: Schematic representation of the structural features found for coarse fibrin clots in length scales ranging from 1 nm to 10 μm.

Figure 1 represents the three-dimensional structure of a coarse fibrin clot over length scales of 1 nm to 10 μm. Fibrin clots are formed when the polymerization of fibrinogen is activated by the enzyme thrombin[2]. After activation, the proteins assemble into highly organized linear arrays

called protofibrils. These protofibrils have a half-staggered linear structure with a repeat distance of half the length of the fully extended fibrinogen molecule (45 nm).2 Under certain solvent conditions, protofibrils can also aggregate laterally to form a larger coarse fiber.[2, 3, 4] It is also known that the inside of these coarse fibers is mostly composed of water that fills the space between the fibrinogen monomers. The internal volume that is occupied by the proteins has been measured using light scattering and refractive index measurements on dilute clots (¡ 0.25 mg/mL).[5, 6] The estimates of the total volume fraction of protein within the fibers vary between 20-30%. However the composition of the fibers has not been determined in a concentrated system.

Thus we propose in this experiment to determine the structure of fibrin networks formed from concentrated solutions of fibrinogen.

Why use SANS?

Generally, static light scattering and small angle X-ray scattering (SAXS) provide the same information about the sample: measurement of macroscopic scattering cross-section $d\Sigma/d\Omega(q)$, as neutron scattering. The contrast in light scattering arises from the difference in the light's refractive index between the particle and water. The wavelength of light limits q < 0.002 Å^{-1} and thus the size range probe to $>\sim$3000. Furthermore, in order to measure the light scattering the sample needs to be dilute and here we wish to study a concentrated network structure. The contrast in X-ray scattering arises from the variation in electron density within the sample. However USAXS does not generally reach as low q as USANS and with protein samples x-rays (particularly at synchrotron sources) can cause damage to the sample as a result of the large amount of energy imparted.

SANS/USANS is therefore an ideal probe for the structure of these biological network structures since it allows measurement at biologically relevant concentrations and conditions over the whole relevant size range with no risk of damage to the sample.

The Objectives of the Experiment

To determine the volume fraction of protein within the fibers Making use of the fact that the absolute scattering cross section is obtained from the SANS experiment, the composition of the fibrin fibers will be determined given the known scattering length density of the fibrinogen and heavy water.

To determine the structure of the fibrin clot A suitable model for the scattering at the various length scales will be chosen and fitted to the SANS and USANS data. The dimensions of the coarse fibers and the structure of the network in terms of the persistence length and mean separation of network cross links will be determined.

To determine the concentration dependence of the structure The models developed will be fitted to data taken at varying concentrations and the dependence of the various key parameters on concentration determined.

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