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α-Mannosidase-Catalyzed Trimming of High-Mannose Glycans in Noninfected and Baculovirus-Infected *Spodoptera frugiperda* Cells (IPLB-SF-21AE). A Possible Contributing Regulatory Mechanism for Assembly of Complex-Type Oligosaccharides in Infected Cells†

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ABSTRACT: Incubation of a *Spodoptera frugiperda* (IPLB-SF-21AE) cell extract with the oligosaccharide $Man_9GlcNAc_2$, the aglucosyl derivative of the glycan that is normally transferred from the dolichol carrier to the relevant Asn residue in the nascent protein, results in its trimming to $Man_6GlcNAc_2$, an intermediate that is relatively stable to further α-D-mannosidase action in these cells. On the other hand, incubation of a similar extract from cells that had been infected for various times with a wild-type baculovirus (*Autographa californica* nuclear polyhedrosis virus) or a recombinant baculovirus (r-BAC)/human plasminogen (HPg) construct employed for expression of HPg led to rapid trimming of $Man_6GlcNAc_2$ to $Man_5GlcNAc_2$ and $Man_3GlcNAc_2$. These latter reactions displayed temporal effects, in that an enhancement of this latter trimming process occurred as a function of the time of infection of the cells with the wild-type and recombinant viral constructs. We have previously demonstrated that the nature of the oligosaccharide assembled on Asn^{289} of HPg expressed in several lepidopteran insect cell lines was dependent on the time of infection of the cells with r-BAC/HPg and that the amount of complex glycan found on this recombinant protein increased with an increase in infection times [Davidson, D. J., & Castellino, F. J. (1991) *Biochemistry 30*, 6167–6174]. A significant contributing explanation for these findings is provided herein, in that the infective process leads to an enhancement of an α-D-mannosidase activity that catalyzes trimming of the $Man_6GlcNAc_2$ that accumulates in noninfected cells, to $Man_5GlcNAc_2$, the preferred substrate for GlcNAc transferase I. This latter process is requisite for further processing of high-mannose oligosaccharide to complex and hybrid glycans. The control of $Man_6GlcNAc_2$ trimming is one potentially important determinant as to whether complex and/or hybrid glycans will be assembled on glycoproteins.

It is now generally accepted that N-linked protein glycosylation is initiated in the rough ER by the transfer of the oligosaccharide $Glc_3Man_9GlcNAc_2$[1] from a dolichol phosphate carrier to an Asn residue contained in a tripeptide consensus sequence of AsnXxxSer/Thr (Hubbard & Ivatt, 1981). After this, a series of glucosidase- and mannosidase-catalyzed trimming reactions occurs in the ER and Golgi complex, followed by further processing catalyzed by a variety of glycosyl transferases, ultimately yielding the mature glycoprotein (Kornfeld & Kornfeld, 1985). The committed step in complex or hybrid oligosaccharide formation is the GlcNAc transferase I catalyzed addition of a residue of GlcNAc to the Man residue linked (α1,3) to the (β1,4)-linked core Man residue on the $Man_5GlcNAc_2$–protein complex that remains after trimming

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[1] Abbreviations: HPg, any form or variant of human plasminogen; r, recombinant; Glc, glucose; GlcNAc, *N*-acetylglucosamine; Man, mannose; pi, postinfection; HPAEC, high-pH anion-exchange chromatography; ER, endoplasmic reticulum; AcMNPV, wild-type *Autographa californica* nuclear polyhedrosis virus; r-BAC/HPg, a recombinant baculovirus containing the human plasminogen cDNA placed under control of the polyhedrin promoter; MOI, multiplicity of infection, DMJ, 1-deoxymannojirimycin.

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of four (α1,2)Man residues from the original $Man_9GlcNAc_2$–protein complex (Tabas & Kornfeld, 1978). The action of mannosidase II on the resulting glycan results in removal of the two remaining (α1,3)- and (α1,6)-linked terminal noncore Man residues, thereby circumventing hybrid oligosaccharide formation and allowing GlcNAc transferase II to function in initiating additional complex oligosaccharide assembly on the (α1,6)-linked core Man residue (Tabas & Kornfeld, 1978).

From these considerations, it is clear that availability of both trimming mannosidases and processing glycosyl transferases is essential for formation of complex oligosaccharides. We have previously demonstrated that lepidopteran insect cells are capable of assembling N-linked complex-type oligosaccharide on the protein HPg when a recombinant baculovirus containing the cDNA for this protein is employed to infect these cells (Davidson et al., 1990; Davidson & Castellino, 1991b,c). Asn-linked complex glycans had not been previously found on glycoproteins derived from such cell types (Luckow & Summers, 1988). It was further demonstrated that the nature of the glycans assembled on insect-expressed r-HPg was dependent on the time of infection of the cells with the r-BAC/HPg, suggesting that availability of relevant trimming and/or processing enzymes was dependent on postinfective temporal events (Davidson & Castellino, 1991b). Since the first important determinant of N-linked complex oligosaccharide formation involves trimming of high-mannose structures by α-D-mannosidase I like enzymes, we have initiated an investigation of these activities in infected *Spodoptera frugiperda* (IPLB-SF-21AE) cells, in order to evaluate whether the presence of such enzyme activities was consistent with experimental findings of the presence of N-linked complex oligosaccharides on r-HPg produced by cells infected in this manner. This paper is a summary of the results of this study.

Materials and Methods

Preparation of Cell Extracts. IPLB-SF-21AE cells (Vaughn et al., 1977) were removed from maintenance spinner cultures and added to cell culture flasks (150 cm^2). Approximately 1×10^7 cells were incubated with 20 mL of serum-free Excell 400 medium (JR Scientific, Woodland, CA). A total of nine independent flasks was established under these conditions, three for uninfected cells and another six for infections. Two hours later, the medium was replaced with fresh medium in all flasks (zero time). Next, the cells from three flasks were infected with AcMNPV at a MOI of 4, and another three flasks were infected in the same manner with r-BAC/HPg (Whitefleet-Smith et al., 1989). The final three flasks served as uninfected controls. At times of 24, 60, and 96 h pi, cells were detached from the flasks by gentle agitation and a total of at least 1×10^6 cells was removed from the infected flasks and the parallel uninfected controls.

For cell disruption, the cells were pelleted by centrifugation and approximately 50–100 μL of a buffer consisting of 50 mM sodium phosphate, pH 7.4, or 50 mM sodium citrate, pH 6.0, was added. The total volume at this point was approximately 250–350 μL. Exact cell counts were then obtained on each suspension. The cells were mechanically disrupted at 4 °C by employing a Potter-Elvehjem homogenizer, and the cell debris was removed by centrifugation at 13600*g* for 2 min. The resulting supernate was retained as the cell extract. Microscopic analysis of the disrupted cells, prior to centrifugation, demonstrated that at least 95% of the cells were broken. The above buffer was then added to each cell extract to adjust the concentration, in terms of the original cell density, to 1×10^6 cells/mL.

Assay of Mannosidase Activity of IPLB-SF-21AE Cell Extracts. A quantity of 5 μL of each cell extract was diluted to 30 μL with either of the above buffers and incubated at 25 °C with 120 μL of high-mannose substrate, viz., Man(α1,2)Man(α1,2)Man(α1,3)[[Man(α1,2)Man(α1,3)[Man(α1,2)Man(α1,6)]Man(α1,6)]]Man(β1,4)GlcNAc(β1,4)-GlcNAc ($Man_9GlcNAc_2$) or Man(α1,2)Man(α1,3)[[Man(α1,3)[Man(α1,6)]Man(α1,6)]]Man(β1,4)GlcNAc(β1,4)-GlcNAc ($Man_6GlcNAc_2$), dissolved in water (30 μM, final concentration). Samples (25 μL) were removed at various times, ranging from 30 min to 12 h, and flash-frozen with liquid N_2 for later analysis.

For evaluation of the action of the α-D-mannosidase activity of the cell extracts on these substrates, the samples were thawed and immediately subjected to quantitative oligosaccharide mapping by HPAEC, according to methodology described earlier (Davidson et al., 1990). Briefly, the samples were added to a Carbopac PA1 column (4 mm × 250 mm), equilibrated with a mixture (v/v) of 50% solvent A (200 mM NaOH)/47% solvent B (H_2O)/3% solvent C (1 M NaOAc). This same mixture was applied for 15 min, after which a linear gradient of the equilibration solvent (start solvent) to 50% A/25% B/25% C (limit solvent) was employed up to 35 min. The limit solvent was then continued for an additional 15 min. The flow rate was 1 mL/min at room temperature.

Identifications of the oligosaccharides were made by comparing the HPAEC elution times of the sample peaks with a library of standard high-mannose-type oligosaccharides. The amount of each glycan in the samples was determined from dose–response curves with the appropriate standard.

All oligosaccharides were purchased from the Dionex Corporation (Sunnyvale, CA) or Oxford GlycoSystems (Rosedale, NY).

Inhibition of Mannosidase Activity of the Cell Extracts with DMJ. A quantity of 5 μL of the 60-h-pi AcMNPV cell extract was incubated with 1 μL (0.1 μg) of DMJ for 5 min at room temperature. This mixture was then added to a solution containing 1 μg of $Man_6GlcNAc_2$ in 20 μL of 50 mM sodium citrate, pH 6.0. After incubation for 15 min, 20 μL of the solution was subjected to HPAEC to quantitate the remaining concentration of $Man_6GlcNAc_2$. A duplicate sample from an incubation not containing DMJ was also analyzed.

Analytical Techniques. All methodology employed for oligosaccharide determinations, including oligosaccharide mapping by alkaline HPAEC, monosaccharide analyses after complete enzymatic digestion of oligosaccharides, and sequential exoglycosidase digestions, has been described previously (Davidson et al., 1990; Davidson & Castellino, 1991a–c). Acetolysis was conducted as described (Bischoff et al., 1986). Separation of components from the acetolysis mixtures was accomplished on HPAEC.

Results

A cell extract obtained from uninfected IPLB-SF-21AE cells, plated for 60 h from a spinner flask maintenance culture, was incubated with $Man_9GlcNAc_2$ for times up to 12 h and the mixture was subjected to analysis on HPAEC. After 6–8 h of incubation at pH 7.4, this substrate was completely converted to a product that comigrated with $Man_6GlcNAc_2$, which was relatively stable to further degradation under these conditions. On the other hand, the same amount of extract from an identical number of cells that had been infected for 60 h with r-BAC/HPg, when added to $Man_9GlcNAc_2$, resulted in its more rapid conversion to products that comigrated on HPAEC with the standard glycans, Man(α1,3)[[Man(α1,3)[Man(α1,6)]Man(α1,6)]]Man(β1,4)GlcNAc(β1,4)-

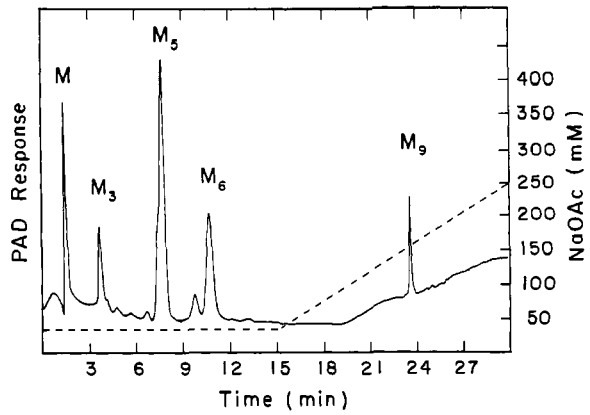
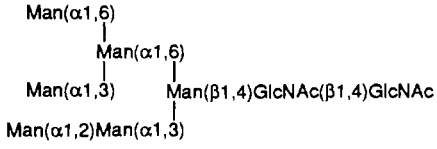


FIGURE 1: HPAEC profile of the reaction products of $Man_9GlcNAc_2$ with IPLB-SF-21AE cell extracts. The example provided here is for the extract from cells infected for 60 h with r-BAC/HPg. The cell extract was incubated for 6 h with 30 μM $Man_9GlcNAc_2$. The resulting sample was applied to a Carbopac PA1 column (4 mm × 250 mm). Elution was accomplished with a gradient of NaOAc, as indicated on the right ordinate, in 100 mM NaOH. The peaks are labeled as follows; M, free mannose; M_3, Man(α1,3)[Man(α1,6)]-Man(β1,4)GlcNAc(β1,4)GlcNAc; M_5, Man(α1,3)[[Man(α1,3)-[Man(α1,6)]Man(α1,6)]]Man(β1,4)GlcNAc(β1,4)GlcNAc; M_6, Man(α1,2)Man(α1,3)[[Man(α1,3)[Man(α1,6)]Man(α1,6)]]Man-(β1,4)GlcNAc(β1,4)GlcNAc; Man_9, Man(α1,2)Man(α1,2)Man-(α1,3)[[Man(α1,2)Man(α1,3)[Man(α1,2)Man(α1,6)]Man-(α1,6)]]Man(β1,4)GlcNAc(β1,4)GlcNAc.

GlcNAc ($Man_5GlcNAc_2$) and Man(α1,3)[Man(α1,6)]Man-(β1,4)GlcNAc(β1,4)GlcNAc ($Man_3GlcNAc_2$). The HPAEC profile of a partial digest of the $Man_9GlcNAc_2$ from early times of incubation (6 h) with the cell extract prepared from 60-h infected cells is illustrated in Figure 1, displaying the separation of the products of the $Man_9GlcNAc_2$ digest. The structures of these products were determined by comparison of their retention times on HPAEC with a standard library of oligosaccharides. The utility of HPAEC for the purpose of oligosaccharide structural determinations has been documented in several studies (Hardy et al., 1988; Townsend et al., 1988, 1989; Basa & Spellman, 1990; Spellman et al., 1989; Davidson et al., 1990; Davidson & Castellino, 1991a–c).

Confirmation of the above structures was obtained from quantitative monosaccharide compositional analyses, determined by HPAEC after hydrolysis with a clam exoglycosidase mixture, and by the observation that treatment of the $Man_6GlcNAc_2$ with *Aspergillus phoenicis* (α1,2)-mannosidase (Oxford GlycoSystems) resulted in liberation of 0.94 mol/mol of Man that existed in (α1,2) linkage in the glycan and comigration on HPAEC of the glycan product of this reaction with the above $Man_5GlcNAc_2$. In addition, one component of the acetolysis products of the Man_6GlcNA_2, formed from $Man_9GlcNAc_2$, was identified by HPAEC, compositional analysis, and specific exoglycosidase digestions to contain Man(α1,2)Man(α1,3)Man(β1,4)GlcNAc(β1,4)GlcNAc. All of the above results are consistent with the following structure of the $Man_6GlcNAc_2$ that accumulates in noninfected cell extracts:



Since these results have such significant implications regarding the ability of IPLB-SF-21AE cells to potentially assemble complex oligosaccharides, a phenomenon that we have established to occur in several infected lepidopteran insect cell lines (Davidson et al., 1990; Davidson & Castellino, 1991b,c), we decided to more thoroughly evaluate the comparative abilities of extracts from uninfected cells and cells infected with AcMNPV and r-BAC/HPg to catalyze further trimming of $Man_6GlcNAc_2$.

Table I: α-D-Mannosidase Activity of Noninfected and Baculovirus-Infected *Spodoptera frugiperda* (IPLB-SF-21AE) Cells

sample	% $Man_6GlcNAc_2$ remaining after incubation for[a]				
	0.25 h	0.5 h	1.0 h	2.0 h	12 h
noninfected[b]					
24 h	100	100	100	100	90.2
60 h	100	100	100	100	96.6
96 h	100	100	100	100	92.3
infected (r-BAC/HPg)[c]					
24 h pi	46.6	30.0	18.3	2.9	0
60 h pi	29.6	17.9	5.5	0	0
96 h pi	20.3	9.6	1.8	0	0
infected (AcMNPV)[d]					
24 h pi	38.7	23.0	15.6	0	0
60 h pi	18.7	9.4	2.8	0	0
96 h pi	13.5	2.6	0	0	0

[a] Incubation of $Man_6GlcNAc_2$ (30 μM, final concentration) with cell extracts from identical numbers of cells for the times indicated. The pH is 6.0 at 25 °C. [b] Noninfected cells. The times listed below in this column indicate the times after plating the cells from the maintenance spinner flasks. [c] Cells infected with recombinant baculovirus/human plasminogen at a MOI of 4. The times listed below in this column indicate the postinfection (pi) times. [d] Cells infected with the wild-type *Autographa californica* nuclear polyhedrosis virus at a MOI of 4. The times listed below in this column indicate the postinfection (pi) times.

After incubation at pH 6.0 of the substrate $Man_6GlcNAc_2$ with extracts obtained from identical amounts of noninfected IPLB-SF-21AE cells and these same cells infected for various times with r-BAC/HPg, the levels of $Man_6GlcNAc_2$ remaining were determined by HPAEC analysis. The data obtained are summarized in Table I. It is clear that while $Man_6GlcNAc_2$ is relatively stable in noninfected cells, extracts from cells infected with r-BAC/HPg contain an enzyme activity that catalyzes the loss of the last remaining (α1,2)-linked Man residue in this substrate, providing $Man_5GlcNAc_2$ and its more extensively trimmed product, $Man_3GlcNAc_2$ (Figure 1 illustrates a sample chromatogram). Of great interest, the level of this putative (α1,2)-D-mannosidase displays a temporal increase with infection times, as does the appearance of complex oligosaccharide on the r-HPg expressed with the plasmid employed for the infection (Davidson & Castellino, 1991b).

When the same experiment was performed with extracts from cells infected with wild-type AcMNPV, a slightly more rapid conversion of $Man_6GlcNAc_2$ to $Man_5GlcNAc_2$ and a small amount of $Man_3GlcNAc_2$ occurred (Table I). Thus, the presence of the α-D-mannosidase activity that catalyzes the further trimming of $Man_6GlcNAc_2$ is likely generally related to the infective process. The fact that this latter enzyme(s) is of the mannosidase I like class is revealed by examination of the inhibition by DMJ of this $Man_6GlcNAc_2$ trimming activity in extracts of AcMNPV-infected cells. Under the experimental conditions detailed in the Materials and Methods section, 100% of added $Man_6GlcNAc_2$ was present in the assay containing DMJ, while 91% of this substrate was trimmed to $Man_5GlcNAc_2$ in a parallel assay performed in the absence of DMJ.

Previous studies have demonstrated that Ca^{2+} was necessary for activity of certain (α1,2)-D-mannosidases (Schweden et al., 1986; Schweden & Bause, 1989; Schutzbach & Forsee, 1990). Thus, we have examined the (α1,2)-D-mannosidase activity in the 60-h AcMNPV infected cell extract after addition of 100 μM Ca^{2+} to the assay mixture at pH 6.0 and

compared the results to those obtained in the same assay in the absence of added Ca^{2+}. After incubation of $Man_6GlcNAc_2$ for 5 min with the cell extracts, we found that 50–53% of $Man_6GlcNAc_2$ remained, with or without added Ca^{2+}. However, when the same assay was performed in the absence of exogenous Ca^{2+} but in the presence of 200 μM EDTA, approximately 95% of $Man_6GlcNAc_2$ remained. These results suggest that divalent cations do play a role in the activity of the particular virally induced mannosidase(s) that is responsible for trimming of $Man_6GlcNAc_2$ and that the cell extracts contain a sufficient level of the required cations for full activity to be displayed.

In order to determine whether a mannosidase inhibitor in the uninfected cells could be responsible for the resistance to cleavage of $Man_6GlcNAc_2$, a mixing experiment was conducted. Here, a 1:1 (v/v) mixture of the extract from the 60-h r-BAC/HPg infected cells was made with buffer (sample 1) and, in a separate preparation (sample 2), with the extract from the 24-h uninfected cells. These samples (5 μL) were incubated with the $Man_6GlcNAc_2$ substrate (30 μM, final concentration) in a total volume of 25 μL. After a 0.5-h incubation at pH 6.0, the amount of $Man_6GlcNAc_2$ decreased to 20% in the sample 1 incubation and to 19% in the sample 2 incubation. These results demonstrate the unlikelihood of a mannosidase inhibitor in the uninfected cells being responsible for the enhanced stability of $Man_6GlcNAc_2$ in these same cells.

The above data have been derived from experiments conducted at pH 6.0, which is nearly optimal for most processing α-mannosidases (Lubas & Spiro, 1987; Schweden & Bause, 1989; Tabas & Kornfeld, 1979), including certain insect mannosidases (Monis et al., 1987). We have also carried out the experiments of Table I at pH 7.4. Under these conditions, the rates of disappearance of $Man_6GlcNAc_2$ were approximately 2–3-fold slower in all cases than what was observed at pH 6.0. However, these rate decreases were virtually in parallel, and studies at this latter pH do not affect the conclusions reached.

Discussion

It is now clear that certain lepidopteran insect cells possess the glycosylation machinery to assemble N-linked complex oligosaccharide on proteins and that this property is induced[2] in these same cells, in one manner, as a result of infection with a baculovirus (Davidson et al., 1990; Davidson & Castellino, 1991b,c). Further, the ability of these cells to assemble N-linked complex oligosaccharides displays a temporal dependency with infection, thereby suggesting an induction of the enzyme(s) required for this process (Davidson & Castellino, 1991b). Since proper glucosidase- and mannosidase-catalyzed trimming of the high-mannose precursor, $Glc_3Man_9GlcNAc_2$, transferred in the ER from the dolichol carrier to the relevant Asn residue of the nascent protein, is requisite for provision of the proper substrates to the late-acting processing glycosyl transferases needed for assembly of complex-type glycans, we have decided to initially investigate the functioning of trimming α-D-mannosidases in infected and noninfected IPLB-SF-21AE cells.

The mannosidase I like enzymes required for more extensive trimming of high-mannose glycans are normally found in the ER and cis and medial compartments of the Golgi complex. Several such enzymes have been identified and partially characterized. One (α1,2)-mannosidase (ER α-mannosidase), found in the ER of Chinese hamster ovary derived UT-1 cells, appears to preferentially catalyze removal of one specific (α1,2)-linked Man, resulting in $Man_8GlcNAc_2$, prior to transport of this glycoform to the Golgi stack (Bischoff et al., 1986). Other (α1,2)-mannosidases, which are presumably of ER origin (termed ER Man_9-mannosidases), from calf liver (Schweden et al., 1986) and pig liver (Schweden & Bause, 1989), have been described, which are capable of removing three of the four (α1,2)-linked Man residues from the $Man_9GlcNAc_2$ substrate, resulting in a mixture of $Man_8GlcNAc_2$, $Man_7GlcNAc_2$, and $Man_6GlcNAc_2$ isomers. Thus, the weight of evidence suggests that trimming of $Man_9GlcNAc_2$ by ER-specific and early-acting Golgi-specific (α1,2)-D-mannosidase-like enzyme(s) proceeds only to the $Man_6GlcNAc_2$ stage. In vivo studies are in agreement with this view. For example, the ER resident protein hydroxymethylglutaryl-CoA reductase is trimmed only to the $Man_6GlcNAc_2$–protein stage (Bischoff et al., 1986). Similarly, only one Man residue was removed from Sindbis viral proteins B and PE2 in the rough ER of chick embryo fibroblasts (Hakimi & Atkinson, 1982). Additionally, in MOPC 315 murine plastocytoma cells, newly synthesized immunoglobulin A α-chains possess glycans identified as Man_8-, Man_7-, and $Man_6GlcNAc_2$, but not $Man_5GlcNAc_2$, when transport of the proteins from the ER to Golgi complex was blocked with carbonyl cyanide *m*-chlorophenylhydrazone (Hickman et al., 1984). Thus, it was concluded that the first three (α1,2)-linked Man residues were removed in the ER of these cells.

The Golgi apparatus in rat liver and other mammalian cell types also contains α-mannosidase I like enzymes. Two rat liver Golgi-associated α-mannosidases (α-mannosidases IA and IB) have been purified, which effectively cleave $Man_9GlcNAc_2$ to $Man_5GlcNAc_2$ (Tabas & Kornfeld, 1979; Tulsiani et al., 1982; Tulsiani & Touster, 1988). At this point, the required substrate for GlcNAc transferase I exists, and processing to complex- and hybrid-type glycans can occur (Schachter, 1986). A rat liver Golgi endo-α-mannosidase I activity has been found which also is functional in HepG2 cells and calf thyroid slices and which liberates at different rates, from $Glc_3Man_9GlcNAc_2$, the saccharides Glc(α1,3)Man, Glc(α1,2)Glc(α1,3)Man, and Glc(α1,2)Glc(α1,2)Glc(α1,3)Man, providing $Man_8GlcNAc_2$ (Lubas & Spiro, 1987, 1988; Moore & Spiro, 1990). This latter glycan has been shown (Lubas & Spiro, 1987) to be a different isomer from the $Man_8GlcNAc_2$ generated from the exo-ER α-D-mannosidase cited above (Bischoff et al., 1986). This alternate processing route allows circumvention of the need for ER glucosidases I and II under certain conditions of glucosidase blockage. Finally, a rat brain microsomal α-D-mannosidase activity has been identified with a novel activity toward (α1,2)-, (α1,3)-, and (α1,6)-linked Man residues and is capable of providing the core glycan, viz., Man(α1,3)-[Man(α1,6)]Man(β1,4)GlcNAc(β1,4)GlcNAc, which presumably is a substrate for GlcNAc transferase I (Tulsiani & Touster, 1985). Within the Golgi stack of some cells, there also appears to be a subcellular distribution of α-mannosidase I like enzymes. When secretion of IgA was inhibited in MOPC 315 cells with monensin, which blocks within the Golgi complex, a $Man_6GlcNAc_2$–IgA accumulated, with the glycan containing one (α1,2)-linked Man residue. From this, it was postulated that the conversion of $Man_6GlcNAc_2$–IgA to $Man_5GlcNAc_2$–IgA occurred in the trans Golgi, just prior to secretion, and it was suggested that a specific (α1,2)-

[2] Since we are uncertain of the mechanism by which the α-D-mannosidase(s) responsible for further trimming of $Man_6GlcNAc_2$ become available in virally infected cells, terms such as "induced" and "activated", when applied to this situation, are not used with their usual mechanistic connotations.

mannosidase that removes the final (α1,2)-linked Man residue from $Man_6GlcNAc_2$-protein is present in the trans Golgi compartment (Hickman et al., 1984).

The above investigations point to processes that lead to trimming of $Glc_3Man_9GlcNAc_2$ to $Man_6GlcNAc_2$ and suggest that formation of $Man_5GlcNAc_2$ requires special consideration. We show herein that extracts of lepidopteran insect IPLB-SF-21AE cells possess relatively lower levels of an enzyme activity allowing trimming of the $Man_6GlcNAc_2$ to $Man_5GlcNAc_2$. This, in itself, may contribute to the findings of the lack of complex N-linked oligosaccharides in lepidopteran insect cell glycoproteins, although other factors, i.e., lack of essential glycosyl transferases, may also play a role in this respect. That all insect cells may not behave similarly in this regard is suggested by the observation that extracts from uninfected *Aedes aegypti* cells do contain an (α1,2)-D-mannosidase that cleaves $Man_6GlcNAc_2$ to $Man_5GlcNAc_2$ but do not contain appreciable levels of GlcNAc transferase I, thus apparently rendering these cells incapable of synthesizing complex-type oligosaccharides (Monis et al., 1987). We also demonstrate that the (α1,2)-D-mannosidase(s) that converts the $Man_6GlcNAc_2$ to $Man_5GlcNAc_2$ is somehow stimulated by infection with AcMNPV and r-BAC/HPg, since this reaction does occur readily in cells infected with this virus (Table I). In this fashion, baculovirus infection of lepidopteran insect cells would allow the possibility of assembly of complex-type glycan on suitable protein acceptors, since the substrate for GlcNAc transferase I thus becomes available. The manner in which this important trimming enzyme is induced by the virus cannot be ascertained from the design of this investigation, but the temporal nature of the appearance of this enzyme suggests a direct relationship to viral infection of insect cells, which may be at the level of insect gene translation and/or cellular alterations accompanying infection.

In conclusion, we have identified a potentially important glycosylation control mechanism which (a) allows the observed switch from high-mannose-type glycan assembly in lepidopteran insect cells to complex-type oligosaccharide consequent to viral infection (Davidson et al., 1990; Davidson & Castellino, 1991b,c), and (b) is consistent with the temporal effects of this process (Davidson & Castellino, 1991b). Thus, while complex-type oligosaccharide assembly on noninfected insect cell glycoproteins would not normally be favored, due to the inability of the cells to further trim the $Man_6GlcNAc_2$ intermediate, viral infection of the cells induces an enzyme activity that allows the release of the final (α1,2)-linked Man on $Man_6GlcNAc_2$, thereby allowing GlcNAc transferase I to function. While other factors, such as the nature of the protein substrate, are probably important as to whether a protein-bound high-mannose-type oligosaccharide would actually be processed to complex-type glycan, the findings described herein are compatible with our previous observations of complex-type oligosaccharide on HPg expressed in infected lepidopteran insect cells and reveal the presence of a potential contributing determinant of importance to the nature of the oligosaccharide assembled on proteins.

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