Experimental report

26/08/2016

Proposal: 8-03-878 **Council:** 4/2016

Title: Neutron Study of Cytochrome P450 Reductase: The Complex With Cytochrome c in Solution

Research area: Biology

This proposal is a new proposal

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Samples: non-toxic recombinant protein

Instrument	Requested days	Allocated days	From	To
D22	1	1	25/06/2016	26/06/2016

Abstract:

Electron transfer from cytochrome P450 reductase to cytochrome P450 can only be observed when both proteins are bound to a membrane, and cytochrome c is widely used as a surrogate electron acceptor for studies in solution. Previous studies have looked at the conformation of CPR in solution alone with respect to redox state, this experiment is aimed to see the conformation adopted by the enzyme in the presence of an electron transfer partner. The work proposed is based off of data obtained in experiment TEST-2457 in May 2015. The data obtained from the experiment was very positive and permitted useful conclusions to be drawn, further extending the understanding of the fundamentals of the system. A publication is currently in the writing stage based on the obtained data. As a result of the data the study can be advanced in to looking at CPR in in a complex with an electron transfer partner, cytochrome c. This will require the use of deuteration facilities (proposal accepted) for deuteration of CPR.

In June of 2016 the proposed experiment on the complex of cytochrome P450 reductase (CPR) with cytochrome c (cyt c) in solution was carried out. This experiment involved the study of the two components alone in solution, followed by the study of the full complex in solution. In order to get a more detailed picture of the complex formation the CPR sample was produced in an 85% deuterated form in the D-lab in order to permit contrast match experiments. In this case the cyt c was matched out and the CPR scattering curve was measured, whilst still complexed. As a result of earlier studies a mutant form of CPR (referred to as PKR) was used for deuteration which is understood to favour a more extended conformation in solution and therefore is more likely to complex with cytochrome c in solution[1].

To avoid aggregation and to separate unwanted species size exclusion chromatography was used shortly before the SANS measurements for each sample.

The scattering curves obtained during the allocated time were:

- Hydrogenated-CPR wildtype
- Hydrogenated-CPR PKR
- Deuterated-CPR PKR
- D-CPR(PKR)-cyt c (43% D_2O cyt c matched out)
- D-CPR(PKR)-cyt c (70% D_2O full complex)

The data appears to be high quality and consistent with hypotheses made before the measurements. Preliminary data analysis has been carried out principally on the non-complexed and cytochrome c matched out complex samples. Figure 1 below shows three of the scattering envelopes (DAMMIF) obtained with a best-fit model based on the known crystal structure (wildtype) superimposed.

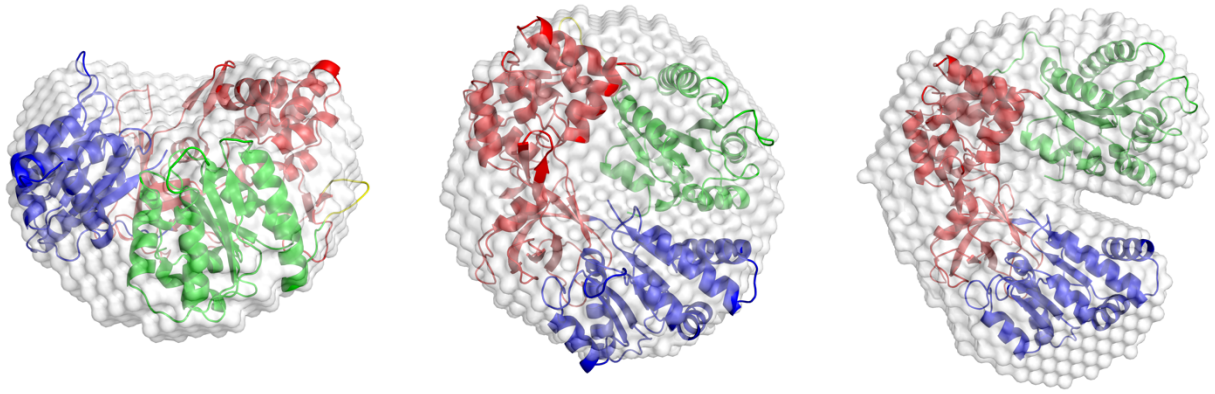

Figure 1 Left and centre show the scattering envelope of H-CPR wildtype in solution, right shows H-CPR PKR in solution, clearly adopting a more extended conformation on average. Green – FMN domain, red – linker domain and blue – FAD/NADPH domain.

Figure 2 (A), below, shows a model for the CPR-cytochrome c complex which is based principally on NMR data obtained from a complex of the FMN-binding domain of CPR and cyt c, rather than intact CPR[2]. This model has been manipulated using Monte-Carlo conformational searches in order to generate a best fit conformation to the experimental curves in a similar way to the structures above. Figure 2 (B) and (C) show the preliminary fits of the manipulated model to the scattering envelope for the full complex. Calculations have been able to reveal that the scattering curve for the full complex correctly represents the particle (CPR + cyt c) yielding the correct molecular weight based on the I(0) value. This alone is very encouraging as it confirms that the full complex was successfully isolated and measured.

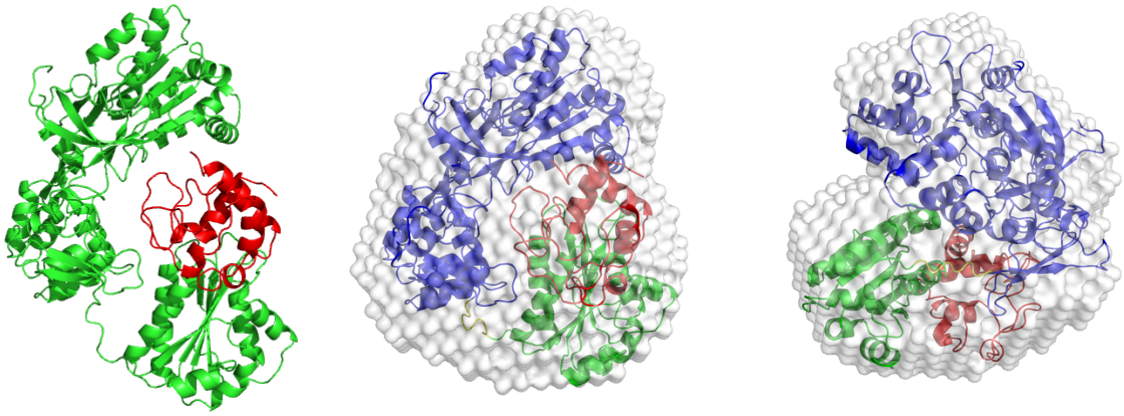

Figure 2 Left - A theoretical model of the CPR-cyt c complex with docking parameters based on NMR data, centre and right (180° rotation) show the manipulated model fit to the experimental scattering envelope.

From this data substantial conclusions can be drawn about the nature and behaviour of the CPR-cyt c complex and will form the basis of a substantial piece of work on CPR. However, in order to get totally complete picture of the system further measurements must be made. This should include the complex with CPR matched out as well as a good scattering curve of cyt c alone. In the conditions used it was found that cytochrome c has a propensity to oligomerise when alone in solution making the collection of a monomeric SANS curve without the presence of a partner enzyme somewhat challenging. Different conditions may have to be used to prevent the formation of dimers/trimers that were encountered in the previous measurements. It may also be beneficial to utilise in-situ FPLC which is now a possibility on ILL D22 and could help with obtaining an even more reliable picture of the complex since there will be no time delay between isolation and measurement. For these reasons we would like to request another day of beamtime on D22 to complete the dataset.

1 Huang, W. C., Ellis, J., Moody, P. C., Raven, E. L. & Roberts, G. C. Redox-linked domain movements in the catalytic cycle of cytochrome p450 reductase. *Structure* **21**, 1581-1589, doi:10.1016/j.str.2013.06.022 (2013).

2 Huang, R., Zhang, M., Rwere, F., Waskell, L. & Ramamoorthy, A. Kinetic and Structural Characterization of the Interaction between the FMN Binding Domain of Cytochrome P450 Reductase and Cytochrome c. *Journal of Biological Chemistry*, doi:10.1074/jbc.M114.582700 (2014).