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A new, quick, highly sensitive ultramicro-analysis method for the identification of fructose removed from fructofuranosyl-containing gluco-oligosaccharides by ESI-CID-MS/MS

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ABSTRACT

An efficient, highly sensitive, and ultramicroscale analytical method for the identification of fructose removed from fructofuranosyl-containing gluco-oligosaccharides, including malto-oligosyl fructofuranosides and oligomeric (1→2)-α-D-glucopyranosyl-(1→2)-β-D-fructofuranosides by ESI-CID-MS/MS has been developed with proven applications far superior to the existing method using NMR. With the established principle of diagnostic fragmentation by ESI-CID-MS/MS, the terminal saccharide (either glucose or fructose) can be readily and unambiguously determined at high sensitivity without a tedious derivatization process. Detection of the A-type fragmentation ^{0,4}A-h type ion, and ^{0,2}A type ion are useful as a diagnostic fragmentation tool to identify whether fructose terminal is removed from oligosaccharides. It will facilitate the efficient production of suitable oligosaccharide microarrays crucial for studies on carbohydrate–protein interaction in seeking functional carbohydrates.

Oligosaccharides and polysaccharides play very important roles in the life process of all living organisms as their essential constituents by: (1) Involving in virus invasion of the host, for example, the oligosaccharides of the HIV virus bind to the DC-SIGN triggering HIV infection.[1–3] (2) Avoiding being killed by the human immune system, for example, α-1,3-glucan of *Histoplasma capsulatum* blocks the Dectin-1 reorganization thus leading to its survival in humans.[4] (3) In the synthesis of carbohydrate: β-1,2-glucan binds to cyclic β-(1,2)-glucan synthase which catalyzes four enzymatic activities: initiation, elongation, phosphorolysis, and cyclization.[5] Consequently, investigating and identifying the carbohydrate–protein interaction conceivably would lead to the understanding of details of the mechanism of oligosaccharides and polysaccharides involved in biomolecular recognition.

Hitherto, there are several methods to explore carbohydrate–protein interaction, including: MALDI-MS,[6] SPR,[7] NMR,[8] as well as carbohydrate microarray.[9] As carbohydrate-microarrays have the advantages of high-throughput analyses requiring only micro amounts of proteins and carbohydrates (oligosaccharides or polysaccharides), carbohydrate microarray has become the leading technology in exploring protein–carbohydrate interactions.

For carbohydrate-microarray analysis, immobilization of the carbohydrate molecules on the chip is the key step for carbohydrate chip preparation, particularly for oligosaccharides. There are two major methods for immobilizing oligosaccharides on the chip: (1) Covalent immobilization via chemical reaction, for example, oligosaccharides are fixed on the chip via an aldehyde group (from the oligosaccharide's reducing terminal) and amine or a semicarbazide-derivatized glass slide.[10,11] (2) Noncovalent immobilization, for example, oligosaccharides are conjugated to the lipid via the aldehyde group (from oligosaccharide's reducing terminal) and the ammonia group (from the lipid). Subsequently, the complex is immobilized on the chip through adsorption between the lipid and the materials (for example, nitrocellulose membrane) on the chip,[12] or using sugar-immobilized gold nano-particles.[13] For oligosaccharides, the aldehyde group is the core for immobilization of oligosaccharides on the chip.

There are different types of fructofuranosyl-containing oligosaccharides,[14] for example, malto-oligosyl fructofuranosides,[15] fructo-oligosaccharides (inulin-FOS type),[16] and oligomeric (1→2)-α-D-glucopyranosyl-(1→2)-β-D-fructofuranosides.[17] In order to immobilize fructofuranosyl-containing oligosaccharides on the chip, the fructose terminal must be removed from oligosaccharides to acquire a reducing terminal sugar containing the aldehyde group. As all of these saccharides are hexose, whether the fructose has been removed from the oligosaccharide terminal

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cannot be identified on the basis of molecular weight (Fig. 1). Although NMR has been successfully used to identify the removal of fructose from oligosaccharides,[18] the required amount of the sample must be at the mg scale and the test is quite time consuming. In addition, only the skilled expert can read and interpret the appropriate NMR profile. Consequently, a new, quick, simple, and highly sensitive reproducible analytical method using only minute amounts of test sample is required to detect the removal of fructose from the oligosaccharides for the preparation of the molecular probes with fructofuranosyl oligosaccharides for the production of the appropriate carbohydrate-microarray.

ESI-CID-MS/MS is a quick, time-saving, highly sensitive methodology which has been widely used for microscale analysis of the oligosaccharide sequence.[19–22] In this work, a novel ESI-CID-MS/MS analytical method has been successfully developed to detect the removal of fructose from the fructofuranosyl-containing gluco-oligosaccharides: malto-oligosyl fructofuranosides and oligomeric (1→2)-α-D-glucopyranosyl-(1→2)-β-D-fructofuranosides.

1. Experimental

1.1. Materials and methods

1.1.1. Chemicals

Sucrose, maltotriose, and kojibiose were purchased from J&K (Shanghai, China). *Cyanobacterium* gluco-oligosaccharide fructosides Cyanobac-Deca and Cyanobac-Nona isolated from *Cyanobacterium*[17] fermentation broth were kindly provided by Professor Eckhard Loos (University Regensburg, Germany). Cyanobac-Glc9 was derived from Cyanobac-Deca. Erlose (*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl β-D-fructofuranoside) was a gift from Professor Javier Moreno (Universidad Autónoma de Madrid, Spain).

1.1.2. Hydrolysis and purification

Cyanobacterium gluco-oligosaccharide fructoside was hydrolyzed using 0.5 M trifluoroacetic acid (TFA) at 95 °C treated for 9 min to release the fructose as previously described.[18] Each hydrolyzate was subsequently separated using Superdex-Peptide FPLC column (Pharmacia Biotech, Uppsala). Deionized water was used as the elution solvent to remove fructose and desalting was conducted at the flow rate of 0.3 ml/min. The resulting eluted solution was collected in tubes with the fraction collector (Gilson Inc, USA) as monitored by the RI detector. The content of each tube was pooled based on the separation profile and lyophilized.

1.1.3. Mass spectrometry

To determine the molecular weight of the oligosaccharide, MALDI-MS analysis was carried out using TOF Spec-2E instrument (Waters, Manchester, UK). The procedure is as follows: Oligosaccharides were dissolved in water at a concentration about 10–20 pmol/μl, and 0.25 μl was used for the analysis of the sample after drying. 0.25 μl matrix of 2-(4-hydroxyphenylazo) benzoic acid was added. The laser energy was set at 60% and resolution at 1000.

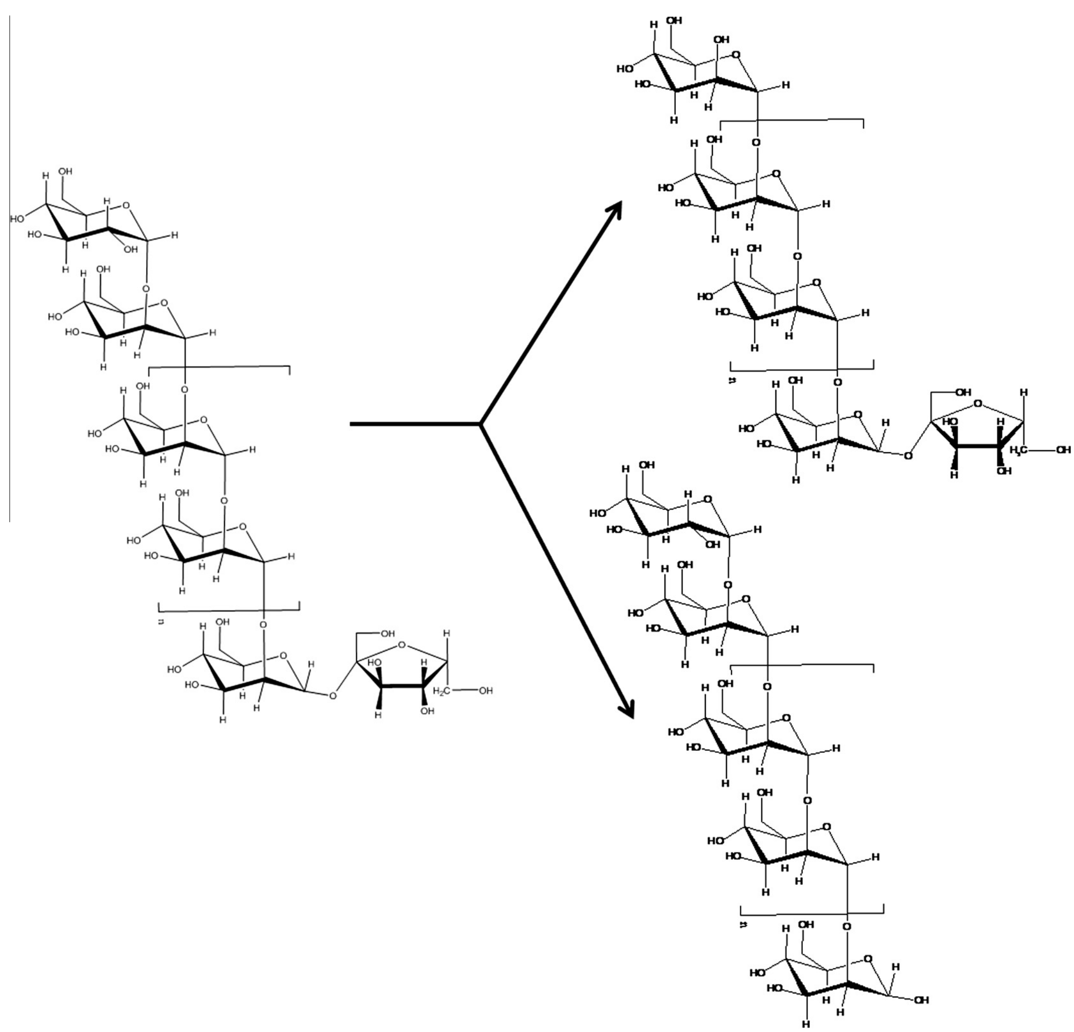

Figure 1. Putative structures of the hydrolyzates from the hydrolysis of fructofuranosyl-containing gluco-oligosaccharides.