Experimental report

03/03/2023

Proposal: 8-04-858 **Council:** 10/2018

Title: Understanding sickle cell disease,its role in oxygen transportation and its genetic cure from the dynamics 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
IN15	10	10	17/05/2021	27/05/2021

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 investigate the dynamics of Hb using NSE. HbS and of the mixture of HbS and therapeutic HbA will be investigated to characterise the anomalous diffusion of HbS induced by its polymerisation and understand how the therapeutic hemoglobin affects the diffusion process. 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 pO_2 (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 pO2 increases again because oxygenated molecules cannot be incorporated in the fiber and neither remaining in it when re-oxygenated.

Fetal hemoglobin (HbF) is a potent inhibitor of HbS polymerization making increasing HbF expression a therapeutic strategy. The administration of hydroxyurea is one of the pharmacological treatments for sickle cell disease; this molecule promotes the synthesis of fetal hemoglobin (HbF), which leads to a mixture of hemoglobin $HbF_xHbS_{(1-x)}$ in red blood cells. Recently, a consortium including the "Red Blood Cell Genetic Diseases Department" of the Henri-Mondor Hospital developed a gene therapy for sickle cell disease and succeeded in curing a first patient [2].

Experiment :

We have developed and installed on the spin-echo a system to control the oxygen partial pressure $p0_2$ in order to control the polymerization of the hemoglobin. We have measured HbA0 sample and then different $HbF_xHbS_{(1-x)}$ with x=0, 0.1, 0.15, 0.24 and 1, at concentration around 200$g.L^{-1}$. For each sample we measured 5 different oxygen partial pressures ($p0_2$=20%, $p0_2$=5%, $p0_2$=2.5%, $p0_2$=0% and back to $p0_2$=20%) and for 3 different wavelengths 6, 8, and 10 Å, in order to cover the wavevector range from 0.05 to 0.25$Å^{-1}$ and a time range from 0.01 to 200 ns (max respectively of 42, 99 and 200ns for the different wavelengths). Data treatment was performed at the ILL with on-site developed tools and we end up with the different I(Q,t) which were analysed by in-house developed software using matlab. We started with the study of the pure HbS solution, the spectra measured at ambient $p0_2$=20% to verify that the polymerization occurs and then the $p0_2$ was decreased progressively down to $p0_2$=0%. Five out of the 21 I(q,t) are presented on figure 1. One can clearly observe two populations, one relaxing in the time window of the spectrometer and another one leading to a plateau in time (elastic in the window of IN15) very clearly located in Q. We attribute this contribution to the polymerized hemoglobin embedded in fibers whose contribution is situated in Bragg peaks around q=0.13 $Å^{-1}$ and q=0.2 $Å^{-1}$. The variation of the plateau contribution as function of the wavevector q is depicted on figure 2. The maximum of the plateau corresponds to the Bragg peak contributions of the fibers in this wavevector range, their intensity is thus related to the number of hemoglobin S polymerized at a certain power. We have then studied the diffusion of the protein remaining in solution (relaxing part of the curve), we performed first a fit with

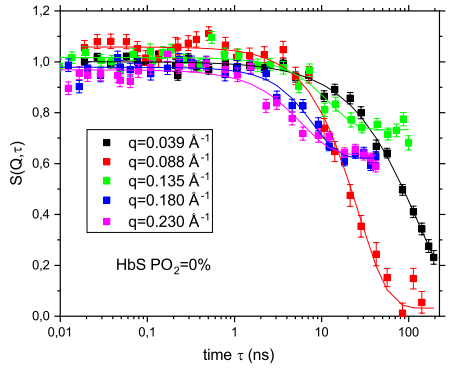

Figure 1 : 5 I(q,t) measured on HbS solution under pO_2=0 out of the 21 measured on IN15.

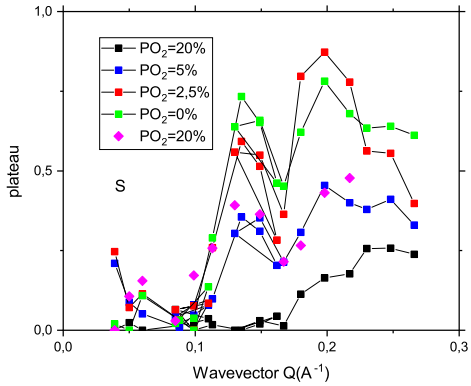

Figure 2: Evolution of the plateau contribution as a function of the wavevector q in pure HbS solution as a function of the oxygen partial pressure pO_2.

a stretched exponential decay to check for a possible anomalous diffusion ($\beta<1$) but over the entire wavevector range β was not significantly different than 1 whatever the value of the oxygen partial pressure. We conclude that the diffusion of the protein remain of Brownian type and performed a second series of fit with a single exponential decay $I(q,t) = \exp(-t/\tau)$. The values of $1/\tau$ is plotted as a function of q^2 on figure 3 for pO_2=20% and pO_2=0. We can draw too conclusion, first the linear nature of $1/\tau$ versus q^2 clearly confirm that the diffusion of hemoglobin in solution remains Brownian and second the diffusion coefficient is lower in fully desoxygenated solution with respect to oxygenated (non polymerized) solution. The last observation can be interpreted by the fact that the concentration of protein in the fiber (0.69 g.cm^{-3}) is higher than in the solution (0.20 g.cm^{-3}), more fiber imply lower solution concentration and hence higher diffusion coefficient (increase of the free volume). The variation of the plateau intensities as a function of the pO_2 is shown on figure 4 for the two peaks at q=0.13 Å^{-1} and q=0.2 Å^{-1}, calculations of the protein fraction in the fiber using the saturation curve of the hemoglobin (fraction of oxygenated protein as a function of the pO_2) is represented as the continuous line, the agreement between theoretical curve and experimental points is reasonable.

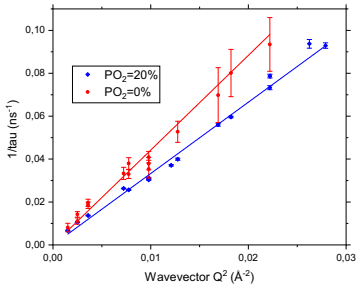

Figure 3 : $1/\tau$ as a function of q^2 of the relaxing population (HbS in solution) for two pO_2=0 and pO_2=20%, the slope are the diffusion coefficients. It is higher in polymerized sample.

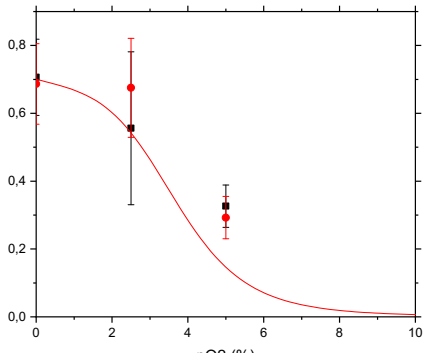

Figure 4 : Variation of the magnitude of the plateau at q=0.13 Å^{-1} and q=0.2 Å^{-1}. The continuous line is a similar calculation using hemoglobin saturation curve.

Finally we performed similar analysis in mixture of foetal and S hemoglobin $HbF_xHbS_{(1-x)}$, we could quantify how the presence of foetal hemoglobin inhibits the polymerization of HbS, confirming the therapeuthics orientation.

Two papers are under redaction.