Experimental report

14/02/2021

Proposal: **INTER-510** **Council:** 4/2020

Title: Hemoglobin aggregation induced bycopper

Research area:

This proposal is a new proposal

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Experimental team:

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Samples: Hemoglobin
Copper

Instrument	Requested days	Allocated days	From	To
D11	1	1	16/09/2020	17/09/2020

Abstract:

Experimental report for INTER-510: Hemoglobin aggregation induced by copper

Scientific background. In the human body, copper is an essential element for a variety of metabolic processes [1] such as the respiratory chain, and its metabolism is thus tightly regulated. In the case of a condition named Wilson's disease, however, copper metabolism is impaired, which leads to an accumulation of copper in the liver [2]. The strong oxidative effects of free copper [3] lead to the formation of reactive oxygen species, thereby destroying liver cells [3,4] and releasing copper ions into the bloodstream [5]. In addition to severe liver damage [2], neurological and psychiatric symptoms can ensue [6,7]. A potentially lethal [8,9] consequence of Wilson's disease is a destruction of red blood cells (erythrocytes) [5,9]. This is, inter alia, due to oxidative damage and a resulting denaturation and aggregation of hemoglobin (Hb) [10,11] inside the erythrocytes. So far, the understanding of the mechanism behind Wilson's disease is rather phenomenological [10]. Therefore, the aim of this study is to apply an interdisciplinary, quantitative approach comprising SANS and dynamic light scattering (DLS) to obtain a comprehensive characterisation of the kinetics as well as Hb form and structure changes throughout copper-mediated Hb aggregation and erythrocyte membrane destruction.

Experiments performed. During beamtime INTER-510 (cycle 203) on D11, SANS measurements on hemoglobin (Hb) solutions in the presence of copper ($CuCl_2$) were performed. Prior to the SANS experiments, commmercially available human hemoglobin was oxidised using a dithionite-loaded column to obtain HbO_2. While a detailed data analysis is still ongoing, example results are shown below.

Preliminary data analysis. A macroscopic observation included a visible solidification and opacification of the hemoglobin solution upon the addition of 100 µM $CuCl_2$ (Fig. 1). This provides a valuable reflection of the pathological aggregation of hemoglobin in patients affected by Wilson's disease.

A dilution series of HbO_2 was measured at first (Fig. 2). The data scale well with HbO_2 concentration and allow for a good fitting with a form factor generated based on a PDB hemoglobin structure (ID 1GZX). Notably, even low HbO_2 concentrations show visible aggregation at low q. This behaviour was not expected and is subject to current analysis.

Fig. 1: Visible opacification of a 100 mg/ml HbO_2 solution.

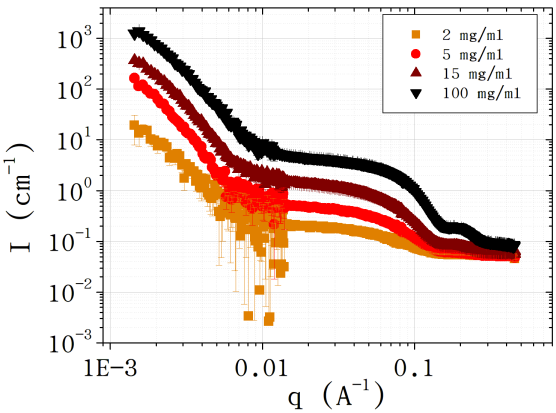

Fig. 2: Form factor measurements of HbO_2. The aggregation at low q is subject to current analysis.

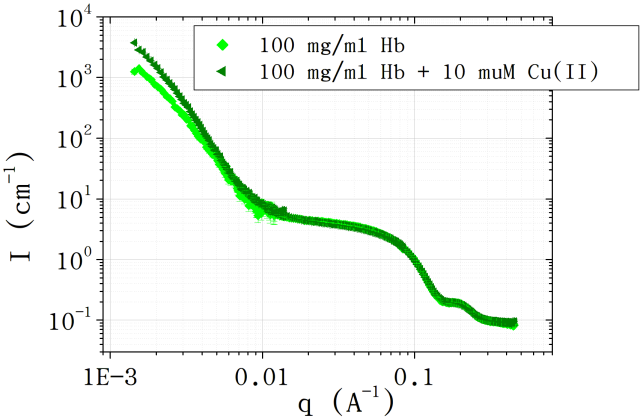

Fig. 3: Aggregation of HbO_2 upon addition of 10 µM $CuCl_2$ (representative data set).

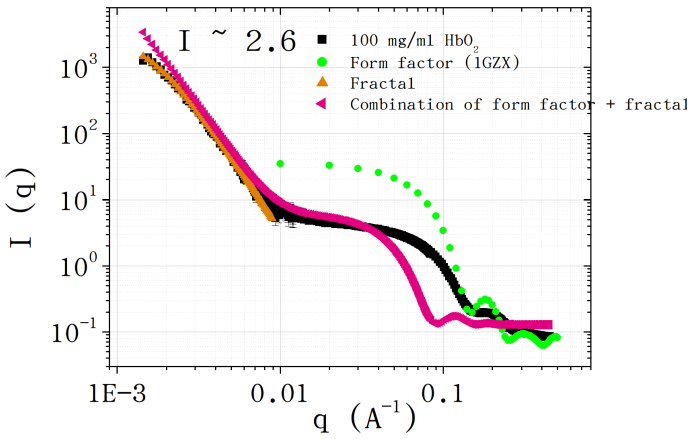

Fig. 4: First iteration of data fitting (example data set with 100 mg/ml HbO_2) using a form factor based on PDB ID 1GZX and a fractal contribution.

The influence of $CuCl_2$ on a highly concentrated HbO_2 solution was also measured (Fig. 3). Upon addition of $CuCl_2$, an aggregation of HbO_2 is seen at low q. This result aligns well with the copper-induced behaviour of HbO_2 in the case of Wilson's disease described in the literature.
Data fitting and modelling is currently ongoing. A first iteration is shown in Fig. 4: a form factor based on PDB ID 1GZX combined with a fractal contribution to account for the aggregate formation visible at low q is used.

Outlook. The preliminary measurements performed during experiment INTER-510 successfully reflect the copper-induced Hb aggregation observed in the case of Wilson's disease. Data analysis is being improved and complemented by DLS data obtained in December 2020. A proposal entitled “Simultaneous SANS and DLS: a powerful combination for elucidating the molecular mechanisms of Wilson's disease” (including the in-situ DLS option of D11) has been accepted for the 2nd ILL cycle of 2021. This beamtime will include erythrocytes obtained from the French Blood Bank (Etablissement Français du Sang) which are also affected by the elevated copper levels of patients with Wilson's disease.

References. [1] Löffler, Petrides, Springer Heidelberg (2007); [2] Riordan, Roger (2001), J. Hepatol. 34, 433-48. [3] Gaetke, Chow (2003), Toxicology 189, 147-63. [4] Gollan, Gollan (1998), J. Hepatol. 28, 28-36. [5] Ferenci (2004), Metab. Brain Dis. 19, 229-39. [6] Ala et al. (2007), The Lancet 369, 397-408. [7] Gitlin (2003), Gastroenterol. 125, 1868-77. [8] Lehninger, Nelson, Cox, W. H. Freeman New York (2005). [9] Lee et al. (1998), J. Kor. Med. Sci. 13, 548-50. [10] Rifkind (1965), Blood 26, 433-48. [11] Jandl, Engle, Allen (1960), J. Clin. Invest. 39, 1818-36.