Introduction

Colorectal cancer (CRC) is the second most common cancer in Europe, and is one of the most curable cancers when detected in its early stages. However, the disease remains undiagnosed due to its aspecific symptoms. Although population-based screenings are effective for early detection and prevention, the current CRC screening methods either lack sensitivity and/or specificity, or cause discomfort and pain due to their invasive character. There is an urgent need for discovering disease biomarkers and personalised treatment. With the exception of CEA (carcinoembryonic antigen) levels in plasma, which have shown limited value, there are many other proposed CRC plasma biomarkers at the DNA, protein and carbohydrate level that have not yet reached clinical application.

By optimizing many of the different method parameters, we have greatly reduced the procedure timeline. Reaction conditions were initially optimized for 21 hours incubation time (Figure 4A). In order to reduce it, samples were incubated in an ultrasonic bath at various temperatures and times (Figures 4B, 4C, 4D, 4E) and the MS signal obtained from each condition was compared. Signal intensity and the areas of the separated O-glycan species from BSM were used for the comparisons. Following these experiments, the incubation at 60°C for 2 hours in an ultrasonic bath was chosen as the standard operating condition as a compromise between structural coverage and incubation time. A comparison of the data generated from each approach is shown in Figure 4. The actual method (Figure 4G) offers shorter timelines, permitting rapid O-glycan release within two hours.

Method

O-glycan analysis:

Glycan release. In order to assay the functionality of our high throughput method for O-glycosylation analysis, bovine fetuin (FET) and bovine submaxillary mucin (BSM) O-glycans were released by reductive β-elimination, using 1M potassium borohydride (KBH₄) solution in 0.1M potassium hydroxide (KOH). After permethylation, all samples were dried down in a centrifugal evaporator and re-constituted in 10 μL of 70% MeOH prior to MALDI-TOF-MS analysis in positive ion mode.

In order to test the accuracy of the high throughput procedure, O-glycans were released from a large range of starting material (5-200 μg of BSM) (Figure 5). Although structural coverage and signal intensity of the O-glycan structures detected for each different sample amount is comparable, with a small decrease in signal intensity for the amounts analysed above 100 μg and non detection of a few small structures at amounts analysed below 50 μg, the O-glycan data generated shows that the optimal amount of starting material to use is 50 μg.

Conclusions and Future Perspectives

The results shown on this poster prove that rapid, accurate, sensitive and reproducible O-glycan data can be generated from a large range of starting material, and that our method is suitable for high throughput studies. The actual glycan workflow is optimized for O-glycosylation analysis of purified glycoproteins. We aim to further validate our method to enable its use as a technique for the high throughput analysis of colorectal cancer samples.

References


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