Experimental Report

Proposal: **6-02-498** **Council:** 4/2012

Title: The hydration structure of chlorophillin: implications for chlorophyll and haemoglobin

This proposal is a new proposal

Researh Area: Chemistry

Main proposer: **NEILSON George**

Experimental Team: NEILSON George
MASON Philip
JUNGWIRTH Pavel
PATEROVA Jana
TAVAGNACCO Letizia

Local Contact: FISCHER Henry

Samples: chlorophillin:C34H36N4O6 and C34H34N4O6Mg in heavy water with N(15)/N(nat) substitution

Instrument	Req. Days	All. Days	From	To
D4	4	4	14/11/2012	18/11/2012

Abstract:

Neutron scattering data will be gathered on 4 samples of chlorophillin, two with and two without the metal ion Mg2+ in heavy water at two different concentrations - 1molal and 2 molal, i.e. 8 sets of scattering data will be obtained The difference method of isotopic substitution will be applied to the geometrically equivalent nitrogen (14N/15N) atoms of the porphin ring in order to obtain structural information regarding how water molecules coordinate within this biologically important molecule. The information obtained will be used to examine the validity of recent MD simulations, and by extension, offer insight into how water coordinates within haemoglobin and myoglobin molecules.

It is OPTIONAL to fill in this part of the Report :

Experiment N° 6-02-498

Date of Experiment - 24/11/12 to 27/04/12

Title The hydration structure of chlorophillin: implications for chlorophyll and haemoglobin

Instrument used D4

Experimental Team Dr. Phil Mason, George Neilson

LOCAL CONTACT Dr. Heny Fischer

Comments on the technical and scientific support received during your stay at I L L :

The technical and scientific support on this instrument was very good.

EXPERIMENTAL REPORT

EXPERIMENT N° 6-02-498 INSTRUMENT D4

DATES OF EXPERIMENT 14/11/12 To : 18/11/12

TITLE Structural studies of large biomolecules in water: pyridine and isopropyl alcohol in water and salt solution.

EXPERIMENTAL TEAM (names and affiliation)

Philip Mason, Department of Food Science, Cornell University, USA

George Neilson, Physics Deaprtment, Bristol University, Tyndall Ave., Bristol, BS8 1TL England, UK

LOCAL CONTACT Henry Fischer

The goal of this study was to examine the structure of chorlophyllins in aqueous solution. Chlorophyllins are heme type compounds that are very soluble in water (Figure 1) and the results will help in the understanding of the processes which occur in photosythesis.

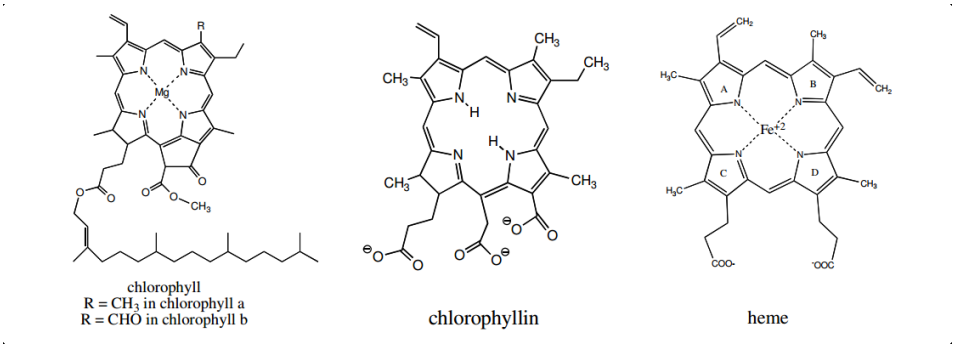

Figure 1. Some biologically important hemes. This study was centered around chlorophyllin due to its high solubility and its similar structure to heme and chlorophyll.

Neutron Diffraction and Isotopic Substitution (NDIS) experiments were carried out on solutions of chlorophyllin near its solubility limit (0.15 molal, ~10 % by mass), and at half this concentration (0.075molal). Solutions were prepared with an identical ratio of solute to water using H_2O, HDO and D_2O, and total raw neutron scattering data were acquired on these solutions. The experimental data were corrected for multiple scattering and absorbtion corrections and were normalized with reference to a standard vanadium rod in order to obtain corrected total neutron structure factors F(Q) for each solution. The functions $S_{HH}(Q)$, $S_{HY}(Q)$ and $S_{YY}(Q)$ were calculated from linear combinations of the F(Q)s. (where Y is any atom in the system that is not the substituted hydrogen nucleus). The functions $S_{HY}(Q)$ and $S_{YY}(Q)$ can be written as follows:

$$S_{HY}(Q) = 24.6\ S_{HC}(Q) + 4.1\ S_{HN}(Q) + 238\ S_{HO}(Q) + 0.76\ S_{HNa}(Q) + 0.54\ S_{HCu}(Q)$$

$$S_{YY}(Q) = 0.36\ S_{CC}(Q) + 0.12\ S_{CN}(Q) + 6.97\ S_{CO}(Q) + 0.034\ S_{CNa}(Q) + 0.025\ S_{CCu}(Q) + 0.0098\ S_{NN}(Q) + 1.16\ S_{NO}(Q) + 0.0057\ S_{NNa}(Q) + 0.0041\ S_{NCu}(Q) + 33.7\ S_{OO}(Q) + 0.34\ S_{ONa}(Q) + 0.24\ S_{OCu}(Q) + 0.0008\ S_{NaNa}(Q) + 0.0012\ S_{NaCu}(Q) + 0.000004\ S_{CuCu}(Q)$$

Examination of the data show that indeed there are significant differences in the hydration of chlorophyllin across this concentration range. The differences are most easily seen in the function $S_{HH}(Q)$ (Figure 2), and as expected the differences appear small, due to the relatively low atomic concentration of the solute. The ability to measure accurately such small differences is crucial in the success of experiments such as this one. This accuracy is only possible with extremely stable diffractometers such as D4C.

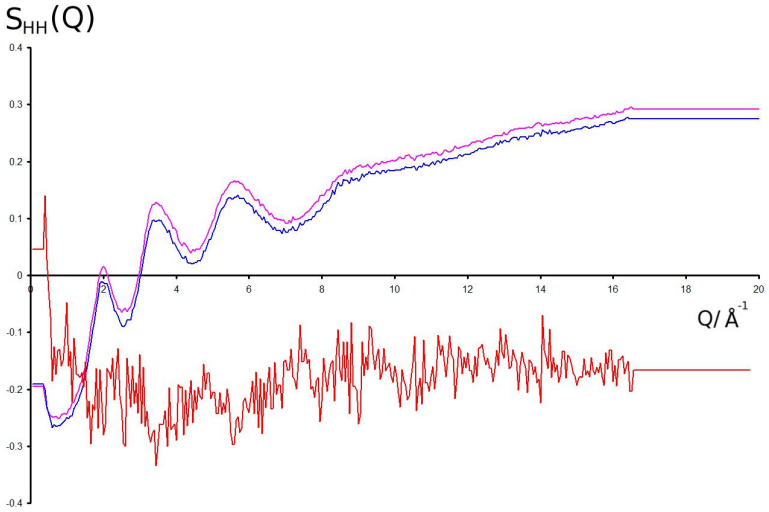

Figure 2. The $S_{HH}(Q)$ as determined for 0.075m (purple) and 0.15m chlorophyllin (blue). Due to the slightly different atomic concentration of hydrogen in these simulations these functions are equal to 224 $S_{HH}(Q)$ and 210 $S_{HH}(Q)$ for the 0.075 and 0.15 molal solutions respectively. Shown in red is the direct difference between these two functions (scaled by a factor of 10). The directly measured signal in this data is related to the different ordering of these large aromatic molecules in aqueous solution.

The experimental data have been fully processed, and we are currently conducting molecular dynamics simulations to better interpret the experimentally observed signals in these complex biological systems.