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Minireview

Is there a "dolichol recognition sequence" in enzymes that interact with dolichols and other polyisoprenoid substrates?*

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Yeast dolichyl-P-mannose synthase and a number of other enzymes that interact with dolichol or dolichyl-P as substrates contain a highly conserved amino-acid sequence that has been proposed as a potential dolichol recognition sequence [Albright, C.F., Orlean, P. & Robbins, P.W. (1989) *Proc. Natl. Acad. Sci. U.S.A.* 86, 7366–7369]. In dolichyl-P-mannose synthase, the most highly conserved amino-acid residues of this domain were modified by site directed mutagenesis, and for one construct the sequence was completely deleted. Enzymes containing the site directed modifications, and the deletion mutant, were found to retain catalytic activity, and all of the modified enzymes had the same apparent affinity for Dol-P as wild type enzyme when assayed in a phospholipid matrix. Based on these results, the amino-acid composition and sequence of the conserved domain are not critically important for the recognition and binding of Dol-P when the synthase is reconstituted in a lipid matrix.

Dolichols are long chain polyisoprenoid alcohols containing between 16 to 20 isoprene units [1]. The α-isoprene unit is saturated and the two isoprene units adjacent to the ω-terminus are of the *trans*-configuration. The remaining isoprene units in dolichol are of the *cis*-configuration. The solution conformation of dolichol has been predicted from molecular mechanic computation and small-angle X-ray scattering [2], but the configuration of dolichol in membranes has not been established. Several studies [3–8] have shown, however, that dolichols destabilize phospholipid bilayers that have compositions approximating membranes of the rough endoplasmic reticulum (RER[1]).

The major biological functions of dolichylphosphates are to serve as carriers of activated-sugars and oligosaccharides in the pathway for biosynthesis of asparagine-linked oligosaccharide chains on yeast and mammalian glycoproteins. The lipophilic dolichyl moiety anchors the growing oligosaccharide chains in membranes of the RER and is probably involved in the translocation of mannose and oligosaccharides across the RER membrane. Details about exact topology of glycosyltransferases involved in the synthesis of Dol-P-Man and Dol-P-P-oligosaccharides, and molecular mechanisms for the translocation of lipid-linked sugars across the hydrophobic interior

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[1]Abbreviation: RER, rough endoplasmic reticulum

of the RER membrane remain speculative at the present time. In addition, dolichyl-phosphate-sugars are important glycosyl-donors for the biosynthesis phosphatidylinositol anchors on membrane proteins, and for the synthesis of *O*-mannosyl linkages in yeast glycoproteins [9]. A number of the enzymes that interact with dolichols and other polyisoprenoid compounds have been shown to contain a highly conserved hydrophobic amino-acid sequence that has been proposed to be involved in the recognition of the hydrophobic substrates [10]. The original consensus sequence for this putative "dolichol recognition site" was defined as LFVxFxxIPFxFY and was identified in the yeast enzymes mannosyltransferase I (*Alg1p*), GlcNAc-1-P transferase (*ALG7p*), dolichyl-P-mannose synthase (*DPM1p*) and dolichol kinase (*SEC59p*). The consensus sequence was later modified (Table 1) to account for homologous sequences identified in GlcNAc-1-P transferase from CHO cells, which contains two copies of the sequence [11–13], and in ribophorin I from canine pancreas which is a component of the oligosaccharyl transferase complex [14]. As defined by Datta & Lehrman [12], all of the proteins in this family match at least 5 of the 7 specified residues in the revised consensus sequence (Table 1). The isoleucine residue at position 7 (253 in Dol-P-Man synthase) is invariant, and the sequence must have aromatic residues at positions 4 and 11 (phenylalanine 250 and tyrosine 257 in Dol-P-Man synthase). A recent study has shown that the amino-acid composition of the putative recognition sequence is essential for the activity of GlcNAc-1-P transferase [12]. Alteration at either site of the GlcNAc-1-P transferase abolished catalytic activity, but the affinity of the mutated proteins towards Dol-P was not directly determined.

Several prokaryotic enzymes that interact with totally unsaturated polyprenols (bactoprenols) have recently been suggested to contain the putative dolichol recognition sequence based on deduced amino-acid sequences from cloned genes [15, 16]. It is important to note that the primary difference between polyisoprene alcohols (bactoprenols) and dolichols is that bactoprenols contain an unsaturated α-isoprene unit. Enzymes involved in the biosynthesis of bacterial polysialic acid capsules, however, do not contain sequences that fit the defined requirements for the putative dolichol recognition sequence. The proteins involved in polysialic acid capsule formation either lack the invariant isoleucine at position 7, or lack the highly conserved residues at positions 4 and 11.

Table 1

The amino-acid sequence of yeast Dol-P-Man synthase from residues 231 to the carboxyl terminus 267 (a) and the consensus sequence of the proposed dolichol recognition sequence (b) as adapted from reference [10]. The potential membrane spanning domain is underlined and the dolichol recognition sequence is double underlined. The asterisks denote residues modified by site directed mutagenesis in DPMFV-250 (c), DPMIF-253 (d), DPMIN-253 (e), and DPMYV-257 (f).

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        231      239        250    257        267
                             *   *   *
a)      LKELYVFK FGANNLILFITFWSILFFYVCYQLY HLVF
                             |   |   |
                          1  4   7   11
b)                        FIXFXXIPFXY   (or)
                          V Y        F
                             |   |   |
c)                        FIXvWSILFFY
                             |   |   |
d)                        FITFWSfLFFY
                             |   |   |
e)                        FITFWSnLFFY
                             |   |   |
f)                        FITFWSILFFv
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An enzyme involved in the biosynthesis of bacterial cell wall peptidoglycan, and which utilizes a polyprenyl substrate, has been shown to have a weak homology to *Alg*1*p* [17], but the deduced amino-acid sequence of MurAc-pentapeptide transferase from *E. coli* does not contain any domain homologous to the consensus sequence.

For a number of years our laboratory has had an interest in enzymes catalyzing the biosynthesis of asparagine-linked oligosaccharide chains of mammalian glycoproteins. Our goal is the isolation of pure enzymes, and reconstitution of these enzymes with phospholipids in well-defined systems that will allow investigations on the activities and the mechanisms of these glycosyltransferases in a membrane environment. We have partially purified Dol-P-Man synthase from rat liver and have characterized many of the properties of the mammalian enzyme [18–20]. The amounts of available protein were limiting, however, for thorough biophysical studies of interaction between the mammalian enzyme and phospholipid matrices. Dol-P-Man synthase has been highly purified from yeast by Haselbeck [21, 22] but the yeast enzyme was only partially characterized and the published procedure did not provide useful amounts of enzyme for detailed characterization of the kinetic and physical properties of the protein. More recently, the structural gene for yeast Dol-P-Man synthase (*DPM*1) was cloned and shown to be expressed as an active protein in *E. coli* [23]. *DPM*1 codes for a protein of 267 amino acids with an apparent molecular mass of 30.36 kDa and a potential membrane spanning domain of 25 amino acids at its carboxyl terminus. The putative dolichol recognition sequence occurs in the membrane spanning domain of the enzyme. In order to more thoroughly characterize the kinetic and physical properties of yeast Dol-P-Man synthase, and to establish the function of the putative dolichol recognition sequence, *DPM*1 was incorporated into a prokaryotic high-expression vector that allowed the purification of milligram quantities of purified enzyme [24]. Three of the most highly conserved residues within the dolichol recognition sequence were altered by site directed mutagenesis (Table 1), and the effect of these substitutions on the apparent affinity of the enzyme for the hydrophobic substrate were assessed. Aromatic and aliphatic residues were exchanged within the sequence to modify the size and properties of the side chains, and the polarity of two of the residues were modified. In addition, a deletion (DPMΔ3) of the hydrophobic C-terminus of the protein from residue 239 to 267 was prepared [25] which therefore lacked the proposed dolichol binding site as well as the potential membrane spanning domain of the enzyme. Kinetic constants for GDP-Man and Dol-P were determined for each of the mutant proteins and for wild type enzyme in both detergent solution and when the enzymes and their hydrophobic

Table 2

K_m values for GDP-mannose and dolichyl-phosphate for wild type and mutated Dol-P-Man synthases when assayed in Nonidet P-40 or in a phosphatidylethanolamine matrix

Enzyme	Reaction mixture			
	NP-40	PE	NP-40	PE
	GDPMan		Dol-P	
	μM	μM	μM	μM
Wild type	1.5[a]	2.5	10.6	2.7
DPMFV-250	1.7	2.3	8.6	2.4
DPMIF-253	1.7	2.5	9.2	3.8
DPMIN-253	2.4	4.3	48	2.7
DPMYV-257	1.5	2.1	8.0	2.2
DPMΔ3	30	31	45	2.6

[a] Data taken from reference [24]. NP-40, Nonidet P-40; PE, phosphatidylethanolamine.

substrate were reconstituted in dispersions of phosphatidylethanolamine.

Our results [24] demonstrated that the putative dolichol recognition site is not required for catalytic activity of Dol-P-Man synthase, or for the recognition and binding of Dol-P. Substitution involving the replacement of one hydrophobic amino acid with another hydrophobic amino acid, or the replacement of tyrosine with valine, had no effect on the affinity of the enzyme for the lipophilic substrate Dol-P, or for the water soluble substrate GDP-Man, as measured by the apparent K_m values for these compounds. The amino acid changes involving substitutions of hydrophobic amino acids were conservative replacements, but equivalent conservative substitutions are not found in any of the family of proteins containing this sequence. The substitution of a polar amino acid (asparagine) for a hydrophobic amino acid (isoleucine) at position 253 (DPMIN-253) resulted in a 5-fold higher value for the apparent K_m for Dol-P when assayed in detergent solutions, but this substitution had no effect on the K_m for Dol-P when the enzyme was reconstituted with phosphatidylethanolamine. The lowered affinity of DPMIN-253 for Dol-P in detergent solution may reflect changes in protein conformation that hinders interaction with the non-ionic detergent rather than with the hydrophobic substrate. Enzyme containing a deletion of the putative membrane spanning domain had an increased K_m for GDP-Man in both detergent and phospholipid suggesting that deletion of the hydrophobic C-terminal domain resulted in a conformational change in the protein. The apparent K_m for Dol-P was increased about 5-fold when DPMΔ3 was assayed in detergent solution, but the K_m for Dol-P was the same as wild type enzyme when DPMδ3 was reconstituted in phosphatidylethanolamine. These results demonstrate that the amino-acid compositions and sequence of the putative "dolichol recognition sequence" of Dol-P-Man synthase are not critically important for the recognition and binding of Dol-P to the enzyme under the assay conditions tested.

In conclusion, it is unlikely that the amino-acid sequence of the putative "dolichol recognition sequence" is involved in the recognition of dolichols. At least one enzyme that utilizes Dol-P as a substrate lacks the putative recognition domain. The deduced amino-acid sequence for GlcNAc-1-P transferase from *Leishmania* is similar to the analogous enzymes in yeast and mammalian cells, but the trypanosome enzyme lacks the dolichol recognition sequence [26]. This should not be totally unexpected since very little homology has been found in the primary sequences among families of cloned Golgi glycosyltransferases [27], and there is no reason to expect *a priori* a closer homology among enzymes that utilize dolichols as substrates. Our recent results [24] demonstrate that the site directed modifications in the proposed recognition domain do not affect affinity of the enzyme for Dol-P when the enzyme is assayed in a phospholipid environment, and finally, the hydrophobic sequence is not required for catalytic activity of Dol-P-Man synthase either *in vitro* [24] or *in vivo* [25]. Since the highly conserved sequence found in the proteins is not involved in binding the hydrophobic substrate, what is the function of this sequence? It is possible that the conserved amino-acid sequence serves some other function in the family of proteins that interact with polyisoprenoid substrates. All of the eukaryotic proteins known to contain this domain are involved in pathways for the synthesis of protein-bound oligosaccharides and are found in the endoplasmic reticulum. This common sequence may therefore represent a retention signal for enzymes in the membranes of this organelle. Alternatively, this sequence may be required for interactions between enzymes that catalyze the highly ordered sequential reactions in the pathways involved in the synthesis of Dol-P and dolichyl-linked oligosaccharides. The function of the highly conserved sequence should become more apparent as additional enzymes in these pathways are cloned and sequenced. A specific dolichol recognition domain(s) must occur in enzymes involved in these pathways, however, because several of the enzymes utilizing dolichols as substrates strongly discriminate against polyprenol substrates as judged by relative reaction rates [28–30]. The sequence of this dolichol recognition domain(s) will be identified as more information about the primary sequences of the enzymes are elucidated, and the structure and function of the domain will be established only when the enzyme are purified thoroughly characterized in membrane environments.

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