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Review

Liquid chromatography/tandem mass spectrometry of dolichols and polyprenols, lipid sugar carriers across evolution☆

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ABSTRACT

Across evolution, dolichols and polyprenols serve as sugar carriers in biosynthetic processes that include protein glycosylation and lipopolysaccharide biogenesis. Liquid chromatography coupled with electrospray ionization mass spectrometry offers a powerful tool for studying dolichols and polyprenols in their alcohol or glycan-modified forms in members of all three domains of life. In the following, recent examples of the how different versions of this analytical approach, namely reverse phase liquid chromatography-multiple reaction monitoring, normal phase liquid chromatography/tandem mass spectrometry and normal phase liquid chromatography-precursor ion scan detection have respectively served to address novel aspects of dolichol or polyprenol biology in Eukarya, Archaea and Bacteria. This article is part of a Special Issue entitled Lipodomics and Imaging Mass Spectrometry.

1. Introduction

Dolichols and polyprenols are specific examples of polyisoprenoid alcohols, a family of hydrophobic polymers containing linearly linked isoprene subunits found in all living organisms [1,2]. Comprising up to 25 isoprene subunits and bearing a hydroxyl group at their α ends, dolichols can be distinguished from polyprenols on the basis of the saturated α isoprene subunit found in the former but not in the latter (Fig. 1).

The main and still best defined role played by dolichols and polyprenols is as a sugar carrier in various biosynthetic processes. In the N-glycosylation pathways of Eukarya and Archaea, the glycans eventually transferred to select Asn residues of target proteins are first assembled on phosphorylated dolichols. In each case, both simple sugars and complex oligosaccharides are attached to these dolichol carriers, with various organisms employing dolichols of different lengths [3,4]. Eukaryal organisms contain families of dolichol species consisting of six to eight members, with C_{80}, C_{90} and C_{95} dolichols predominating in yeast, rat and man, respectively [1]. Multiple dolichol species are also involved in archaeal N-glycosylation [5]. By contrast, the bacterial N-glycosylation pathway predominantly relies on a single polyprenol species, C_{55} undecaprenol, as glycan carrier [6]. The same polyprenol carrier is employed in the assembly of peptidoglycans [7,8], and covalent modification of lipid A (endotoxin), the hydrophobic anchor of lipopolysaccharide (LPS[1]) in Gram-negative bacteria [9].

Despite the wealth of information on N-glycosylation and LPS biosynthesis available, outstanding questions remain. Often, these unknowns are centered on those pathway steps involving the biosynthesis and assembly of dolichol or polyprenol, as well as their glycan-charged derivatives. Liquid chromatography (LC) coupled with electrospray ionization tandem mass spectrometry (ESI-MS/MS) has proven to be a highly sensitive and specific tool for studying dolichols and polyprenols in their alcohol or glycan-modified forms in members of all three domains of life [10–17].

In the following, recent examples of the use of LC-ESI/MS to provide novel insight in dolichol and polyprenol biology are reviewed. Specifically, we describe: 1) reverse phase LC-multiple reaction monitoring (MRM) detection of polyprenols and dolichols in mouse embryos; 2) normal phase LC ESI-MS/MS of dolichol phosphate-linked glycans in Archaea; 3) normal phase LC-precursor ion scan

Abbreviations: ESI-MS/MS, electrospray ionization tandem mass spectrometry; GalN, galactosamine; GalNAc, N-acetyl galactosamine; Glu, glucose; LC, liquid chromatography; LPS, lipopolysaccharide; MRM, multiple reaction monitoring; SRD5A3, steroid 5α-reductase 3; S-layer, surface layer

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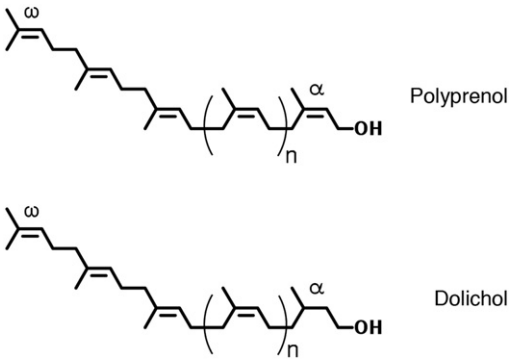

Fig. 1. Chemical structures of polyprenol and dolichol. The positions of the α and ω isoprene units are indicated.

detection of undecaprenyl phosphate-linked monosaccharide donors in the covalent modification of lipid A in Bacteria.

2. Reverse phase liquid chromatography-multiple reaction monitoring (LC-MRM) detection of polyprenols and dolichols in mouse embryos

Although the role of phosphorylated forms of dolichol as glycosyl carrier lipids in eukaryal N-glycosylation was described over 40 years ago [18], the enzymes responsible for several biosynthetic steps remain unknown. Recently, the gene responsible for catalyzing reduction of the α isoprene of polyisoprenol and giving rise to dolichol was described. Humans bearing mutations in the steroid 5α-reductase 3 (SRD5A3) gene suffer from a congenital disorder of glycosylation, characterized by mental retardation, and eye and cerebellar defects [11]. Since yeast cells lacking *DFG10*, encoding the ortholog of human SRD5A3, fail to fully reduce the α isoprene, as required to convert polyprenol to dolichol, it was proposed that SRD5A3 corresponds to the long-elusive dolichol reductase in mammals. To confirm this hypothesis, as well as to unequivocally identify the last step of dolichol biosynthesis, *Srd5α3* knock-out mice were generated and reverse phase LC-MS was used to analyze polyprenols in wild type and SRD5A3-lacking mice embryos at the 11-day stage, representing the last day of viability of the mutant mice. However, the small size of the embryos presented an obstacle for the detection of polyprenols by MS in the full scan mode using a QSTAR XL quadrupole time-of-flight tandem mass spectrometer (Applied Biosystems, Foster City, CA), as previously described [10]. Accordingly, LC coupled with MRM, offering maximum sensitivity for the detection of dolichols and polyprenols, and especially valuable when only small amounts of cells or tissues are available, was employed.

MRM is primarily performed on triple quadrupole mass spectrometers, where the first quadrupole (Q1) isolates the precursor (parent) ion, Q2 acts as a collision cell and the third quadrupole (Q3) selects a specific fragment of the precursor ion. The two mass filters, Q1 and Q3, produce a very specific and sensitive response for the selected analyte that can subsequently be used to detect and integrate a peak in a

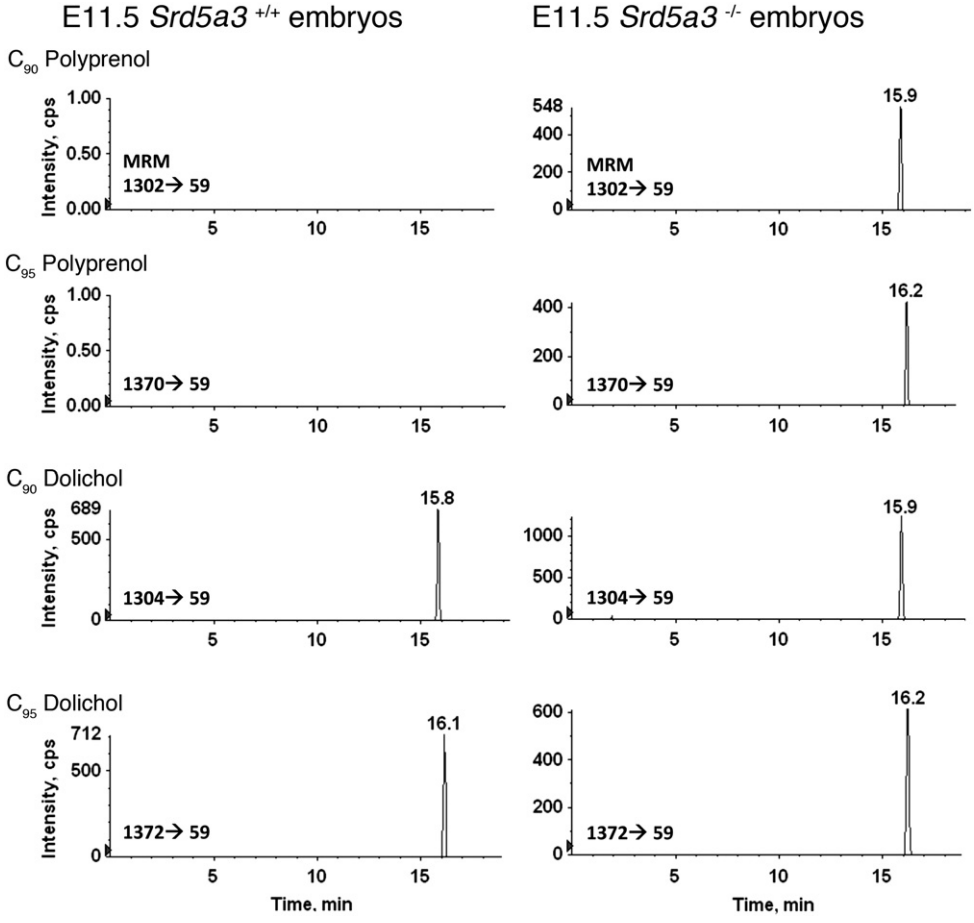

Fig. 2. Reverse phase LC-MRM of dolichols and polyprenols in mouse embryos. Homozygous SDR5A3 mutation in mice causes the death of embryos and accumulation of polyprenols. The C_{90} polyprenol and C_{95} polyprenol are detected in the 11-day old (E11.5) *Srd5α3*$^{-/-}$ mutant embryos, but not in the E11.5 *Srd5α3*$^{+/+}$ wild type embryos. C_{90} polyprenol and C_{95} polyprenol are detected through the MRM pairs of 1302/59 and 1370/59, respectively. C_{90} dolichol and C_{95} dolichol are detected through the MRM pairs of 1304/59 and 1372/59, respectively. C_{90} dolichol and C_{95} dolichol are known to be retained at 15.5–16.5 min [28].