Cj1411c GENE OF CAMPYLOBACTER JEJUNI 11168 ENCODES FOR A CYTOCHROME P450 INVOLVED IN BACTERIAL CAPSULE SUGAR METABOLISM

GENA Cj1411c, DIN GENOMUL BACTERIEI CAMPYLOBACTER JEJUNI 11168, CODIFICA UN CITOCROM P450 IMPLICAT IN METABOLISMUL ZAHARURILOR CE INTRA IN COMPONENTA CAPSULEI BACTERIENE

CORCIONIVOSCHI N.*, REID G.**

* University College Dublin, The Children's Research Centre
** University of Edinburgh, Institute of Structural and Molecular Biology

After isolation in 1970s, Campylobacter jejuni become the most commonly recognized cause of bacterial gastroenteritis in man. In animals is frequently found in bovines on ovines. Publishing of the genome sequence of Campylobacter jejuni 11168 (Parkhill, 2000) revealed the presence of only one cytochrome P450 in an operon involved in sugar and cell surface biosynthesis. The gene name is Cj1411c, is 1359 bp long and encodes 453 aa. The sequence is strictly conserved in Campylobacter jejuni RM221. Similarities with two cytochrome P450s, one form Silicobacter sp. and one form Poloromonas sp., were identified. These two enzymes are known to be involved in ascorbate and aldarate metabolism. The recombinant construct allowed the expression of active P450 enzyme with a 450 nm peak when binds CO. The protein was purified in proportion of ~ 70 %. By deleting the P450 gene from the Campylobacter jejuni 11168 genome clear changes in cell morphology were identified cells becoming wider and shorter. The capsular sugar profile of the NCI strain reveals the presence of arabinose which was not found in the wild type strain. The arabinose was identified by both High Performance Liquid Chromatography (HPLC) and Nuclear Magnetic Resonance (NMR).

Key words: P450, carbohydrate metabolism, Campylobacter jejuni 11168

Introduction

Cytochromes P450 are b-type heme containing monooxygenases which catalyse the insertion of an oxygen atom into a wide range of organic substrates using molecular oxygen (figure1 and 2). These enzymes are involved in the metabolism of a wide variety of both exogenous and endogenous compounds. Their physiological functions vary enormously. The P450 gene is part of an cluster which hosts genes involved in the synthesis of cell surface components (capsula). Campylobacter capsule are important in adhererence, invasion and colonisation of host cells [1,2] and for maintenance of cell surface charge and serum resistance.
These capsule are thought to cause autoimmunity leading to Guillan-Barre and Miller-Fischer syndromes [3].
The structure of the lipooligosaccharides and capsule polysaccharide was published [5, 6] revealing that the strain possessed a type II/III capsule locus found in other microorganisms such *Nisseria meningitidis*.

**Figure 1. Classes of P450s**

**Figure 2. Catalytic cycle of P450**

**Material and Methods**

**Capsular sugar extraction**

After centrifugation *Campylobacter* cells were resuspended in 70 % ethanol and were stirred for few minutes. Cells were harvested again and resuspended in acetone and air dried after collection. For hydrolysis was used 2 M trifluoroacetic acid (TFA) and samples were heated at 120 °C for 1 hour (10 mg acetone powder take 1 ml TFA), and dried in a speed vacuum. The resulted pellet was resuspended in 1 ml distilled water.

**High Performance Liquid Chromatography (HPLC)**

The hydrolysed sugars were analysed by HPLC. Samples were filtered in 0.45 μm filters and eluents filtered degassed. Water has not been filtered because ultra pure water was used instead. The HPLC was performed in a Dionex HPLC with a CarboPacPA1 column using the following program for NaOH concentrations: 0-5 min, 10 mM, 5-30 minutes, 0 mM, 30-70 min, 0-800 mM (linear gradient), 70-75 min, 800 mM, 75-76 min, 800-10 mM, 76-85 min, 10 mM.

**Nuclear Magnetic Resonance (NMR)**

 Sugars were analysed on a Dionex HPLS with a CarboPac PA1 column. The eluent flow-rate was 1 ml/min (0.5 M, 0.6 ml/min) was added post column, and sugars were quantified with a pulsed amperometric detector (with gold electrode). The 1 Dimensional (1D) $^1$H spectra were acquired on a 600 MHz Bruker Avance spectrometer equipped with a cryoprobe. The purified peak 2 from the NC1 spectra was dissolved in D$_2$O and spectra collected at 25 °C (16 scans were used to acquire the data). The HOD signal (4.78 ppm) was used as an internal standard to reference the spectra. By comparison of the standard probe spectrum (L – Arabinose from Sigma-UK) with the sample spectrum a perfect identity in profile was identified.
All NMR experiments were performed on a 600 MHz BRUKER Avance spectrometer equipped with a cryoprobe.

**Results and Discussions**

The aim of this study was to characterise this novel enzyme and to explore its possible role in carbohydrate metabolism. The localization of the P450 gene in a cluster in which most gene products are apparently involved either in biosynthesis of cell surface components or in sugar biosynthesis was the starting point in identification of the P450 enzyme function. The only P450 enzyme involved in sugar metabolism was previously identified in mammalian system and seems to be involved in conversion of glucuronic acid to glucaric acid. In this mechanism the cytochrome P450 system is more likely to be involved than a “glucuronolactone dehydrogenase” [4]. There is no other published evidence about any other P450s being involved in carbohydrate metabolism.

Analysing the capsular sugar profile by HPLC two main differences were identified between the wild type and NCI (mutant strain-the P450 gene was replaced with a kanamycin cassette). Rhamnose, arabinose, galactose and glucose were used as components in the marker mix sample (fig. 3). The wild type and NCI show similar elution profiles with two peaks making the difference between strains. Figure 4 shows that the wild type contains a sugar, which is eluted after 13 minutes (peak 2), that was not identified in the NCI strain. However, the NCI strain produce a sugar, eluted at 11.5 minutes, close to the elution times of the rhamnose and arabinose markers (fig. 5). Figure 6 shows the increase in the amount of arabinose produced when arabinose was injected into the sample.

![Figure 3. HPLC spectra of the marker mix sample. Rhamnose, arabinose, galactose and glucose were used as markers. (individual sugar peaks are labelled, elution time being indicated)](image-url)
Figure 4. HPLC spectra of the NCI sugar profile. Arrow indicates the extra sugar present in to NCI profile. (individual sugar peaks are labelled, elution time being indicated)

Figure 5. HPLC spectra of the NCI sugar profile with injected arabinose. Arrow indicates the arabinose peak. 50 mg / ml arabinose were injected into the sample. The arabinose peak matches perfectly the NCI peak 2. (individual sugar peaks are labelled, elution time being indicated)

Figure 6. HPLC spectra of the wild type sugar profile. Arrow indicates the extra sugar present in to wild type profile. (individual sugar peaks are labelled, elution time being indicated)
The 1 Dimensional (1D) $^1$H spectra were acquired on a 600 MHz Bruker Avance spectrometer equipped with a cryoprobe. The purified peak 2 from the NC1 spectra was dissolved in D$_2$O and spectra collected at 25 °C (16 scans were used to acquire the data). The HOD signal (4.78 ppm) was used as an internal standard to reference the spectra. By comparison of the standard probe spectrum (L – Arabinose from Sigma-UK) with the sample spectrum a perfect identity in profile was identified. The peak 2 from the NCI strain was identified to be arabinose (fig. 7).

![Figure 7](image_url)

Figure. 7. 1D 1H spectrum of the NCI peak 2. Standard arabinose was used to compare the NMR spectrum of the peak 2. The two spectra mach perfectly arabinose being identified as a new sugar produced by the NCI strain.

**Conclusion**

Arabinose was identified by HPLC and NMR to be the monosaccharide produced by the NC1 strain but undetected in the wild type. As previously stated the only P450 enzyme previously known to be involved in sugar metabolism was identified in mammalian system and seems to be involved in conversion of the glucuronic acid to glucaric acid. Under the same mechanism the arabinose might be changed to arabinonic acid (figure 8) which might be the explanation for the absence of arabinose in the Wt strain. The wild type extra peak, in sugar profile, was partially characterised by NMR and further work is necessary.
Figure 8. Proposed reaction type.
A – mammalian system, B – bacterial system

Bibliography

Dupa ce a fost izolat in anii ’70, Campylobacter jejuni a fost caracterizat ca fiind cauza principală a gastroenteritelor de natura bacteriana la oameni. La animale cel mai frecvent este intalnita la bovine si ovine. Odata cu publicarea hârtii genomice a bacteriei Campylobacter jejuni 11168 s-a descoperit prezenta unui singur citocrom P450 localizat într-un operon implicat în biosinteza zaharurilor și a diferitelor structuri de la suprafața celulară. Numele genei este Cj1411c, are o lungime de 1359 bp și este codificată de 453 aminoacizi. Aceasta gena este conservată și în alte tulpini cum ar fi Campylobacter jejuni RM1221. Scopul acestei lucrări a fost de a identifica rolul acestui citocrom P450. Pe parcursul cercetărilor gena a fost clonată în E.coli identificându-se în acest fel caracteristicile generale ale unui citocrom P450: absorbție la 450 nm cand interacționează cu dioxidul de carbon. Proteina a fost purificată în proporție de ~ 70 %. Pentru a atinge acest scop gena Cj1411c a fost înlocuită în genomul bacteriei Campylobacter jejuni 11168 cu o gena ce confera rezistență la antibiotice (kanamicina). Prin analiza monoazaharidelor ce intră în componenta capsulei bacteriene s-a descoperit că în cazul tulpinii mutante există un nou monoazaharid produs: arabinoza. Acest monoazaharid nu a fost identificat în cazul tulpinii salbatice. Absența arabinozei din capsula tulpinii salbatice sugerează faptul că pe parcursul biosintezei arabinoze este trasformată cu ajutorul enzimei P450 în acid arabinonic utilizat de către microorganism în alte scopuri metabolice.

Cuvinte cheie: P450, metabolism glucidic, Campylobacter jejuni 11168