A Review on Study of Mannosyltransferases in Glycosylation

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Abstract

Glycosyltransferase in general plays a very important role in the post-translational modification of number of biomolecules including glycoproteins. The glycosylation of the protein occurs in the endoplasmic reticulum and is carried out by at least 14 different types of membrane-bound glycosyltransferases. It is now well established that glycosylation of protein occurs either at serine or threonine residues (O- glycosylation) or at asparagine residue (N-glycosylation) of proteins.

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In higher eukaryotes the N–glycosylation is the major post-translational process, initiated by the synthesis of a large lipid oligosaccharide, Glc_3–Man_9–$GlcNAc_2$–PP–Dolichol. This represents the immediate precursor of the carbohydrate unit involved in the biosynthesis of glycoproteins. The large oligosaccharide is then transferred from the lipid oligosaccharide to the acceptor lipid by the oligosaccharide transferase.

The glycosyltransferases involved in the initial processes are therefore of great interest. The enzymes of the dolichol pathway of N– N-glycosylation have been studied in detail. The glycosyltransferases like mannosyltransferases that transfer the first mannose residues from Dol-P-Man to Dol-PP-Glc NAc_2 have been studied in great detail, and these enzymes appear to be involved in the regulation of the dolichol pathway.

Enzymology Involved In Sugar Transfer

α -linked mannose residues in the heptasaccharide-lipid are donated directly by GDP-mannose

Lehle and Tanner (1978) presented in their study that particulate fraction isolated from baker yeast catalyzes the formation of mannose from GDP-mannose into a series of acid-labile lipid-linked oligosaccharides up to 12 mannose units long, which appear to have GlcNAc at the reducing terminus. Chambers et al (1977) prepared a particulate enzyme preparation from the intimal layer of the pig aorta, it catalyzed the transfer of mannose from mannosyl-phosphoryl-polyprenol (MPP) into a series of oligosaccharides that were linked to lipid. Chapman et al (1980) concluded at least two mannosyl donors are involved in the synthesis of lipid-linked oligosaccharides. GDP-mannose was the probable donor for the formation of the $Man_{1-5}GlcNAc_2$ species, while dolichol-P-mannose donates the 6^{th}mannosyl residue. Kornfeld (1980) worked on Thy $^{-1}$ mutant mouse lymphoma cells of the class E-complementation group and found that they lack GDP man: Dol-P mannosyltransferase and therefore are unable to interconvert GDP-Man and Dol-P- Man. Babczinski et al (1980) solubilized enzymes transferring mannose from GDP-mannose to dolichol phosphate and from dolicholposphomannose to protein. Spencer and Elbein (1980) solubilized mannosyltransferases by treatment of the pig aorta particulate enzyme with the non-ionic detergent followed by centrifugation .This enzyme preparation catalyzed the transfer of mannose from GDP-[^{14}C]mannose (but not from [^{14}C] mannosyl phosphoryl dolichol) to form a pentasaccharide-lipid. The synthesis of this heptasaccharide-lipid required the addition of an acceptor lipid that was isolated from pig liver. The oligosaccharide portion of the acceptor lipid appeared to be a mixture of trisaccharide and pentasaccharide. These data demonstrate that at least some of the alpha-linked mannose residues in the heptasaccharide-lipid were donated directly from GDP-mannose.

Purification and Solubilization of Mannosyltransferase

Lehle (1980) developed a new membrane preparation from *Saccharomyces cerevisiae*, which catalyzes the synthesis of large oligosaccharide lipids from GDP-Man and UDP-Glc following a detailed study of their formation and size, the major compound formed had the composition $(Man)_9(GlcNAc)_2$. Upon incubation with UDP-Glc, three oligosaccharides corresponding to the size of $(Glc)_{1-3}$ $(Man)_9$ $(GlcNAc)_2$ are formed. The oligosaccharides generated *in vitro* by yeast membrane appeared to be identical in size to the oligosaccharides found in the animal systems. Jensen and Schutbach (1981) purified a mannosyltransferase that catalyzes transfer from GDP-manose to tetrasaccharide pyrophosphoryl lipid from rabbit liver microsomes. Jensen and Schutbach (1982) reported the activation of mannosyltransferases II by phospholipid and presented evidence for the formation of the enzyme phospholipids complex. Although many membrane proteins do not require specific phospholipids for activity, maximal mannosyltransferase activity was obtained only in the presence of phosphatidylethanolamine containing unsaturated acyl chains. Sharma et al (1982) solubilized the three enzymes required for the first three reactions of the dolichol pathway from yeast membrane with detergents, the reactions required divalent metal ions. Substrate of enzyme I is Dol-P, for enzyme II it is Dol-P-GlcNAc and for enzyme III the substrate is Dol-P-P-chitobiose. Herscovics et al (1983) solubilized alpha (1'!3) mannosyltransferases from calf pancreas microsomes. Prakash et al (1984) presented in their communication, the solubilization of the enzyme that catalyzes the transfer of the first five mannose residues in the biosynthetic pathway. The enzyme that catalyzes the elongation of the tetrasaccharide to the heptasaccharide product was also studied in some detail. . Arcadia (1985) investigated the role of phospholipids in the activity of inner mitochondrial mannosyltransferases. Kaushal and Elbein (1986) solubilized from pig aorta microsomal preparations, the â-mannosyl transferase that adds mannose, from GDP-mannose, to GlcNAc-GlcNAc-pyrophosphoryl dolichol, to form Man-â-GlcNAc-GlcNAc-pyrophosphoryl dolichol . Hasselbeck (1989) modified the method of purification of the enzyme GDP-Man:Dol-P- mannosyltransferase to obtain better recovery and stability of the enzyme. Sharma et al. (1990) solubilized dolichyl-P-mannose : dolichyl-P-P-heptasaccharide alpha mannosylransferase which catalyzes the transfer of mannose from dolichyl-P-mannose to the $Man_5(GLc\ NAc)_2$-PP-Dol acceptor glycolipid, from pig aorta microsomes. Sharma et al (1991) partially purified the mannosyltransferase that catalyzes the transfer of mannose from dolichol phosphate mannose (Dol-P-Man) to the hydroxyl group of serine/threonine residues in the acceptor peptide (Tyr-Asn-Pro-Thr-Ser-Val) from the microsomal membrane fraction of *S. cerevisiae*. Strahl & Tanner(1991)

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solubilized enzyme Dol-phosphate-D-mannose : protein O-D mannosyl transferase from *S .cerevisiae* membrane and its enzyme activity demonstrated, using short peptides. The mannosyl transferase activity is influenced in transfer specificity by amino acid next to serine and threonine within the acceptor peptide. Bolsinger and Tanner (1991) described the purification and characterization of the membrane protein from *Saccharomyces cerevisiae* which catalyzes the reaction Dol-P-Man + protein'!protein-Man+Dol P. The solubilized enzyme shows sequence specificity *in vitro* for the peptides used as mannosyl acceptors.

Glycosylation at Serine and Threonine Residues

Rush et al (1993) investigated the specificity of Man-P-Dol:$Man_{5-8}GlcNAc_2$-P-P-Dol mannosyltransferase activity in pig brains by comparing a variety of mannosyl phosphoryl isoprenoids as mannosyl donors. He suggested that the saturated á - á-isoprene unit of dolichyl moiety is critical for the recognition of the lipophilic mannosyl donors by the ER-associated mannosyltransferases. The anomeric configuration of the mannosyl moiety is apparently essential because the brain mannosyltransferase exhibited a strong preference for â-Man-P-Dol over the corresponding chemically synthesized á-stereoisomers. Mudgapalli et al (1994) purified the enzyme mannosyltransferase which catalyzes the transfer of [^{14}C]Man from GDP-[^{14}C]Man to Man â1—>4GlcNAc â 1—>4GlcNAc-P-P-Dol in á1,3-linkage to give [^{14}C]Man á 1—>3Man â 1—>4GlcNAc â 1—>4GlcNAc-P-P-Dol as the product. Gentzsch et al (1995) investigated that protein O- mannosyltransferase Pmt 1 P and Pmt 2 P catalyze the O-glycosylation of serine and threonine residues in the ER of yeast. Deletion of each of these proteins by disruption of the corresponding gene leads to dramatic decrease in mannosyltransferase activity in vitro. Gentzsch and Tanner (1996) described that the transfer of mannose to seryl and threonyl residues of secretory protein is catalyzed by a family of protein mannosyltransferases coded for by seven genes (PMT 1-7). Sharma et al (2001) stated that mannosyltransfer from Dol-P-Man to [^{3}H] $Man_5GlcNAc_2$-PP-Dol with the formation of all intermediates up to $Man_9GlcNAc_2$-PP-Dol occurred in a rapid time and protein-dependent fashion. The Alg^3 mutant was described to accumulate $Man_5GlcNAc_2$-PP-Dol. Dotson et al (1995) in a report described a procedure for obtaining a highly stable, detergent–solubilized partially purified preparation of PMT1.The specificity of the partially purified preparation of PMT1 for the structural features of Man–P-Dol was examined by testing a variety of Man-P-polyisoprene as substrate. Girbach et al (2000) in their study performed a structural function analysis of ScPmt1P.They demonstrated that the N-terminal third of the protein is essential for ScPmt1P-ScPmt2P complex formation. Gentzch et al (1995) characterized a new protein mannosyltransferase activity which differed in part from the enzyme encoded by the PMT1 gene.

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