Analysis and role of oligosaccharides in milk

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Milk is an important fluid in glycobiology because it contains a number of short carbohydrate chains either free or as glycoconjugates. These compounds as a class are the most abundant component and benefit the infant by developing and maintaining the infant's gut flora. New and emerging methods for oligosaccharide analysis have been developed to study milk. These methods allow for the rapid profiling of oligosaccharide mixtures with quantitation. With these tools, the role of oligosaccharide in milk is being understood. They further point to how oligosaccharide analysis can be performed, which until now has been very difficult and has lagged significantly those of other biopolymers. [BMB Reports 2012; 45(8): 442-451]

INTRODUCTION

Human milk is a unique fluid that is composed of lactose, lipids, free oligosaccharides and proteins, of which the free oligosaccharides are important constituents, at a concentration ranging from 5 to 23 g/L (1-3). Free oligosaccharides have the important role of establishing the gut flora of infants. Determining the role of these compounds has been led primarily by the analytical tools that made the rapid analysis and quantitation possible.

Free oligosaccharides in human milk can either be linear or branched, consisting of 3 to 14 monosaccharides (4, 5) (Fig. 1). It was initially believed that there are potentially thousands of structures. More recent analyses employing nanoflow liquid chromatography suggest perhaps a couple of hundred structures. With these tools, the role of oligosaccharide in milk is being understood. They further point to how oligosaccharide analysis can be performed, which until now has been very difficult and has lagged significantly those of other biopolymers. [BMB Reports 2012; 45(8): 442-451]

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Fig. 1. Structural features of HMOs. Monosaccharide building blocks, together with their figurative representation (A). Examples of HMO structures, which can be linear or branched and may be decorated with fucoses or sialic acids (NeuAc) (B).

Assumption is not specific for bifidobacteria, but can also be observed for bacteriodes species (26, 29). In general, it is now established that human milk oligosaccharides have a strong influence on the composition of the gut microflora. It is proposed that a well-balanced intestinal microflora is important for the development of the infant’s immune system (30), indicating that HMO play an important role in the infants well-being.

ANALYSIS OF HMOs

The key to the advancement in understanding the role of HMOs, has been the development of sensitive and quantitative methods for analysis. In this regard, a number of recent advancements have made this possible. The development of new mass spectrometry methods for ionization such as matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization allowed rapid determination of accurate mass as well as obtain structural information through tandem MS. The coupling of liquid chromatography with mass spectrometry allowed the profiling of oligosaccharide mixtures. Furthermore, nanoflow liquid chromatography yielded high sensitivity while microchip based devices yielded highly reproducible retention times.

Compositional profiling of HMOs

Complicated mixtures of milk oligosaccharides can be readily profiled by MALDI MS. Profiling of human milk oligosaccharides using MALDI-TOF-MS was first described by Stahl et al. (31), who were able to observe neutral oligosaccharides in positive mode as monosodium adducts as well as acidic oligosaccharides in both the positive and negative modes. It was noticed that desialylated fragments could be observed in the acidic fraction. This approach has been applied recently for the determination of lewis blood group by HMO fingerprinting. Following an automated oligosaccharide purification, HMO were analyzed using MALDI-TOF with 6-aza-2-thiothymine (ATT) as the matrix (9). Neutral oligosaccharides and sialyllactose could be observed as sodium- and potassium adducts in the positive mode, while other sialylated HMO were detected as deprotonated molecular ions in the negative mode. Using this method, 93.8% of the samples could be assigned the right blood group.

MALDI-FTICR-MS of oligosaccharides coupled mixture analysis with high mass accuracy and allowed the rapid determination of accurate compositions (32). Using 2,5-dihydroxybenzoic acid (DHB) as the ionizing matrix, neutral oligosaccharides were observed as sodiated adducts in the positive mode. The high-resolution analysis made the determination of composition relatively fast providing the identification of fucosylated and sialylated species. Furthermore, the high resolution of the FTICR-MS allowed application of deuterium labeled internal standards, which was shown to be simple and effective for rapid and accurate relative quantitation (28, 32, 33). Employing just this method, allowed the determination of the specific consumption of Bifido strains from infant gut (28).

Compound profiling of HMOs

The profiling of HMO structures requires the separation of compositions into individual components. Despite the advancements in liquid chromatography in normal phase (34, 35) and reverse phase (36-38) as well as in capillary electrophoresis (37, 38), there remains no single separation method sufficiently effective for separating HMO mixtures. The problem lies in the heterogeneity of the structures. While oligosaccharides are generally hydrophilic, the degree of hydrophilicity depends on the constituent monosaccharides. Those containing sialic acids tend to be slightly more ionic compared to those containing “neutral” components.

Reverse phase (RP)-HPLC has long been used for oligosac-