Experimental report

09/02/2016

Proposal: 8-02-741 **Council:** 4/2015

Title: Surface Studies of Lytic Polysaccharide Monooxygenase Action on Polysaccharides

Research area: Chemistry

This proposal is a new proposal

Main proposer: Paul WALTON

Experimental team: Giovanna FRAGNETO
Trevor FORSYTH
Luisa CIANO
Esther JOHNSTON

Local contacts: Richard CAMPBELL

Samples: Lytic polysaccharide monooxygenase enzyme
Electron transfer protein (e.g. CDH)

Instrument	Requested days	Allocated days	From	To
FIGARO	2	2	23/11/2015	25/11/2015

Abstract:

Lytic polysaccharide monooxygenases are enzymes which were discovered in 2010/11 have been heralded as a breakthrough in biomass degradation and in the future of sustainable biofuels. The enzymes work by oxidising the polysaccharide chains in what otherwise would be highly recalcitrant materials (e.g. cellulose, chitin) and, as such, make the substrate susceptible to further degradation by normal enzymatic or chemical methods. The mode of action of LPMOs is poorly understood, in particular the need for LPMOs to act in concert with other proteins, many of which are simple electron-transfer proteins. It is possible that these electron-transfer proteins form "super-complexes" with LPMOs on the surface of polysaccharide substrates. We aim to use neutron reflectometry to establish the nature of this super-complex interaction. Dynamic measurements of deuterated LMPOs (supplied as discussed by the D-Lab) both with and without hydrogenous electronic transfer proteins will be carried out by exploiting the high flux of FIGARO in combination with isotopic contrast variation. The structure of the binding combined with the kinetics of film breakup will be resolved.

FIGARO Experiment #8-02-741: Preliminary Report, January 2016

Introduction

Lytic polysaccharide monooxygenases (LPMOs) are enzymes which were discovered in 2010/11 have been heralded as a breakthrough in biomass degradation and in the future of sustainable biofuels. The enzymes react with molecular oxygen after being activated by a reducing agent (*e.g*. ascorbate), to then oxidise the polysaccharide chains in what otherwise would be highly recalcitrant materials (e.g. cellulose, chitin) and, as such, make the substrate susceptible to further degradation by normal enzymatic or chemical methods. The active site of LPMOs contains an unprecedented mono-nuclear copper ion within a *histidine brace*. This active site is currently attracting much attention for its ability to carry out very highly energetic oxidation reactions, in particular the role of hydrogen atoms within the ligands that surround the copper ion are of critical importance.

The aim of the proposal was to investigate the degradation of a thin cellulose film by LPMO and/or cellulases. Fully deuterated LPMO (dLPMO) was produced by the DLab at ILL and used in one of the experiments.

This was the first neutron experiment of the Walton group and it was used to assess the viability of measurements of the system prior to a more extensive study in 2016.

Formation of Cellulose Films

Cellulose films were spin coated and the thickness was measured at different spot locations using the phase modulated ellipsometer in the Partnership for Soft Condensed Matter.

Results: demonstrating film uniformity

NR measurements on FIGARO were carried out using 3 different isotopic contrasts of the solvent: D_2O, H_2O and CMSi which is 'contrast matched silicon' with a scattering length density (SLD) equal to that of the substrate. The films were clearly very smooth on the nanometre scale because low values of the interfacial roughnesses (all just 4 Å) were required to match the Fresnel falloff of the reflectivity. However, uniform layer models all produced sharp Keissig fringes in the data and a model averaging the reflectivities of a range of film thicknesses was required, i.e., the films were clearly not uniform on the micrometre scale above the coherence length of the measurement. The following distribution of film thicknesses with its weighting was attempted:

Thickness / Å	130	135	145	160	180	210	250	300	360
Weighting	0.05	0.08	0.11	0.16	0.20	0.16	0.11	0.08	0.05

Excellent agreement between the fits and measured data were achieved. The parameters included cellulose SLDs of 1.64, 2.64 and 3.39 x 10^{-6} $Å^{-2}$ in H_2O, CMSi and D_2O due to the exchange of 3 labile protons per unit, and a film solvation of 56% in line with that of a study in the literature.

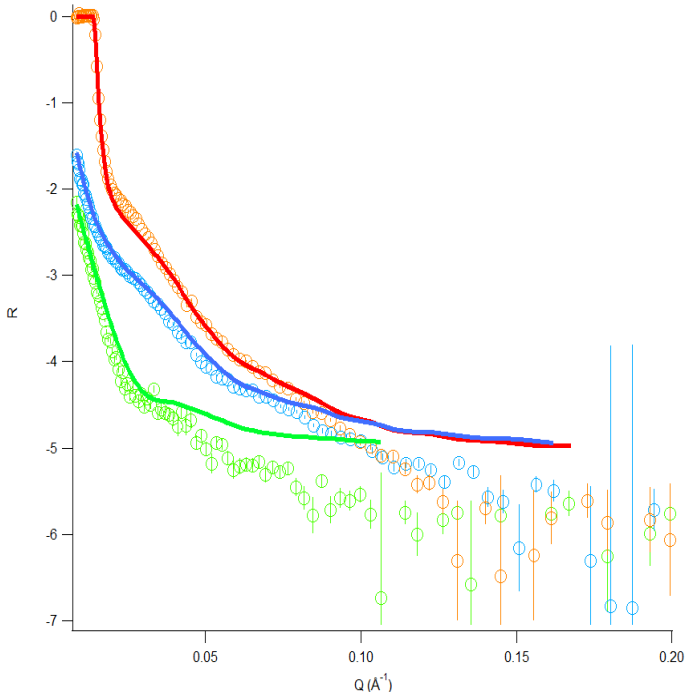

Figure 1. Cellulose films with a median thickness of 180 Å in D_2O (red), H_2O (blue) and CMSi (green).

A reaction was then carried out for the sample in D_2O involving hLPMOs mixed with cellulases and ascorbate. There were pronounced changes in the reflectivity data, which afterwards was possible to fit using a homogenous layer model (unlike the cellulose film alone). The parameters of the model included a film of 310 Å, a hydration of 80% and outer interfacial roughness values of 36 Å. This experiment taught us that the action of the cellulases in the presence of LPMO with ascorbate on the cellulose film resulted in its thickening with increased hydration, roughness and lateral uniformity, resulting in loss of about one quarter of the material.

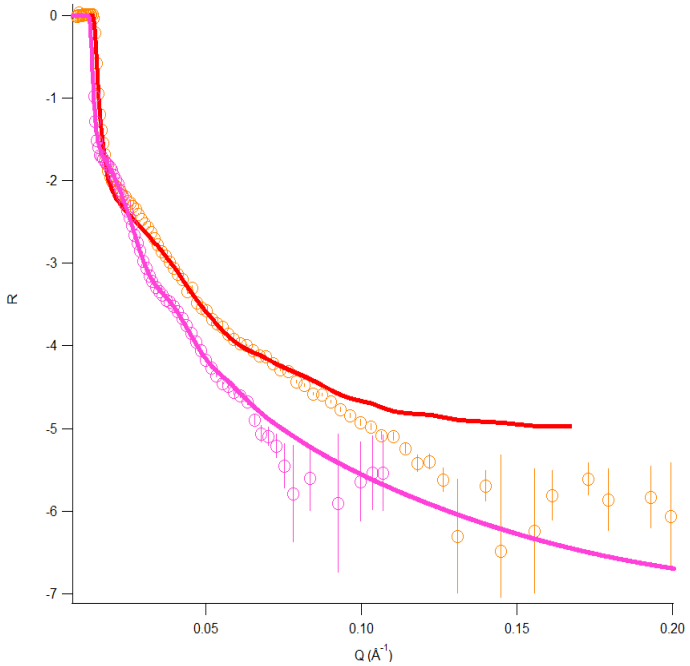

Figure 2. A cellulose film with a median thickness of 180 Å in D_2O before (red) and after (pink) its reaction with hLPMOs, cellulases and ascorbate.

Data were also recorded after the LPMO/cellulase/ascorbate reactions in H_2O and CMSi, and the results were broadly consistent with the films becoming more hydrated, thicker and rougher, although differences were observed probably due to the fact that the films were all slightly different.

Lastly, a two-step reaction was carried out to examine the synergy of action of LPMOs with ascorbate: first with adsorption of LPMOs to the cellulose film and then addition of ascorbate. While it was possible to see the LPMO sitting on top of the cellulose film prior to ascorbate addition, surprisingly no changes were observed upon addition of ascorbate, which was injected into the cell in the presence of the LPMO solution. An equivalent reaction with the same conclusion was carried out with dLPMO in H_2O, as provided by the ILL's Deuteration Laboratory. Even more surprisingly, when the cellulases were added alone the cellulose film was completely degraded. The implication is that the LPMO protected the film from degradation, an hypothesis which will require further testing. The fit in figure 3 of the cell after addition of cellulases involves no cellulose at all.

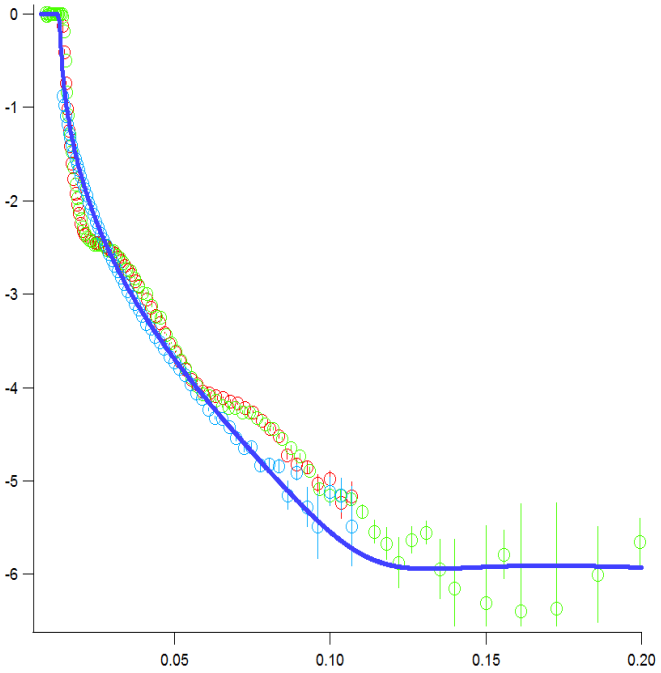

Figure 3. A cellulose film with a median thickness of 140 Å exposed to hLPMOs in D_2O before (green) and after (red) the injection of ascorbate which resulted in negligible changes in the film; the same reaction was carried out with the cellulases (blue) resulting in complete destruction of the film.

Future Work: A Continuation of This Project on FIGARO in 2016

The very promising results obtained during this first set of NR experiments clearly demonstrated the feasibility of the technique for this project. A continuation project would be highly recommended in order to further the experiments and gain high quality data to feed in a publication. The continuation experiments will require further development of the cellulose films to overcome the problems due to non-uniformity on the micrometre scale. Furthermore, a more detailed study on the interaction and synergy between LPMO and cellulases in the degradation of cellulose films will be carried out.