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Characterization of Lipid-Linked Octa- Through Undecasaccharides Implicated in the Biosynthesis of *Saccharomyces cerevisiae* Mannoproteins†

CHANDRA PRAKASH, ALBINE KATIAL, AND INDER K. VIJAY*
Department of Dairy Science, University of Maryland, College Park, Maryland 20742

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The lipid-linked oligosaccharide $Glc_3Man_9(GlcNAc)_2$ (Glc, glucose; Man, mannose; GlcNAc, *N*-acetylglucosamine) serves as a precursor for the biosynthesis of the inner core portion of the asparagine-linked polysaccharide of *Saccharomyces cerevisiae* mannoproteins. It has been shown previously that incubation of a microsomal preparation from this organism with UDP-*N*-acetylglucosamine and GDP-[^{14}C]mannose gives rise to a series of lipid-linked oligosaccharides of the general structure $Man_n(GlcNAc)_2$, with n from 1 to 9. A structural characterization of Man_1- to $Man_5(GlcNAc)_2$ oligosaccharides indicated that the major structures among these were identical to the intermediates proposed for the biosynthesis of animal glycoproteins (C. Prakash and I. K. Vijay, Biochemistry 21:4810–4818, 1982). In the present study, the structural characterization of the Man_6- through $Man_9(GlcNAc)_2$ species was conducted. The Man_6- through $Man_8(GlcNAc)_2$ species have two isomers, whereas $Man_9(GlcNAc)_2$ is monoisomeric. One isomer each of Man_6- through $Man_8(GlcNAc)_2$ and the monoisomeric $Man_9(GlcNAc)_2$ are identical to the intermediates for the biosynthesis of asparagine-linked glycoproteins in animal systems. It is proposed that the steps of the lipid-linked assembly of the carbohydrate precursor for *S. cerevisiae* mannoproteins are identical to those of the major pathway in animal systems. A lack of acceptor substrate specificity by the mannosyltransferases, as observed with in vitro studies with animal systems, also might be responsible for the biosynthesis of multiple isomers reported here.

Studies from several laboratories have established that the inner core of the asparagine-linked polymannose chains of the mannoproteins of *Saccharomyces cerevisiae* are synthesized analogously to the assembly of the carbohydrate unit of animal glycoproteins on a lipid (dolichol) carrier and then are transferred en bloc as the oligosaccharide $Glc_3Man_9GlcNAc_2$ (Glc, glucose; Man, mannose; GlcNAc, *N*-acetylglucosamine) (6, 11, 16, 17). Subsequently, all of the glucose and at least one mannose residue are removed before or concomitant with the transfer of mannose residues, directly from GDP-mannose, for the construction of the outer region (1, 6, 9–11). An elegant structural analysis led Nakajima and Ballou (8) to propose that the inner core of *S. cerevisiae* mannan consists of approximately 12 to 17 mannose residues linked at the reducing terminus to an *N,N'*-diacetylchitobiose unit. A somewhat similar region of 150 or more mannose residues, constituting the outer portion, is attached at the nonreducing end of the inner core. Except for the first mannose residue, which is linked β1,4 to the *N,N'*-diacetylchitobiose, the entire polymannose component is built on an α1,6-linked backbone. Short branches of one to four mannose units having α1,2 and α1,3 linkages are attached to the backbone. Additionally, the outer region also has occasional mannosyl and mannobiosyl phosphate side chains joined to the primary branches (1).

Recently, the structure of the carbohydrate units of many asparagine-linked animal glycoproteins (5) and the major pathway for the lipid-linked assembly of the precursor oligosaccharide for these glycoproteins have been elucidated (2, 18-20, 22). From these studies, it appears that the inner core of *S. cerevisiae* mannoproteins is similar, if not quite the same, as the high-mannose units of animal glycoproteins. There have been no details for the sequential assembly of the lipid-linked precursor $Glc_3Man_9GlcNAc_2$ for the biosynthesis of the

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inner core of *S. cerevisiae*. We therefore undertook to study this process. In a previous report (12), it was shown that the major lipid-linked intermediates up to the heptasaccharide, $Man_5(GlcNAc)_2$, synthesized by a membrane preparation were identical to the intermediates for the biosynthesis of animal glycoproteins. In this communication, we have extended this work to include a structural investigation of lipid-linked octa- through undecasaccharides belonging to the general formula $Man_n(GlcNAc)_2$, with n from 6 to 9. Two major isomers are present in the octa-, nona-, and decasaccharides, whereas undecasaccharide is monoisomeric. One of the isomers of the octa-, nona-, and decasaccharides and the undecasaccharide are identical to the intermediates in the animal systems.

MATERIALS AND METHODS

Organism and growth conditions. The following *S. cerevisiae* strains were used: wild type X-2180-1B(α) (obtained from Yeast Genetics Stock Center, University of California, Berkeley) and ATCC 24297 (isolated from Fleischmann's brand of baker's yeast). The organisms were grown at 25°C to early-logarithmic phase in 2% glucose–2% Bacto-Peptone (Difco Laboratories)–0.5% yeast extract. Slants of the organisms were maintained on the same medium containing 2% agar.

The particulate enzyme fraction was prepared according to the procedure of Lehle (6).

Other materials and methods, including the sources of oligosaccharide standards, enzymatic digestions, chromatographic procedures, and acetolysis, have been documented in detail in earlier reports (18–20, 22). The following description pertains to the preparation of oligosaccharides investigated for structural analysis in this study.

Octa-, nona-, deca-, and undecasaccharides. The [^{14}C]mannose-labeled octa-, nona-, deca-, and undecasaccharides were prepared from a large scale incubation of the yeast membranes as described previously (12). Briefly, 300 mg of the membrane fraction, 40 mM Tris-hydrochloride, pH 7.4, 150 mM NaCl, 8 mM $MgCl_2$, 0.1 mM UDP-GlcNAc, and 12 μCi of GDP-[^{14}C]mannose in a total volume of 30 ml were incubated at 37°C for 20 min. After quenching the reaction with $CHCl_3CH_3OH$ (2:1), further processing of the reaction mixture by multiple solvent extraction, mild acid hydrolysis, and purifications by combinations of repeated paper and gel filtration chromatography were performed as described previously. The octa- through undecasaccharides reported here correspond to radioactive peaks that cochromatographed with Man_6-, Man_7-, Man_8-, and $Man_9(GlcNAc)_2$ standards upon paper and gel filtration chromatography (compare Fig. 2B, reference 12, with Fig. 5B, reference 22).

RESULTS

General characteristics of octa- through undecasaccharides. To rule out any possibility of strain-to-strain differences in the biosynthetic pathway that could potentially be exhibited in the final structure of the asparagine-linked polymannose unit mannoproteins, we analyzed the lipid-linked oligosaccharides synthesized by strains X-2180-1B(α) and ATCC 24297. Of the two, strain X-2180-1B(α) is the same one used by Nakajima and Ballou (8) for structural studies of its mannan. Strain ATCC 24297 is the commonly employed baker's yeast and was included for comparison. Earlier, it had been shown that the structure of lipid-linked intermediates up to the heptasaccharide synthesized by the two strains were essentially similar (12). Since identical results were obtained for the higher intermediates reported here, only data on the glycolipids synthesized by the strain X-2180-1B(α) are reported below.

When treated with jack bean α-mannosidase, each [^{14}C]mannose-labeled octa- through undecasaccharide gave mannose and a trisaccharide that cochromatographed with a $Man^{\beta 1,4}\rightarrow GlcNAc^{\beta 1,4}\rightarrow GlcNAc$ standard as the only products (Fig. 1). The trisaccharide was resistant to acetolysis, indicating the absence of any 1,6 linkages in this portion of the molecule. The trisaccharide was susceptible to cleavage by endo-β-*N*-acetylglucosaminidase L and yielded a disaccharide that cochromatographed with a $Man^{\beta 1,4}\rightarrow GlcNAc$ marker. Upon digestion with β-mannosidase, the disaccharide gave radioactive mannose as the labeled product. On the basis of these results and earlier studies with the lower lipid-linked oligosaccharides from both *S. cerevisiae* (12) and lactating bovine mammary tissue (18–20, 22), the overall structures of the octa- through undecasaccharide would be $Man_{5\text{-}8}{}^{\alpha}\rightarrow Man^{\beta}\rightarrow GlcNAc^{\beta}\rightarrow GlcNAc$.

Due to endogenous unlabeled lipid-linked oligosaccharides in the membrane preparation, the labeling of different mannosyl residues within each oligosaccharide was not uniform. This was evident from the α-mannosidase results given above, since the percent distribution of the label in mannose and the trisaccharide was not consistent with uniform distribution of radioactivity. It was also illustrated by detailed structural analysis, including methylation, in a previous study on the lipid-linked intermediates up to the heptasaccharide (12). The lack of even distribution of the label gets progressively pronounced as the degree of polymerization of the oligosaccharide increases, with maximum concentration of the label occurring in the outermost residues within each oligosaccharide, as these are added onto the preexisting endogenous unlabeled oligosaccharides. We therefore utilized the specificity of α1,2-mannosidase from *Aspergillus saitoi* to cleave only α1,2-linked mannose residues within

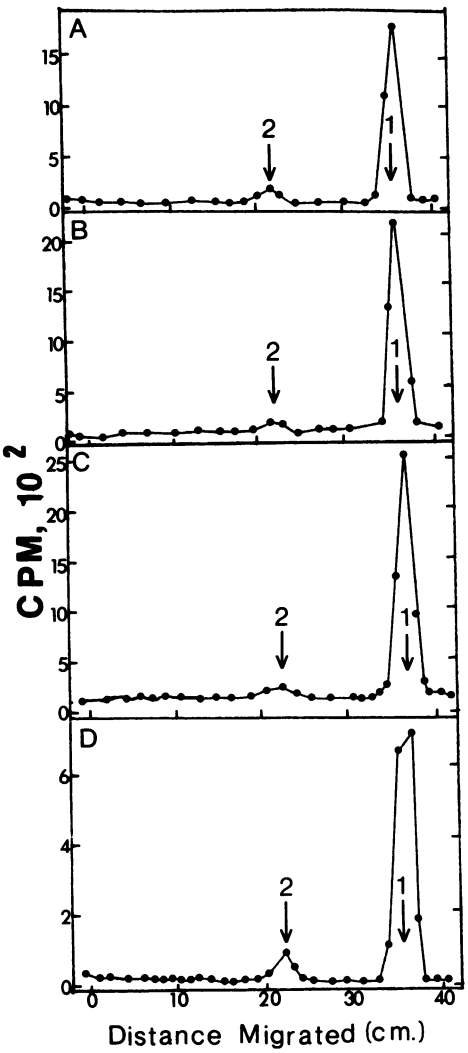

FIG. 1. Products of α-mannosidase digestion of [^{14}C]mannose-labeled octa- (A), nona- (B), deca- (C), and undecasaccharide (D). Paper chromatograms were developed in ethyl acetate-pyridine-acetic acid-water (5:5:1:3) for 24 h. Arrows indicate the positions of standards: 1, mannose; 2, Manβ1→4(3)GlcNAcβ1 →4(3)GlcNAc.

the oligosaccharides and aid in the structural analysis.

All of the oligosaccharides characterized in this study were resistant to the action of endo-β-*N*-acetylglucosaminidase D. Since a prime requirement for the cleavage by this enzyme of the *N*,*N*′-diacetylchitobiose linkage in the $Man_n \xrightarrow{\alpha} Man \xrightarrow{\beta} GlcNAc \xrightarrow{\beta} GlcNAc$ type of substrates is the presence of a free mannose linked α1,3 to the β-mannose (15), it would appear that such a residue is further substituted with additional mannose residues. This would also be predicted by the structure of the major lipid-linked heptasaccharide, a presumed precursor of the oligosaccharides analyzed here, as Manα1→2Manα1 →2Manα1→3(Manα1→6)Manβ1→4(3)GlcNAcβ1 →4(3)GlcNAc (12).

Characterization of [^{14}C]mannose-labeled octasaccharide. Upon digestion with endo-β-*N*-acetylglucosaminidase H, ~70% of the [^{14}C]mannose-labeled octasaccharide was cleaved to yield a product that migrated as a GlcNAc unit smaller in size than the starting material (Fig. 2A); this was also checked on a calibrated column of BioGel P-4 (22). The resistant octasaccharide did not undergo cleavage when redigested with endo-β-*N*-acetylglucosaminidase H. Treatment of [^{14}C]mannose-labeled octasaccharide with α1,2-mannosidase yielded radioactivity in products that cochromatographed with mannose and $Man_4(GlcNAc)_2$ markers (Fig. 3A). Both the endo-β-*N*-acetylglucosaminidase H-cleaved (presumably $Man_6GlcNAc$) and the endo-β-*N*-acetylglucosaminidase H-resistant [^{14}C]mannose-labeled octasaccharide were reduced with $NaBH_4$ and subjected to acetolysis. The former gave mannobiose and $Man_4GlcNAc_{OH}$ ($GlcNAc_{OH}$, *N*-acetylglucosaminitol) as the major products of degradation along with the original, undegraded starting material (Fig. 4A). The slight amount of mannose and mannotriose present were probably due to overdegradation. The reduced, endo-β-*N*-acetylglucosaminidase H-resistant, [^{14}C]mannose-labeled octasaccharide gave products that cochromatographed with mannose and the Manα1→2Manα1→2Manα1 → 3Manβ1 → 4(3)GlcNAcβ1,4(3) → $GlcNAc_{OH}$ markers (Fig. 4B).

On the basis of the results given above, an earlier characterization of the lower lipid-linked oligosaccharides, and the major intermediates characterized for the lipid-linked assembly of the carbohydrate precursor for asparagine-linked glycoproteins in animal systems, the following structures may be proposed for the two isomers of octasaccharide: Manα1→ 2Manα1 → 2Manα1 → 3(Manα1 → 3Manα1 → 6)Manβ1,4(3)GlcNAcβ1,4(3)GlcNAc (the endo-β-*N*-acetylglucosaminidase H-susceptible fraction, saccharide VIII-1), and Manα1→2Manα1 →2Manα1→3(Manα1→6Manα1→6) Manβ1→ 4(3)GlcNAcβ1→4(3)GlcNAc (the endo-β-*N*-acetylglucosaminidase H-resistant fraction, saccharide VIII-2). Of these, saccharide VIII-1 is the major intermediate for the lipid-linked assembly of the precursor oligosaccharide for the biosynthesis of animal glycoproteins (2, 20). Saccharide VIII-2 is most likely derived by the addition of an α1,6-linked mannose to the lipid-

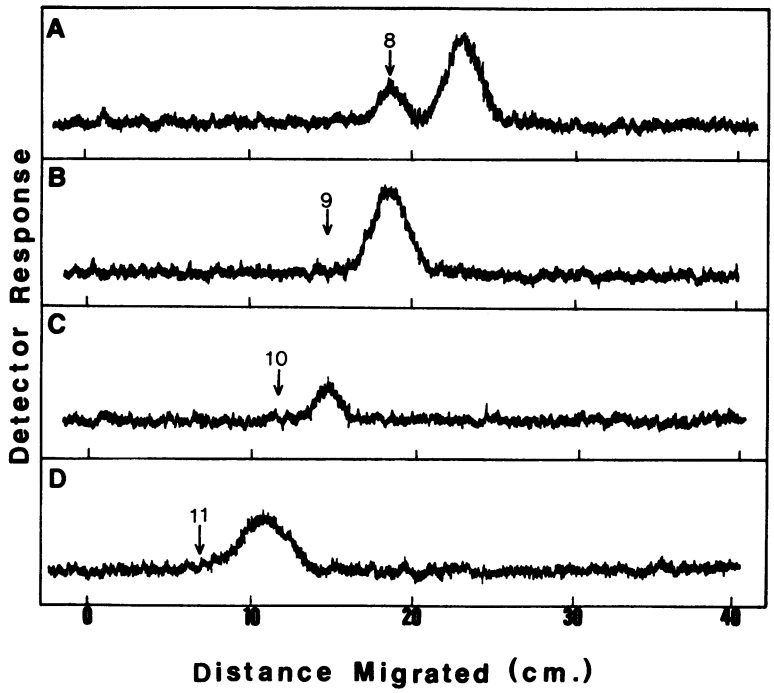

FIG. 2. Paper chromatography of the products obtained upon the digestion of [^{14}C]mannose-labeled octa- (A), nona- (B), deca- (C), and undecasaccharide (D) with endo-β-*N*-acetylglucosaminidase H. The chromatograms were developed in butanol-pyridine-water (4:3:4) for 88 h. The standards shown are: 8, $Man_6(GlcNAc)_2$; 9, $Man_7(GlcNAc)_2$; 10, $Man_8(GlcNAc)_2$; 11, $Man_9(GlcNAc)_2$.

linked mannose to the lipid-linked heptasaccharide Manα1→2 Manα1→2 Manα1→3 (Manα1→6) Manβ1→4 (3) GlcNAcβ1→4 (3) GlcNAc, previously characterized as an intermediate for the biosynthesis of *S. cerevisiae* mannoproteins (12) and animal glycoproteins (2, 22). Since this structure lacks a mannose residue that is linked α1,3 to the mannose residue that is further linked α1,6 to the β-mannose residue, it would not be expected to be susceptible to the action of endo-β-*N*-acetylglucosaminidase H (14, 22).

Characterization of [^{14}C]mannose-labeled nonasaccharide. [^{14}C]mannose-labeled nonasaccharide was fully susceptible to cleavage by endo-β-*N*-acetylglucosaminidase H and yielded a product that chromatographed a GlcNAc unit smaller in size than the starting material (Fig. 2B). Upon digestion with α1,2-mannosidase (Fig. 3B) it gave mannose and two oligosaccharides that cochromatographed with Manα-1→3Manα1→6 (Manα1→3) Manβ1→4 (3) GlcNAcβ1→4 (3) GlcNAc and Manα1→6 (Manα1→3) Manα1→6 (Manα1→3) Manβ1→4 (3) GlcNAcβ1→4 (3) GlcNAc markers. The endo-β-*N*-acetylglucosaminidase H-cleaved, [^{14}C]mannose-labeled nonasaccharide was reduced with $NaBH_4$ and subjected to acetolysis. It gave mannose, mannobiose, mannotriose, and $Man_4GlcNAcOH$ as the major products (Fig. 4C).

The above data are consistent with the following proposed structures for the two isomers in the nonasaccharide: Manα1→2Manα-1→2Manα1→3(*Manα1→2Manα1→3Manα1→6) Manβ1 → 4(3)GlcNAcβ1 → 4(3)GlcNAc (saccharide IX-1) and Manα1→2Manα1→2Manα-1→3 (Manα1 → 6 [* * Manα1 → 3] Manα1 → 6) Manβ1→4 (3) GlcNAcβ1→4 (3) GlcNAc (saccharide IX-2). Saccharide IX-1 could be derived by an α1,2 addition of a *Man residue to saccharide VIII-1. It is also the major intermediate for the assembly of the precursor oligosaccharide in animal systems (2, 20). Saccharide IX-2 is presumably synthesized by the transfer

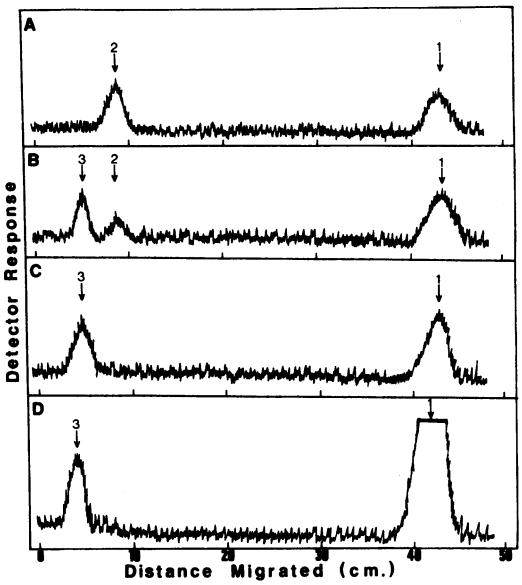

FIG. 3. Profile of the products obtained by α1,2-mannosidase digestion of [^{14}C]mannose-labeled octa- (A), nona- (B), deca- (C), and undecasaccharide (D). The conditions of chromatography were the same as those described in the legend to Fig. 1. The arrows indicate the migration positions of the standards: 1, mannose; 2, $Man_4(GlcNAc)_2$ of the structure Manα1 → 3Manα1 → 6(Manα1 → 3)Manβ1 → 4(3)GlcNAcβ1 → 4(3)GlcNAc; 3, $Man_5(GlcNAc)_2$ of the structure Manα1 → 6(Manα1 → 3)Manα1 → 6(Manα1 → 3)Manβ1 → 4(3)GlcNAcβ1 → 4(3)GlcNAc (21).

Characterization of [^{14}C]mannose-labeled decasaccharide. [^{14}C]mannose-labeled decasaccharide was totally susceptible to the action of endo-β-*N*-acetylglucosaminidase H and gave a product with the chromatographic characteristics of $Man_8GlcNAc$ (Fig. 2C). Upon digestion with α1,2-mannosidase it gave mannose and an oligosaccharide that comigrated with a $Man_5GlcNAc_2$ standard (Fig. 3C). The $Man_8GlcNAc$ product obtained after digestion of [^{14}C]mannose-labeled decasaccharide with endo-β-*N*-acetylglucosaminidase H was reduced with $NaBH_4$ and subjected to acetolysis. It gave mannose, mannobiose, mannotriose, and $Man_4GlcNAcOH$ along with undegraded $Man_8GlcNAcOH$ as the major products (Fig. 4D).

It appears that the decasaccharide also has two isomers, viz., Manα1 → 2Manα1 → 2Manα1 → 3(*Manα1 → 6[Manα1 → 2Manα1 → 3]Manα1 → 6)Manβ1 → 4(3)GlcNAcβ1 → 4(3)GlcNAc (saccharide X-1) and Manα1 → 2Manα1 → 2Manα1 → 3(**Manα1 → 2Manα1 → 6[Manα1 → 3]Manα1 → 6)Manβ1 → 4(3)GlcNAcβ1 → 4(3)GlcNAc (saccharide X-2). Saccharide X-1 is the major intermediate for the

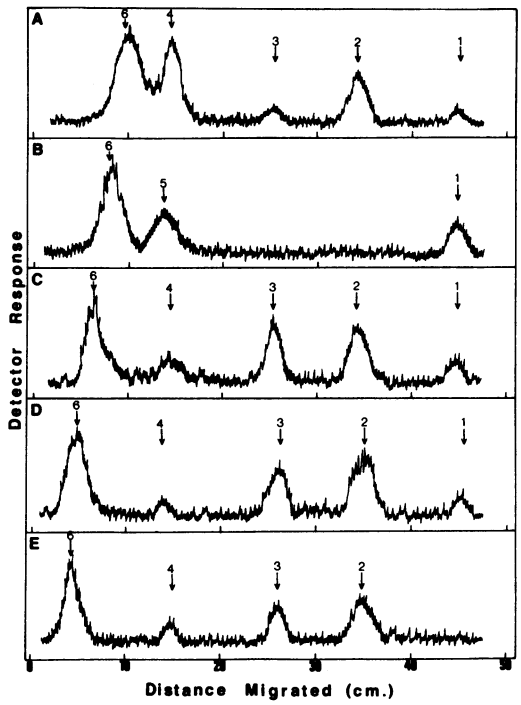

FIG. 4. Products obtained upon acetolysis of [^{14}C]mannose-labeled oligosaccharides. Panels A, C, D, and E are the fragmentation patterns obtained from endo-β-*N*-acetylglucosaminidase H-cleaved and $NaBH_4$-reduced octa-, nona-, deca-, and undecasaccharide, respectively. Panel B represents the profile of products obtained from endo-β-*N*-acetylglucosaminidase H-resistant and $NaBH_4$-reduced octasaccharide. Paper chromatograms were developed in ethyl acetate-pyridine-acetic acid-water (5:5:1:3) for 33 h. The migration of standards is shown as: 1, mannose; 2, mannobiose; 3, mannotriose; 4, Manα1→2Manα1→ 2Manα1 → 3Manβ1 → 4GlcNAcOH; 5, Manα1 → 2Manα1 → 2Manα1 → 3Manβ1 → 4(3)GlcNAcβ1 →4(3)GlcNAcOH. The arrow under 6 represents the undegraded starting material in all of the panels.

major intermediate for the lipid-linked assembly of the precursor oligosaccharide for the biosynthesis of asparagine-linked glycoproteins in animal systems (2, 20). Saccharides X-1 and X-2 appear to result from α1,6-transfer of *Man to saccharide IX-1 and α1,2-transfer of **Man to saccharide IX-2, respectively.

Characterization of [^{14}C]mannose-labeled undecasaccharide. [^{14}C]mannose-labeled undecasaccharide was fully susceptible to cleavage by endo-β-*N*-acetylglucosaminidase H and gave a product that migrated as $Man_9GlcNAc$ upon paper chromatography (Fig. 2D). Upon treatment with α1,2-mannosidase, it released mannose and an oligosaccharide equivalent to $Man_5GlcNAc_2$ (Fig. 3D). The $Man_9GlcNAc$ fragment obtained after endo-β-*N*-acetylglucosaminidase H digestion of undecasaccharide was reduced with $NaBH_4$ and subjected to acetolysis. It yielded mannobiose, mannotriose, $Man_4GlcNAcOH$ and the undegraded $Man_9GlcNAcOH$ as the major products of the reaction (Fig. 4E).

All of the evidence for structural analysis can be combined to propose the following structure for undecasaccharide: Manα1→2Manα1→2 Manα1→3 (Manα1→2Manα1→6 [Manα1→2 Manα1→3] Manα1→6) Manβ1→4 (3) GlcNAc-β1→4 (3) GlcNAc. This structure is identical to the lipid-linked intermediate synthesized by the membrane preparation from lactating bovine mammary tissue (22).

DISCUSSION

From the results of this study and previous work (12), it appears that the major reaction for the lipid-linked assembly of the carbohydrate unit for attachment to the asparagine residues in *S. cerevisiae* mannoproteins and animal glycoproteins is quite similar. All of the major isomers up to the heptasaccharide, one of the isomers in each of the octa-, nona-, and decasaccharides, and the monoisomeric undecasaccharide are common to both of the systems. Because of the non-uniformity of the labeling of mannosyl residues within these intermediates, caused by the presence of endogenous lipid-linked oligosaccharides at various stages of assembly, it is difficult to draw a definitive conclusion regarding the precise relative proportion of each isomer within the octa-, nona-, and decasaccharides. Multiple isomers have also been observed during in vitro assembly of the lipid-linked intermediates in animal systems (13, 18–20). Supplementation of in vitro incubations of bovine mammary membranes with dolichol phosphate (18, 20) to drive the biosynthetic process significantly reduced the number of isomers in favor of an ordered sequence similar to the major in vivo pathway in Chinese hamster ovary cells (12). However, inclusion of Triton X-100- or Nonidet P-40-dispersed dolichol phosphate failed to stimulate the biosynthesis of lipid-linked oligosaccharides by *S. cerevisiae* membranes, even though it caused a severalfold increase in the biosynthesis of mannosylphosphoryldolichol (not shown). Possibly, the detergents Triton X-100 and Nonidet P-40 are inhibitory to one or more mannosyl transferases in the yeasts. In results not reported here, we were also unable to detect any α-mannosidase activity in *S. cerevisiae* membranes under conditions that were employed for the biosynthesis of lipid-linked oligosaccharides characterized here by using [^{14}C]mannose-labeled $Man_9(GlcNAc)_2$ or characterized in a previous study (22), or by using a mixture of [^{14}C]mannose-labeled lipid-linked tetra- through undecasaccharides and monitoring for the release of [^{14}C]mannose. If the mannosyltransferases for the assembly of the intermediates are not very specific for the acceptor substrates, as has been reported with lymphoma membranes (13), then these enzymes could catalyze the biosynthesis of multiple products. The monoisomeric nature of lipid-linked undecasaccharide, as well as the major isomers in the lower intermediates reported here and in the systems of animal membranes, indicate that there is a certain degree of directed or ordered assembly even in the in vitro systems.

Based on the structure of the undecasaccharide reported here and considering that the lipid-linked precursor for the wild type as well as the *mnn1* and *mnn2* mutants were shown to have the same overall structure, i.e., $Glc_3Man_9(GlcNAc)_2$ (6, 9, 11), it would appear that the structure of the glucosylated oligosaccharide intermediates in *S. cerevisiae* may be identical to the same intermediates in animal systems. However, this remains to be established.

A major assumption in the analysis reported here concerns the presence of α1,3 linkages within the oligosaccharides. This is based on the relative stability of these linkages to cleavage by acetolysis, a technique that preferentially clips 1,6 linkages (1), and the resistance of these linkages to digestion by α1,2-specific mannosidase. The assignment of α1,3 linkages instead of the only other possibility, i.e., α1,4 linkages for pyranosyl residues, is based on the overall structure of *S. cerevisiae* mannan characterized by Nakajima and Ballou (7), the similar presence and location of these linkages in the lipid-linked oligosaccharides (2, 18–20, 22), and the high-mannose glycoproteins in animal systems (5).

The extremely high degree of nonuniform labeling of the oligosaccharides prevented any meaningful analysis of these oligosaccharides by methylation.

It should be noted that the structure of the lipid-linked undecasaccharide reported here is fully consistent with the structure of the inner core of the double mutant *mnn1 mnn2* recently characterized by Cohen et al. (3) and reported as Manα1→6 (Manα1→2) Manα1→6 (‡Manα1→3) Manα1 → 6(Manα1 → 2Manα1 → 2Manα1 → 3)Manβ1→4GlcNAc. The outermost α1,6-linked mannose residue on this structure probably represents the mannose residue left uncleaved by endo-α1,6-mannanase (employed along with endo-β-*N*-acetylglucosaminidase to obtain the core), probably due to steric hindrance from the branched core. Then the only difference between this inner core and the undecasaccharide characterized in this study is the lack of a mannose, presumably trimmed off during processing, linked α1,2 to the ‡Man. Our linkage assignments are also compatible with the interpretation of Cohen and co-workers (3) that the terminal α1,3-linked mannose units are most likely transferred to the core while it is attached to the protein component.

A previous characterization of the lipid-linked precursor $Glc_3Man_9GlcNAc_2$ (4) involved in the biosynthesis of plant glycoproteins has indicated that it is also structurally similar to the precursor for animal glycoproteins. It would then appear that the lipid-linked assembly pathway for the biosynthesis of precursor oligosaccharide for asparagine-linked glycoproteins has been well conserved during evolution. Since the biosynthesis of asparagine-linked glycoproteins is so ubiquitous throughout the phylogeny and since these macromolecules play fundamental roles in the structure and function of eucaryotic cells, it is possible that a mutation that causes deletion of the lipid-linked pathway for the assembly of the carbohydrate unit would be lethal to the cell.

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