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Glucose Trimming and Mannose Trimming Affect Different Phases of the Maturation of Sindbis Virus in Infected BHK Cells

WILLIAM McDOWELL,[1] PEDRO A. ROMERO, ROELF DATEMA, AND RALPH T. SCHWARZ*

*Institut für Virologie, Justus Liebig Universität Giessen, Frankfurter Strasse 107, D-6300 Giessen, Federal Republic of Germany, and *Laboratoire de Chimie Biologique, Université des Sciences et Techniques de Lille, 59655 Villeneuve D'Ascq Cedex, France*

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The roles of glucose and mannose trimming in the maturation of Sindbis virus in BHK cells have been investigated using inhibitors of glycoprotein oligosaccharide processing. In the presence of the glucosidase inhibitor *N*-methyl-1-deoxynojirimycin the viral glycoproteins were equipped with oligosaccharides of the composition $Glc_3Man_{8,9}(GlcNAc)_2$ and the yield of virus in the extracellular medium was reduced as a result of a block in the proteolytic cleavage of the precursor (pE2) of the E2 viral envelope glycoprotein. The mannosidase I inhibitor 1-deoxymannojirimycin (dMM) also inhibited the appearance of virus in the medium and the oligosaccharides on the viral glycoproteins had the composition $Man_9(GlcNAc)_2$. However, pE2 was cleaved to E2 under these conditions, and it was found that when the yield of virus from the cells and medium together was considered, there was no difference between untreated and dMM-treated cultures, suggesting the presence of intracellular virus particles in the dMM-treated cultures. When examined by electron microscopy, the dMM-treated cultures were found to contain intracellular virus particles. In addition, nucleocapsids were found lining intracellular membranes. These observations taken together with the plaque test data intimate that Sindbis virus preferentially buds from internal membranes in BHK cells treated with dMM. The results confirm the essential role of glucose trimming in the Sindbis virus–BHK cell system and suggest that the initial stages of mannose removal may be important too. © 1987 Academic Press, Inc.

INTRODUCTION

Sindbis virus, a Togavirus, is a simple RNA-containing virus consisting of a nucleocapsid surrounded by a membrane in which the two viral glycoproteins, E1 and E2, are embedded. The viral proteins are generated by sequential proteolytic cleavage of the 26 S mRNA translation product (Garoff *et al.*, 1982). The E1 and E2 glycoproteins of Sindbis virus each contain two glycosylation sites (Hsieh *et al.*, 1983a). Whether a particular glycosylation site carries complex or high-mannose oligosaccharides is presumably determined to a large extent, if not exclusively, by the folding of the polypeptide chain, since accessibility to the processing enzymes determines the amount of processing that can take place (Hsieh *et al.*, 1983b). One glycosylation site on E1 and E2 carries exclusively complex-type oligosaccharides irrespective of whether the virus is grown in avian or mammalian cells (Hsieh *et al.*, 1983a).

Complex-type oligosaccharides are generated via the trimming pathway (Kornfeld and Kornfeld, 1985). Trimming of the precursor oligosaccharide $Glc_3Man_9(GlcNAc)_2$, of the complex and high-mannose oligosaccharides characteristic of N-linked glycoproteins, is initiated in the endoplasmic reticulum by the removal of the three glucose residues by glucosidases I and II (Grinna and Robbins, 1979; Burns and Touster, 1982). Some mannose trimming may also occur in the endoplasmic reticulum (Bischoff and Kornfeld, 1983). Subsequently, the action of mannosidase I in the Golgi apparatus completes the removal of up to four mannose residues (Tabas and Kornfeld, 1979). Addition of GlcNAc by GlcNAc transferase I (Harpaz and Schachter, 1980), the subsequent removal of two more mannose residues by mannosidase II (Tulsiani *et al.*, 1982a), and the addition of the peripheral GlcNAc, Gal, and NeuAc residues by specific glycosyltransferases results in the formation of complex-type oligosaccharides (Kornfeld and Kornfeld, 1985).

The biological significance of the trimming pathway and the relative importance of high-mannose and complex oligosaccharides for the proper functioning of glycoproteins can be studied with specific inhibitors of the trimming glycosidases (Schwarz and Datema, 1984). Several inhibitors of the trimming glycosidases are now available and proteins glycosylated in the presence of the glucosidase inhibitors 1-deoxynojirimycin (Saunier *et al.*, 1982), *N*-methyl-1-deoxynojirimycin (Romero *et al.*, 1983), or castanospermine (Pan *et al.*, 1983) have oligosaccharides of the structure $Glc_3Man_{7,8,9}(GlcNAc)_2$, whereas in the presence of bromoconduritol (Datema *et al.*, 1982) the oligosaccharides have the composition $Glc_1Man_{7,8,9}(GlcNAc)_2$. The mannose analog of 1-deoxynojirimycin, 1-deoxy-

[1] To whom requests for reprints should be addressed.

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mannojirimycin (dMM),[2] inhibits Golgi mannosidase I (Fuhrmann *et al.*, 1984; Bischoff and Kornfeld, 1984) and proteins are equipped with oligosaccharides of the composition $Man_9(GlcNAc)_2$ (Elbein *et al.*, 1984). Swainsonine, on the other hand, inhibits Golgi mannosidase II (Tulsiani *et al.*, 1982b) leading to the formation of hybrid-type structures.

In Sindbis virus-infected BHK cells in which glucose trimming is inhibited by bromoconduritol (Datema *et al.*, 1984), 1-deoxynojirimycin, or castanospermine (Schlesinger *et al.*, 1985), the cleavage of pE2 to E2 is inhibited. As expected from the requirement of cleavage of pE2 for virus formation, the release of virus particles is decreased in the inhibitor-treated cells. This effect can be reversed by lowering the incubation temperature from 37 to 30° (Schlesinger *et al.*, 1985). This infers that pE2 equipped with glucosylated high-mannose oligosaccharides has a conformation resistant to proteolytic cleavage. It was therefore of interest to determine whether glucose trimming alone was sufficient to allow virus maturation or whether mannose trimming is also required. Thus the relative roles of glucose and mannose trimming in Sindbis virus maturation in BHK cells were investigated by the use of glucosidase (MdN) and mannosidase I (dMM) inhibitors.

MATERIALS AND METHODS

Virus and tissue culture

Confluent monolayers of BHK cells were grown in Dulbecco's medium supplemented with 5% fetal calf serum. The cells were infected with Sindbis virus strain Sa-AR-86 at a multiplicity of infection of 50 plaque-forming units per cell. After infection the cells were maintained in either Earle's medium or Dulbecco's medium containing 10 m*M* fructose instead of glucose as indicated in the text. When labeling with [^{35}S]methionine was performed, Dulbecco's medium lacking methionine was used. After labeling the cells were washed three times with ice-cold phosphate-buffered saline and either suspended in chloroform–methanol (2:1 v/v) for extraction of lipids or lysed with a buffer comprising 62.5 m*M* Tris–HCl, pH 6.8, 3% SDS, 5% mercaptoethanol, and 10% glycerol for SDS–PAGE. Virus in the medium was quantitated by plaque assay and by the incorporation of [^{35}S]methionine into virus particles isolated by sucrose density centrifugation (Schwarz *et al.*, 1976).

Chemicals and enzymes

N-Methyl-1-deoxynojirimycin (MdN) was prepared by methylation of 1-deoxynojirimycin (supplied by Dr. E. Truscheit, Bayer AG, Wuppertal, FRG) (Romero *et al.*, 1983) and 1-deoxymannojirimycin (1,5-dideoxy-1,5-imino-D-mannitol) was obtained from Dr. G. Kinast (Bayer AG). MdN and dMM were added to the culture medium at final concentrations of 1 and 2 m*M*, respectively. Swainsonine was from Dr. P. Dorling, Murdoch University, Western Australia, and was used at a final concentration of 0.5 μg/ml. Tunicamycin was purchased from Calbiochem (Frankfurt am Main, FRG) and used at a final concentration of 2 μg/ml. Inhibitors were added to cultures 1 hr after infection and maintained throughout the incubation period.

[2-^{3}H]Mannose (19 Ci/mmol), U-^{14}C-labeled protein hydrolysate (57 mCi/mAtom) and [^{35}S]methionine (1100 Ci/mmol) were bought from Amersham Buchler (Braunschweig, FRG). Pronase, endo β-*N*-acetylglucosaminidase H, and Jack Bean α-mannosidase (type III) were purchased from Serva (Heidelberg, FRG), Seikagachu Kogyo Co., Ltd. (Tokyo, Japan), and Sigma (Munich, FRG), respectively. Rat liver microsomal α-glucosidase was prepared and assayed according to Grinna and Robbins (1979). Oligosaccharide standards labeled in their glucose or mannose moieties were prepared as described previously (Datema and Schwarz, 1981; Schwarz and Datema, 1982a) and characterized by chromatography on columns of Bio-Gel P4 and their resistance or susceptibility to digestion with α-glucosidase and α-mannosidase (Datema and Schwarz, 1981; Datema *et al.*, 1982).

Separation techniques

Columns of Bio-Gel P4 and P6 were calibrated and eluted as described previously (Datema *et al.*, 1980, 1982). SDS–polyacrylamide gel electrophoresis (SDS–PAGE) of labeled viral glycoproteins was performed in 10% gels using the Laemmli discontinuous system (Schwarz and Klenk, 1974).

Other procedures

Extraction of lipid-linked oligosaccharides and the preparation of glycopeptides have been described elsewhere (Datema *et al.*, 1982; Schwarz and Datema, 1982a). Pronase digestion and α-mannosidase diges-

[2] Abbreviations used: MdN, *N*-methyl-1-deoxynojirimycin; dMM, 1-deoxymannojirimycin; BHK, baby hamster kidney; SW, swainsonine; Tun, tunicamycin; PFU, plaque forming units; endo H, endo β-*N*-acetylglucosaminidase H; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; SEM, standard error of mean.

tion were carried out as described earlier (Datema and Schwarz, 1981). Electron microscopy was performed using published procedures (Scholtissek *et al.*, 1978).

RESULTS

Effect of trimming inhibitors on the glycosylation of Sindbis virus glycoproteins

It was necessary to determine whether the two inhibitors MdN and dMM were specific trimming inhibitors in Sindbis virus-infected BHK cells. Chick embryo cells were not used because in our hands dMM inhibited the formation of lipid-linked oligosaccharides in these cells (Elbein *et al.*, 1984). However, no effect on the assembly of $Glc_3Man_9(GlcNAc)_2$-PP-Dol was observed in BHK cells in the presence of 2 m*M* dMM. At higher concentrations (5 m*M*) an inhibitory effect was observed (data not shown). The structures of the oligosaccharides of the Sindbis viral glycoproteins synthesized in the presence of either 1 m*M* MdN or 2 m*M* dMM were analyzed, after labeling with [^{3}H]mannose, pronase digestion, and endo H treatment, by gel filtration on Bio-Gel P4 (Fig. 1). It can be seen that both inhibitors blocked the formation of complex-type oligosaccharides. Analysis of the oligosaccharides formed in the presence of MdN showed the occurrence of $Glc_3Man_{8,9}(GlcNAc)_2$ (Fig. 1B), whereas in the presence of dMM (Fig. 1C) only one major oligosaccharide of the composition $Man_9(GlcNAc)_2$ was detected. Therefore the inhibitors exert specific blocks in the trimming pathway in infected BHK cells.

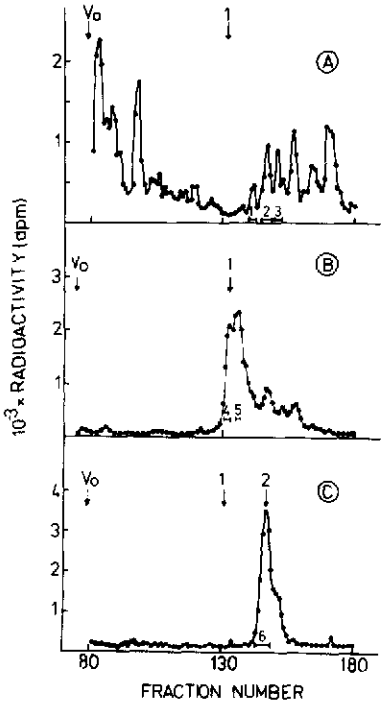

FIG. 1. Bio-Gel P4 analysis of [^{3}H]mannose labeled oligosaccharides and glycopeptides derived from Sindbis virus-infected BHK cells. BHK cells infected with Sindbis virus were (A) untreated, (B) treated with 1 m*M* MdN, or (C) treated with 2 m*M* dMM at 1 hr postinfection, labeled with 100 μCi [^{3}H]mannose from 4 to 5 hr postinfection, and then chased for 2 hr. The cells were extracted with chloroform–methanol (2:1 v/v) and chloroform–methanol–water (10:10:3 v/v) to remove lipid-linked oligosaccharides and the lipid-free residue was digested with Pronase. The resulting glycopeptides were then desalted by passage through a column of Bio-Gel P6, treated with endo H, and analyzed by gel filtration through Bio-Gel P4. Vo signifies the void volume and the arrows indicate the elution positions of (1) $Glc_3Man_9GlcNAc$ and (2) $Man_9GlcNAc$ reference compounds. Peaks 1–6 were characterized following digestion with α-glucosidase and α-mannosidase as described by Datema *et al.* (1982) and are as follows: (1) $Glc_1Man_9GlcNAc$; (2) and (6) $Man_9GlcNAc$; (3) $Man_8GlcNAc$; (4) $Glc_3Man_9GlcNAc$; (5) $Glc_3Man_8GlcNAc$.

Virus formation in the presence of trimming inhibitors

To assess the effects of MdN and dMM on the release of virus into the extracellular medium, the infectivity was determined by plaque assay and in addition the incorporation of [^{35}S]methionine into virus particles, isolated by sucrose density centrifugation, was measured. Both inhibitors significantly suppressed virus particle formation (Table 1; Student's *t* test $P < 0.001$ for MdN and $P < 0.02$ for dMM). In contrast to dMM the mannosidase II inhibitor swainsonine had no inhibitory effect on virus formation. To check for possible toxic (nonspecific) effects of MdN or dMM, BHK cells were infected with a strain of influenza virus adapted to growth in these cells, and treated with MdN or dMM. Previous studies had shown that MdN (Romero *et al.*, 1983) and dMM (Elbein *et al.*, 1984) do not affect the formation of influenza virus. Indeed, no change in the hemagglutination titer in MdN- or dMM-treated cultures with respect to the untreated control was observed (data not shown). When the infected cells were incubated at 33° instead of 37°, the inhibitory effects of MdN on the yield of virus were reduced (Table 1), thus confirming that the block induced by inhibiting glucose removal is temperature sensitive (Schlesinger *et al.*, 1985). However, this was not so for the inhibition caused by dMM treatment (Table 1).

Post-translational processing of Sindbis viral glycoproteins

Proteolytic cleavage of pE2 to E2 is a late event, occurring just prior to budding of the virus, either in the Golgi or during transport to the cell surface or at the cell surface itself, and is necessary for virus particle

TABLE 1

EFFECTS OF TRIMMING INHIBITORS ON THE RELEASE INTO THE EXTRACELLULAR MEDIUM OF SINDBIS VIRUS FROM INFECTED BHK CELLS

Addition	Infectivity (PFU/ml ± SEM)	Radioactivity in particles (dpm)	Infectivity at 33° (PFU/ml ± SEM)
None	$1.7 \times 10^{10} \pm 0.1$	255,260	$2.5 \times 10^{10} \pm 0.2$
1 m*M* MdN	$3.4 \times 10^{8} \pm 0.3$	26,345	$2.7 \times 10^{10} \pm 0.2$
2 m*M* dMM	$1.6 \times 10^{9} \pm 0.3$	53,400	$4.0 \times 10^{9} \pm 1.1$
500 ng/ml SW	$2.6 \times 10^{10} \pm 0.4$	262,918	n.d.
2 μg/ml Tun	$2.9 \times 10^{5} \pm 0.9$	6,640	n.d.

Note. Sindbis virus-infected BHK cells were treated with inhibitors of protein glycosylation as indicated from 1 hr postinfection. At 4 hr postinfection 50 μCi [^{35}S]methionine was added and labeled virus particles present in the medium were harvested at 24 hr postinfection. The virus was purified by sucrose density centrifugation and the incorporation into the virus band was measured. Virus infectivity in the extracellular medium was determined by plaque assay. The experiment at 37° was performed five times and that at 33° was performed three times. The results shown are from representitive experiments. n.d., not determined; SEM, standard error of mean.

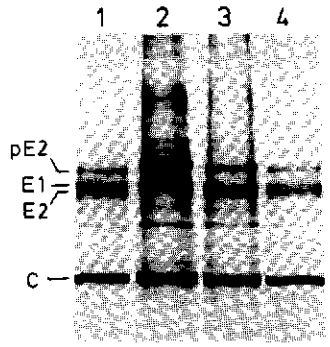

FIG. 2. Formation of Sindbis virus proteins in the presence of trimming inhibitors. Sindbis virus-infected BHK cells were either untreated (lane 1) or treated with 1 m*M* MdN (lane 2), or 2 m*M* dMM (lane 3) or 500 ng/ml SW (lane 4) and were labeled with 10 μCi ^{14}C-labeled protein hydrolysate at 37° for 2 hr from 4 hr postinfection. Lysates were prepared (see Materials and Methods) and the proteins were separated by SDS–PAGE in 10% gels (Schwarz and Klenk, 1974).

production (Garoff *et al.*, 1982). Previous studies (Datema *et al.*, 1984; Schlesinger *et al.*, 1985) have shown that in Sindbis virus-infected BHK cells treated with the glucosidase inhibitors bromoconduritol and 1-deoxynojirimycin, the pE2 to E2 cleavage is blocked, resulting in a reduction in the yield of virus. To investigate if proteolysis was blocked in cells treated with the trimming inhibitors in the present study, treated cells were labeled with ^{14}C-labeled amino acids for 2 hr at 4 hr postinfection and the cell lysates were analyzed by SDS–PAGE (Fig. 2). Untreated cultures showed the formation of pE2, E1, and E2 glycoproteins and the capsid protein C (Fig. 2, lane 1). In the presence of 1 m*M* MdN it appeared that no E2 was present (Fig. 2, lane 2), indicating that the pE2 to E2 cleavage was impaired in these cells. In the presence of 2 m*M* dMM no block is evident (Fig. 2, lane 3). The E1 and E2 proteins synthesized in the presence of dMM migrate very close together on SDS–PAGE due to alterations in the oligosaccharide side-chains of these glycoproteins induced by the mannosidase inhibitor. Under normal conditions in virus produced in BHK cells the E1 protein contains two complex oligosaccharides, whereas the E2 protein contains one complex oligosaccharide and one high-mannose oligosaccharide (Hsieh *et al.*, 1983a). In the presence of dMM both proteins are glycosylated exclusively with high-mannose oligosaccharides ($Man_9(GlcNAc)_2$) as shown in Fig. 1. It was to be expected that the change in molecular weight of E1 due to the conversion of two complex oligosaccharides to two high-mannose oligosaccharides would be greater than that in E2 in which only one complex oligosaccharide is converted. Cultures treated with the mannosidase II inhibitor swainsonine also showed no effect on the pE2 to E2 cleavage (Fig. 2, lane 4). The suspected impairment in the pE2 to E2 cleavage in MdN-treated cultures was further investigated by pulse–chase labeling with [^{35}S]methionine in the absence and presence of the trimming inhibitors (Fig. 3). Thus in the untreated cultures and in those treated with dMM and SW the pE2 precursor formed during the pulse is absent after chasing with unlabeled medium for 60 min (Fig. 3, lanes 1, 3, and 4). However, in the MdN-treated culture (Fig. 3, lane 2), pE2 is still present after the 60-min chase. Thus in the presence

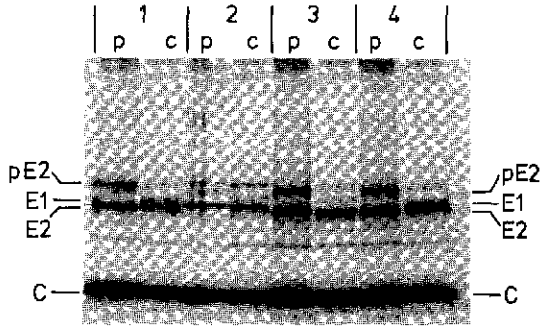

FIG. 3. Effect of trimming inhibitors on the proteolytic cleavage of pE2 to E2. Duplicate plates of Sindbis virus-infected BHK cells treated with trimming inhibitors as described in the legend to Fig. 2 were labeled with 50 μCi [^{35}S]methionine for 20 min from 4 hr postinfection. The cells from one set of plates were then harvested and prepared for SDS–PAGE (p). In the other set the medium was removed, the cells were washed three times with warm phosphate-buffered saline, and fresh medium containing the appropriate inhibitor and 1 m*M* unlabeled methionine was added. The cells were incubated at 37° for 60 min and then harvested for SDS–PAGE (c). Lanes 1–4 refer to the lysates from cultures treated as follows: (1) untreated, (2) treated with 1 m*M* MdN, (3) treated with 2 m*M* dMM, (4) treated with 500 ng/ml SW. p, pulse; c, chase.

of MdN the proteolytic cleavage of pE2 to E2 is impaired, like with the other glucosidase inhibitors, and as result the formation of virus is inhibited. dMM, however, does not affect the pE2 to E2 cleavage and therefore the inhibition of virus release observed in the presence of dMM must be due to another cause.

In a similar experiment Sindbis-infected cells were labeled with [^{3}H]palmitic acid to investigate whether acylation of the viral glycoproteins was affected by the trimming inhibitors. No effects on acylation were observed since all the Sindbis viral glycoproteins were labeled (data not shown).

Is there intracellular budding of Sindbis virus in the presence of dMM?

One possible cause of the reduction in virus release into the extracellular medium in dMM-treated cultures is that virus is budding intracellularly. This phenomenon occurs in the presence of monensin (Johnson and Schlesinger, 1980) and is the major route for Sindbis virus production in insect cells (Gliedman *et al.*, 1975). In order to test for this possibility, the yields of infectious virus in the medium alone and in the medium plus cells taken together after freezing and thawing three times to release any intracellular virus particles were determined (Table 2). Comparison of the virus titers between untreated and dMM-treated cultures shows that when only the medium is considered, the yield of virus in the dMM-treated cultures is significantly lower than that in the control (Student's t test $P < 0.05$). However, there are no significant differences between untreated and dMM-treated cultures when the yield of virus in medium plus cells is considered (Student's t test $P > 0.1$). Tunicamycin which inhibits protein glycosylation by blocking the assembly of the lipid-linked oligosaccharide precursor (Schwarz and Datema, 1982b) does not show this effect (Table 2). It is significant that only freeze–thaw treatment of the dMM-treated cultures resulted in an increase in virus titer.

TABLE 2

EFFECT OF FREEZING AND THAWING ON THE VIRUS TITER OF THE CULTURE MEDIUM ALONE COMPARED WITH THAT OF THE CELLS PLUS MEDIUM TOGETHER IN CULTURES OF SINDBIS VIRUS-INFECTED BHK CELLS UNTREATED OR TREATED WITH 1-DEOXYMANNOJIRIMYCIN OR TUNICAMYCIN

	Virus infectivity (PFU/ml ± SEM)	
Addition	Medium alone	Medium + cells
Control	$1.6 \times 10^{10} \pm 0.3$	$7.3 \times 10^{9} \pm 1.3$
2 m*M* dMM	$4.6 \times 10^{9} \pm 0.3$	$7.0 \times 10^{9} \pm 0.5$
2 μg/ml Tun	$3.5 \times 10^{8} \pm 0.1$	$2.3 \times 10^{7} \pm 0.2$

Note. Sindbis virus-infected BHK cells were treated with inhibitor from 1 hr postinfection as indicated. After 15 hr incubation virus in either the medium alone or the medium and cells together (after freezing and thawing three times) was determined by plaque assay. The lowered titer in the medium + cell control is probably a consequence of the sensitivity of the virus to the freezing and thawing treatment.

These results are suggestive of the presence of intracellular virus particles in the cultures treated with dMM. However, a similar result would be obtained if virus was adhering to the cell surface. Therefore to distinguish between these two possibilities electron microscopy of untreated and dMM-treated Sindbis virus-infected BHK cells was performed (Figs. 4a–4d). In the untreated cells virus was seen budding from the cell surface (Fig. 4a), whereas in the dMM-treated cells intracellular virus particles were seen (Figs. 4b–4d). In addition, nucleocapsids were found lining intracellular membranes (Fig. 4d) providing evidence that virus assembly is actually taking place at the intracellular site. These findings coupled with those of the plaque assays intimate that in the presence of dMM Sindbis virus buds preferentially from intracellular membranes.

DISCUSSION

As previously observed (Datema *et al.*, 1984; Schlesinger *et al.*, 1985), glucose trimming is essential for the effective maturation of Sindbis virus in infected BHK cells. At least one critical step is impeded when glucose trimming does not occur, the proteolytic cleavage of pE2 to E2. This cleavage has been previously shown to be required for virus release (reviewed by Garoff *et al.*, 1982) and it has been postulated that pE2 equipped with glucosylated high-mannose oligosaccharides does not go through a conformational rearrangement which makes the protein susceptible to cleavage (Schlesinger *et al.*, 1985). The question remained, where in the trimming pathway does this conformational change take place? Here it has been shown that when glucose trimming is allowed to occur and mannose removal is prevented by inhibiting Golgi mannosidase I, pE2 is cleaved to E2. This is an example of the essential role for glucose trimming in glycoprotein biosynthesis. Allowing glucose trimming, but preventing subsequent mannose trimming, restores the yield of infectious virus to control levels, although the virus in dMM-treated cultures appears to bud from intracellular membranes to a greater extent than virus in untreated cultures as demonstrated by a decrease in the yield of cell-free virus and by electron microscopy. These findings support the proposal (Rott *et al.*, 1975) that intracellular membranes represent a site of second choice for the formation of enveloped viruses as has been demonstrated for influenza virus (Rott *et al.*, 1975; Stitz *et al.*, 1977). It is interesting

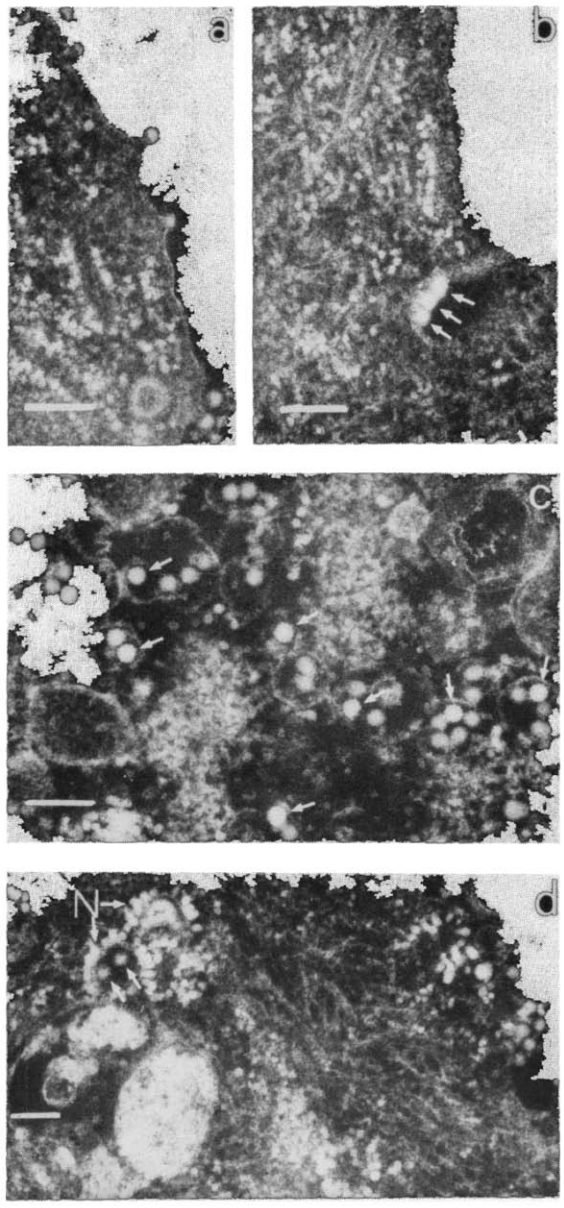

FIG. 4. Electron microscopy of untreated and 1-deoxymannojirimycin-treated Sindbis virus-infected BHK cells. Virus-infected cells either untreated (a) or treated (b–d) with 2 m*M* dMM from 1 hr postinfection were incubated for 8–15 hr, and harvested and prepared for electron microscopy (Scholtissek *et al.*, 1978). (b–d) Virus particles (arrows) inside dMM-treated Sindbis virus-infected BHK cells at 8 hr (b) and 15 hr (c, d) postinfection. Viral nucleocapsids (N) are seen lining the internal membranes in (d), an effect reminiscent of that observed in monensin-treated cells (Johnson and Schlesinger, 1980). (a) Virus particles budding from the cell surface in untreated cultures. The bar represents 0.2 μM.

that small alterations in carbohydrate structure of the Sindbis viral glycoproteins, as brought about by an inhibitor of oligosaccharide processing, can lead to the choice of such a second site. The oligosaccharide composition of the glycoproteins from virus budding from intracellular membranes in untreated cultures is not known, but it is possible that such virus contains a higher proportion of glycoproteins with less processed oligosaccharide than virus released by budding from the plasma membrane.

The phenomenon of intracellular budding virus has been observed in cultures treated with the ionophore monensin (Johnson and Schlesinger, 1980), which blocks intracellular transport between the *cis* and *trans* compartments of the Golgi apparatus (Tartakoff, 1983). It is interesting that when mannose trimming is blocked later in the pathway by inhibiting the action of Golgi mannosidase II with swainsonine (Table 1) or by growing the virus in mutant cells lacking GlcNAc transferase I (Schlesinger *et al.*, 1976), no effect on virus maturation was observed. Thus it would appear that the initial stages of mannose trimming may be important for determining the final destination of the Sindbis viral glycoproteins in infected BHK cells. This apparent observation of preferential intracellular budding when preventing mannose trimming may be analogous to the result that in Sindbis virus-infected mosquito cells, the virus buds from internal membranes (Gliedman *et al.*, 1975). Mosquito cells lack the terminal glycosyltransferases necessary for the formation of complex-type oligosaccharides (Butters and Hughes, 1981; Butters *et al.*, 1981) and the glycoproteins are equipped with oligosaccharides of the composition $Man_3(GlcNAc)_2$ at the glycosylation sites which would carry exclusively complex oligosaccharides in avian or mammalian cells (Hsieh and Robbins, 1984). The remaining glycosylation sites carry high-mannose oligosaccharides containing five to nine mannose residues (Hsieh and Robbins, 1984).

The essential role of glucose trimming for Sindbis virus formation observed in this study corresponds with observations made with vesicular stomatitis virus (Schlesinger *et al.*, 1984) and mouse hepatitis virus (Repp *et al.*, 1985) and a consensus suggests that removal of glucose residues is essential for the establishment of a functional conformation for some viral glycoproteins. Thus the formation of some influenza A viruses (Romero *et al.*, 1983; Elbein *et al.*, 1984) and Rous sarcoma virus (Bosch and Schwarz, 1984; Bosch *et al.*, 1985) is not affected by either glucosidase or mannosidase inhibitors with the exception of the influenza A virus, fowl plague virus (H_7N_1), which has a cleavable hemagglutinin (Bosch *et al.*, 1981). When equipped with $Glc_1Man_9(GlcNAc)_2$ oligosaccharides, the hemagglutinin is metabolically unstable (Datema *et al.*, 1982, 1984), but it is stable with oligosaccharides of the composition $Glc_3Man_9(GlcNAc)_2$ (Romero *et al.*,

1983). Clearly, as the biological role of trimming cannot be predicted, each system must be examined in its own right.

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