Experimental report

03/03/2023

Proposal: 8-04-859 **Council:** 10/2018

Title: Understanding sickle cell disease and its genetic cure from the structure of haemoglobin

Research area: Physics

This proposal is a new proposal

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Samples: purified human blood

Instrument	Requested days	Allocated days	From	To
D11	0	0		
D22	3	1	11/10/2019	12/10/2019

Abstract:

Sickle cell disease is a group of blood disorders, the most common is the sickle cell anemia. It is a genetic mutation of the oxygen-carrying protein hemoglobin found in red blood cells. This leads to a rigid, sickle-like shape. This hemoglobin (HbS) is a variant of normal human hemoglobin (HbA). From a molecular point of view, the mutation induces polymerization of HbS, when the protein is in its deoxygenated state and very concentrated. Recently, the Henri-Mondor hospital developed a genetic cure for sickle cell anemia and successfully cured a first patient. After 15 months, a mixture of HbS and HbA composes the total amount of patient hemoglobin and no sickle cell anemia crises are observed. In this experiment, we want to characterise using SANS the structure of HbS and of the mixture of HbS and therapeutic HbA to verify at which concentration the polymerization is not taking place and, more important, to compare the structure of cured patient with the one of a sane patient. This is a crucial step in the understanding of the genetic cure. Different oxygen partial pressure will be investigated to simulate different body environment (lungs, heart, tissues).

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Sickle cell disease (SCD), or sickle cell anemia, is a blood disorder. It results from a genetic mutation of hemoglobin, the oxygen-carrying protein present in red blood cells (RBC), which, when deoxygenated, will polymerize and cause deformation and stiffening of cells. This leads to a rigid, sickle-like shape under certain circumstances (high concentration, low pO2). SCD was the first molecular disease identified by Linus Pauling in 1949 [1]. The mutation constitutes a substitution of a hydrophilic glutamic acid by hydrophobic valine which binds to another molecule and will induce the polymerization of HbS, when the protein is in its deoxygenated state. Humans homozygous for the HbS gene (inherited from both parents) suffer from severe anemia which can induce early death. Populations in sub-Saharan Africa, Afro-America and around the Mediterranean are particularly concerned because up to 10% of the population carries the gene. The polymerization occurs when the concentration is greater than the solubility, which is dependent on pO2 (160 g.L^{-1} at pO_2=0). In red blood cells, whatever the initial hemoglobin concentration (c=330+/-25 g.L^{-1}), the polymerization will stop when the concentration of HbS remaining in solution reaches the solubility. The polymerization process is very fast and reversible if the pO_2 increases again because oxygenated molecules cannot be incorporated in the fiber and neither remaining in it when re-oxygenated.

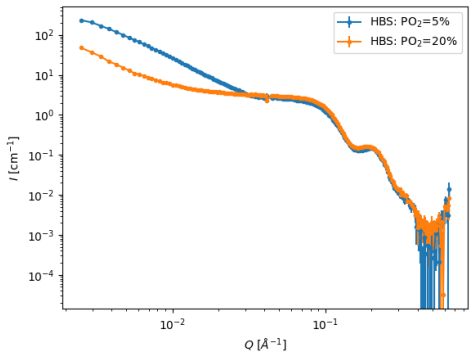

Figure 1 : Scattering intensity of an HbS solution at two different partial pressures, $p0_2$=2.5% and pO_2=0.

Experiment :

We planned to measure different cycling of HbS polymerization as a function of the pO_2 changes between 20% to $p0_2$=0. And then different $HbF_xHbS_{(1-x)}$ with x=0, 0.1, 0.15, 0.24 and 1, at pO_2=0 and pO_2=20% at concentration around 200g.L^{-1}. In fact due to an experimental problem, not related to D22, but to our oxygen control system we were not able to measure continuously the polymerization in-situ but we have to choose some defined oxygen partial pressure and some defined samples.

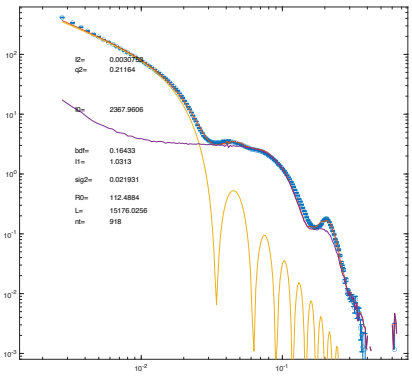

Figure 2 : Scattering intensity I(q) for fully gelyfied sample, the continuous line is the refinement using the form factor of a cylinder , the measured scattering of a solution at 130 g.L^{-1} and a Bragg peak around q=0.2 Å^{-1}.

The figure 1 depicts the SANS spectra of hemoglobin S solutions at oxygen partial pressure of pO_2=20% and pO_2=5%. The form factor of hemoglobin is clearly observable in the wavevector range from q=0.05 Å^{-1} to q=0.5 Å^{-1} in both spectra. The small increase observed in the regime pO_2=20% (red circles) below q=0.01 Å^{-1} means that either some other species of rather large size are still present in solution (membrane residues ...) or that a small number of hemoglobin molecules are aggregated or polymerized into fibers. When lowering the oxygen partial pressure down to pO_2=5% the signal at small wavevectors (q < 0.05 Å^{-1}) significantly increases and follows a I(q)~q^{-1}

dependence, as is usually observed for cylinder shape macromolecules. Figure 2 shows the spectra obtained for a strongly aggregated solution at pO_2=0, which has gelyfied. It has been fitted using the sum of the contributions from the theoretical form factor of a cylinder and the measured scattering intensity of an hemoglobin solution whose concentration was below the initial concentration of HbS (130 $g.L^{-1}$ versus 190 $g.L^{-1}$). An additional peak (Bragg peak) around $q\sim0.2$ $Å^{-1}$ which corresponds to (an harmonic) hemoglobin-hemoglobin correlation in the fibers is also observed. The broad peak around $q\sim0.04$ $Å^{-1}$ corresponds to the first oscillation of the cylinder form factor. The fits are of rather good qualities except for the contribution of the hemoglobin solution around $q\sim0.1$ $Å^{-1}$, this is rather obvious because the scattering intensity in this q range depends on the hemoglobin solution concentration after fiber formation. The measured I(q) that is used for fitting the data was measured on a hemoglobin F solution of ~ 130 $g.L^{-1}$, whereas the solubility for pure hemoglobin S solution is closer to $c_s\sim160$ $g.L^{-1}$. A small change in the structure factor due to the change of protein concentration can explain the small discrepancies between experimental data and fitting function. Using such a combination of different components (fiber, protein solution and a Bragg peak whose origin is the hemoglobin-hemoglobin correlations in the fibers) we are aware that we neglect the cross terms between hemoglobin in solution and the fibers. From the refinements, the cylinder has a length that is undefined in the wavevector range of the measurement (L>15000 Å) and the radius of the cylinder is R~112.5± 1 Å. The fiber structure strongly depends on the way and the kinetics the polymerization occur but the building blocs are always the same a 14 strands fiber. The diameter of the fibers with 14 strands was measured by electron microscopy, and it is given in the literature the value D=217 ± 9 Å in very good agreement with the value given above.