Characterising an esterase common to humans and their gut microbes

Thomas M. Kidder, Dr Elisabeth C. Lowe, Dr David N. Bolam.

Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle Upon Tyne.

Newcastle University

Introduction

One of the main functions of the microbes of the human gut is the ability to breakdown complex carbohydrates that humans are unable to metabolise. This is done through a range of very specific biochemical reactions carried out by enzymes. Deacetylation is one of these reactions, an acetyl group is removed from a mono/oligo or polysaccharide by an acetylesterase, enabling downstream metabolism of the sugars. *YdjC* is a highly evolutionarily conserved gene which encodes a monodeacetylase in *E.coli* which acts upon one of the acetate groups on chitobiose. Homologs of YdjC are found in many gut microbes and also in mammals. A homolog from *Clostridium difficile* also removes a single acetate from chitooligosaccharides, and the structure has been solved (below)

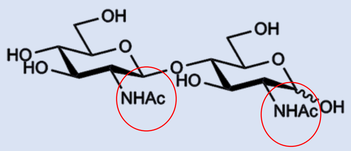


Figure 1. Chitobiose with the acetate groups circled.

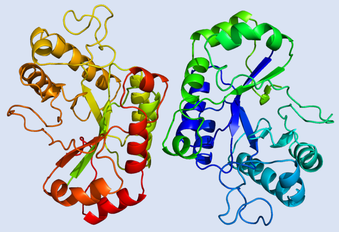


Figure 2. 3 dimensional ribbon structure of *Clostridium difficile* YdjC enzyme coloured by secondary structure.

It is not known whether the activity of the mammalian homologs of YdjC is conserved or what its function is *in vivo*.

Aims

- To express and purify the human and mouse homologues of the YdjC protein (hYdjC and mYdjC) for crystallisation trials.
- Test the deacetylation activity of the proteins.
- Attempt to crystallise the hYdjC and mYdjC enzymes.

Methods

YdjC enzyme expression and purification

- Transformation of tuner cells with each mYdjC and hYdjC plasmids and expression by cultured growth in LB nutrient broth with Kanamycin to act as a selection barrier. The culture was grown at 37°C to an optical density of 1.0 before being induced by the allolactose mimic IPTG at 16°C overnight.
- The his-tagged YdjC enzymes were then extracted from the cells and run on a metal ion affinity column to purify from the other proteins in the tuner cells. The enzymes were eluted using imidazole at 10mM and 100mM.

Activity Assays

- The deacetylase activity of the YdjC enzymes was tested using P-nitrophenyl-acetate (PNP). This colourless chemical turns yellow when acetate group is removed by an enzyme, releasing the yellow phenol group.

Crystalisation Trials

- For crystallography trials proteins were further purified using a Superdex S75 16/600 gel filtration column.
- 96 well crystallisation trials were set up with 10 mg/ml YdjC protein and standard crystallisation screens (JCSG, PACT and Morpheus) using a Mosquito nanolitre pipetting robot.

Results

Metal Ion Affinity Chromatography

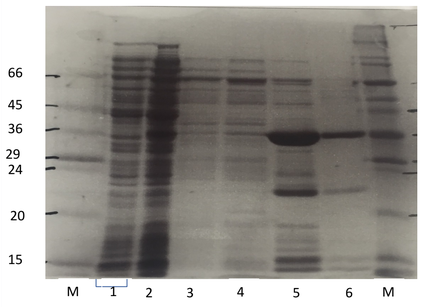


Figure 3. An SDS PAGE of the His-tagged hYdjC.
M – Marker
1 – Cell free extract
2 - Flow through
3 – Buffer wash
4 – Elution with 10 mM imadazole
5 – Elution with 100 mM imadazole
6 – Elution with 100 mM imadazole
7 - Marker

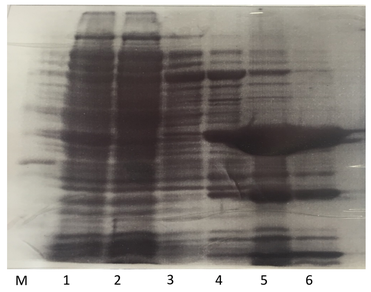


Figure 4. An SDS PAGE of the His-tagged mYdjC.
M – Marker
1 – Cell free extract
2 – Flow through
3 – Buffer wash
4 – Elution 10 mM imadazole
5 – Elution 100 mM imadazole
6 – Elution 100 mM imadazole

Both hYdjC and mYdjC enzymes express solubly in E.coli and could be purified with a metal affinity column.

The enzymes show a similar pattern of expression: a strong band of protein at approximately 36 kDa, which is close to the enzymes actual mass of 34.5 kDa; and two bands at approximately half and a quarter the molecular weight, initially thought to be contaminants.

Gel Filtration

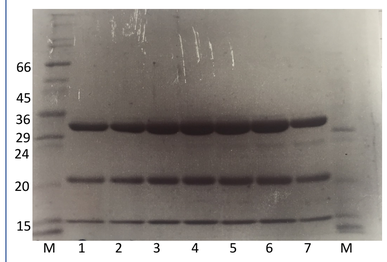


Figure 5. SDS PAGE of gel filtrated hYdjC fractions.
M – Marker
1-7 – Fractions containing hYdjC.

2ml of hYdjC was loaded onto a Superdex S75 16/600 gel filtration column, equilibrated in 50 mM Tris pH 7.5, 400 mM NaCl. Fractions were collected with an absorbance above 50 mAu.
The mYdjC was still too contaminated with other proteins, so only the hYdjC was used in crystalisation trials.

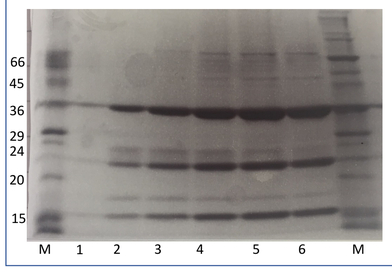
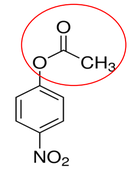


Figure 6. SDS PAGE of gel filtrated mYdjC fractions.
M – Marker
1-6 – Fractions containing mYdjC.

Activity Assays and Crystalisation trials

Fractions 1-5 of hYdjC were pooled and concentrated to 10 mg/ml for crystallisation trials. Unfortunately, after checking the crystal trays after a few days, a week, and two weeks, there was no sign of crystallisation.



1 µM of mYdjC and hYdjC was incubated with 1 mM P-NP acetate at 37°C and a slight colour change was observed after 10 minutes, indicating a small amount of deacetylase activity.

Figure 7. PNP-Ac with the acetate circled in the red.

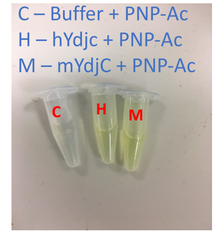


Figure 8. Assays of a control, hYdjC, and mYdjC with PNP-Ac.

Conclusions

Both enzymes were soluble and successfully purified. However, the presence of multiple bands at approximately the same level of expression suggests they are being degraded at some point in expression. This could be the reason for the low level of deacetylase activity observed in the assay. Future investigations could include the use of protease inhibitors in the purification to prevent the degradation of the protein. Another possibility is to reclone the YdjC gene and express the enzyme into different vectors such as yeast species Pichia pastoris or Saccharomyces cerevisiae. Changing the location of the His-tag from the C-terminus to the N-terminus may also improve the level of activity of the enzyme.