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Research paper

Synthesis and biological evaluation of chemical tools for the study of Dolichol Linked Oligosaccharide Diphosphatase (DLODP)

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ABSTRACT

Citronellyl- and solanesyl-based dolichol linked oligosaccharide (DLO) analogs were synthesized and tested along with undecaprenyl compounds for their ability to inhibit the release of [^{3}H]OSP from [^{3}H] DLO by mammalian liver DLO diphosphatase activity. Solanesyl (C45) and undecaprenyl (C55) compounds were 50–500 fold more potent than their citronellyl (C10)-based counterparts, indicating that the alkyl chain length is important for activity. The relative potency of the compounds within the citronellyl series was different to that of the solanesyl series with citronellyl diphosphate being 2 and 3 fold more potent than citronellyl-PP-Glc*NAc*$_2$ and citronellyl-PP-Glc*NAc*, respectively; whereas solanesyl-PP-Glc*NAc* and solanesyl-PP-Glc*NAc*$_2$ were 4 and 8 fold more potent, respectively, than solanesyl diphosphate. Undecaprenyl-PP-Glc*NAc* and bacterial Lipid II were 8 fold more potent than undecaprenyl diphosphate at inhibiting the DLODP assay. Therefore, at least for the more hydrophobic compounds, diphosphodiesters are more potent inhibitors of the DLODP assay than diphosphomonoesters. These results suggest that DLO rather than dolichyl diphosphate might be a preferred substrate for the DLODP activity.

1. Introduction

N-glycans play crucial roles in cell growth, differentiation and communication [1]. Protein *N*-glycosylation occurs by transfer of Glc$_3$Man$_9$Glc*NAc*$_2$, from dolichol-linked oligosaccharide (DLO, Glc$_3$Man$_9$Glc*NAc*$_2$-PP-dolichol), onto polypeptides containing an Asn residue in the Asn-X-Ser/Thr glycosylation sequon. Dolichyl-diphosphate, the by-product of this reaction, is recycled into DLO. This sequence of reactions constitutes the dolichol cycle, and because dolichol-P (DolP) is rate limiting for protein glycosylation, its interruption leads to hypoglycosylation of glycoproteins. In man, mutations in genes encoding proteins of the dolichol cycle lead to type I congenital disorders of glycosylation (CDG-I) [2], a group of rare inherited diseases, manifesting multisystemic clinical pictures, whose hallmark is the presence of hypoglycosylated serum glycoproteins [3]. Of particular interest for the study of these diseases are DLO regulation and the fate of truncated DLO intermediates often seen in CDG-I. In fact, data show that truncated DLO species are cleaved by a DLO diphosphatase (DLODP), to yield DolP and oligosaccharyl-phosphates (OSP) in cells derived from CDG-I patients [4,5] and it has been hypothesized that such a mechanism may restrict truncated DLO accumulation while at the same time allowing DolP recycling [6]. In order to understand the role of a recently described Co^{2+}-dependent DLODP activity [7,8] (Fig. 1) we initiated a chemistry program aimed at generating chemical tools that are required for DLODP characterization.

In particular, we were interested in defining the structural elements required for molecules to interact with DLODP. Thus, the synthesis of simplified DLO analogs (Fig. 2) has been carried out taking the simplest Glc*NAc*-diphosphoryl-dolichol

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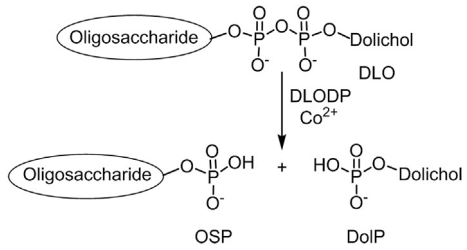

Fig. 1. Role of the DLODP.

(Glc*N*Ac-PP-dolichol, R^1-*O*-P-*O*-P-*O*R^2) as a model. In order to investigate the relative importance of each part of the DLODP substrate, the R^1 position has been substituted with either a Glc*N*Ac residue or a di-*N*-acetylchitobiose moiety, which occur in the natural substrate, and the dolichol residue at the R^2 position has been replaced by the shorter citronellyl or solanesyl moieties. Such R^2 groups were intended to probe the possible requirement of the diphosphatase activity for long polyprenyl chains. Furthermore, to evaluate the importance of the sugar chain for recognition by DLODP, the corresponding citronellyl and solanesyl diphosphates have also been synthesized (R^1 = H).

Additionally, the related monophosphates (R^1-*O*-P or R^2-*O*-P) were also prepared to assist the identification of the reaction products. Finally, the synthesis of citronellyl medronate in which a methylene group replaces the central oxygen atom of the diphosphate moiety has also been achieved as a non-cleavable substrate. Results concerning the biochemical characterization of the Co^{2+}-dependent DLODP have recently been published [7]. Here we report the full results dealing with the efficient synthesis of these complex compounds in pure form and the complete comparison of their inhibition of DLODP activity.

2. Results and discussion

2.1. Chemistry

The preparation of the targeted disubstituted diphosphates **A** has been envisaged through the coupling of the corresponding phosphosugars **B** and phosphodolichol mimics **C** (Fig. 3).

The peracetylated Glc*N*Ac **1** (Scheme 1) was prepared from commercially available Glc*N*Ac by treatment with acetic anhydride in excess in pyridine and the pure α anomer was isolated in 89% yield after flash chromatographic purification. Peracetylated $GlcNAc_2$ **2** was obtained by acetolysis of chitin by H_2SO_4 and acetic

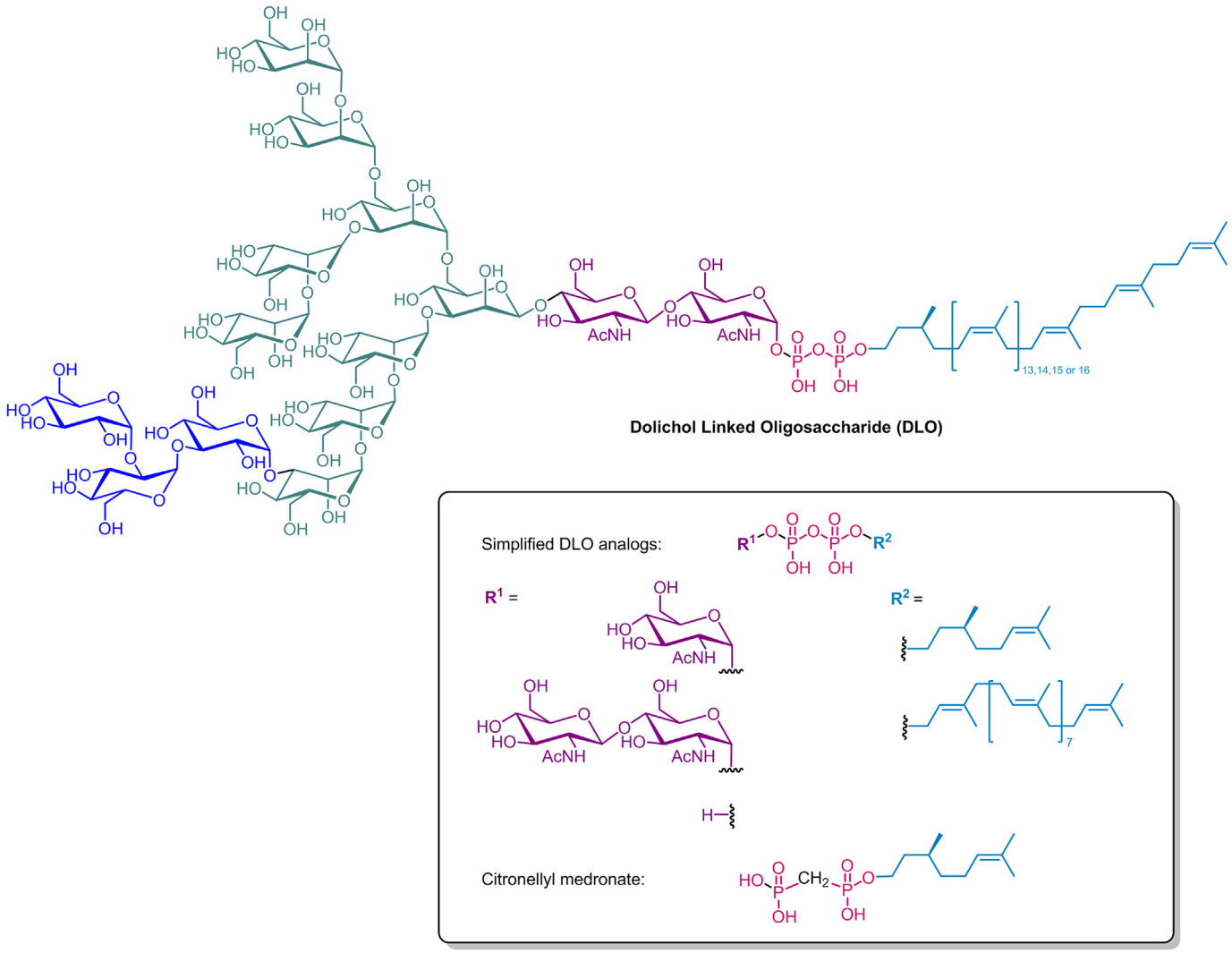

Fig. 2. Structure of DLO and of the targeted compounds.