

Inhibition of formation of complex oligosaccharides by the glucosidase inhibitor bromoconduiritol

(glycoprotein processing/ α -glucosidase/influenza virus)

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ABSTRACT The α -glucosidase inhibitor bromoconduiritol (6-bromo-3,4,5-trihydroxycyclohex-1-ene) inhibits trimming of the innermost glucose residue from the $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ precursor of high-mannose and complex oligosaccharides. This inhibition occurs both in intact cells and with a microsomal enzyme preparation. The formation of lipid-linked oligosaccharides was increased in glucosidase-inhibited cells. Inhibition of transfer of high-mannose oligosaccharides to protein was not observed. In bromoconduiritol-treated virus-infected cells, trimming of mannose can occur despite incomplete removal of glucose. The glucosylated high-mannose oligosaccharides $\text{GlcMan}_9\text{GlcNAc}$, $\text{GlcMan}_8\text{GlcNAc}$, and $\text{GlcMan}_7\text{GlcNAc}$ were released from viral glycoproteins after digestion with Pronase and *endo*- β -N-acetylglucosaminidase H. The formation of complex oligosaccharides was concomitantly inhibited. The release of infectious fowl plague virus particles (an influenza virus) was inhibited from bromoconduiritol-treated infected chicken-embryo cells.

The formation of asparagine-linked oligosaccharides starts with transfer of the oligosaccharide $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$ from dolichol diphosphate to the protein (1, 2). This oligosaccharide is a precursor of two basic types of asparagine-linked oligosaccharides—i.e., the high-mannose oligosaccharides (containing mannose and GlcNAc) and the complex oligosaccharides (containing also galactose, fucose, and NeuNAc).

The glucose residues in the precursor occur in the sequence $\alpha\text{Glc}(1\rightarrow2)\alpha\text{Glc}(1\rightarrow3)\alpha\text{Glc}(1\rightarrow3)\alpha\text{Man} \dots$ (2). Two neutral glucosidases have been reported to be involved in the removal of the glucose residues that occurs after transfer of the oligosaccharide from the lipid to the protein (2). These are glucosidase I (or Glc_3 -glucosidase), which removes the $\alpha(1\rightarrow2)$ -linked glucose, and glucosidase II (or Glc_2 -glucosidase), which cleaves the two $\alpha(1\rightarrow3)$ -linked glucose residues. The subsequent removal of up to four mannose residues gives rise to high-mannose oligosaccharides, whereas mannose trimming followed by addition of GlcNAc, galactose, fucose, and NeuNAc from the appropriate sugar donors results in the formation of complex oligosaccharides (3). Thus, trimming of glucose residues is an obligatory step in glycoprotein processing.

Most inhibitors of protein glycosylation interfere with the assembly of the dolichol-linked oligosaccharide, but some drugs are known to interfere with processing (4, 5). Bromoconduiritol (6-bromo-3,4,5-trihydroxycyclohex-1-ene) is an active-site directed covalent inhibitor of α -glucosidases (6, 7). We have tested conduiritol derivatives as inhibitors of glucosidases involved in glucose trimming of asparagine-linked oligosaccharides and, therefore, as inhibitors of formation of the high-mannose and complex oligosaccharides found on mature

glycoproteins. For these investigations, cells infected with influenza virus were used. In these cells, host glycoprotein synthesis is shut off and, after infection, viral glycoprotein synthesis *de novo* can be followed.

EXPERIMENTAL PROCEDURES

Chemicals. Bromoconduiritol was prepared as described (6). Unless indicated otherwise, a 1:1 mixture of bromoconduiritol A (3,5/4,6-6-bromo-3,4,5-trihydroxycyclohex-1-ene) and bromoconduiritol B (3,5,6/4-6-bromo-3,4,5-trihydroxycyclohex-1-ene) was used. The material was dissolved in cold water and used immediately. The following isotopic precursors were obtained from Amersham Buchler: [^1H]fucose, [^2H]mannose, [^1H]galactose (all at 2–5 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), and [^{35}S]methionine (600 Ci/mmol). Pronase P (from *Streptomyces griseus*) was from Serva, α -mannosidase (type III from jack beans) was from Sigma, and *endo*- β -N-acetylglucosaminidase H (*endo* H) was from Miles. Tunicamycin was a gift from G. Tamura, University of Tokyo.

$\text{Glc}_3\text{Man}_9\text{GlcNAc}$ labeled in its glucose or mannose moiety was prepared from lipid-linked oligosaccharides formed in intact cells after labeling with [^3H]galactose or [^3H]mannose as described (8). $\text{Glc}_3\text{Man}_9\text{GlcNAc}$, $\text{Man}_9\text{GlcNAc}$, $\text{Man}_8\text{GlcNAc}$, and $\text{Man}_7\text{GlcNAc}$ labeled with [^{14}C]mannose were prepared from their respective dolichol diphosphate derivatives followed by treatment with *endo* H as described (8). [^3H]Glc $_1\text{Man}_9\text{GlcNAc}$ was isolated after Pronase treatment and digestion with *endo* H of glycoproteins from virus-infected cells that had been labeled with [^3H]galactose for 1 hr and then further incubated for 2 hr. Characterization was done by chromatography on Bio-Gel P-4 columns, resistance against α -mannosidase (9), and susceptibility to α -glucosidase (see *Results*, Fig. 6A). β -ManGlcNAc was obtained by α -mannosidase digestion of $\text{Man}_9\text{GlcNAc}$ as described (10).

Tissue Culture and Labeling Procedures. For labeling, *in vivo* confluent monolayer cultures of primary chicken embryo cells in 9-cm-diameter Petri dishes were used. The cells were infected with fowl plague virus (strain Rostock) or PR8 (a human influenza virus) as described (9). After infection, the cells were maintained in 2.5 ml of Earle's medium containing 10 mM pyruvate. Radioactive isotopes were added 4 hr after infection.

Separation Techniques. Extraction of lipid-linked oligosaccharides and preparation of glycopeptides was as described (8). Bio-Gel P-4 columns were calibrated as described (10). Round gel electrophoresis was as described (8). Separation of virus particles on sucrose density gradients was as described (11).

Other Procedures. Release of glucose from oligosaccharides was accomplished as described by Grinna and Robbins (12), except that the assay mixture was modified by addition of 10 mM

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Abbreviation: *endo* H, *endo*- β -N-acetylglucosaminidase H.

EDTA to inhibit the residual α -mannosidase activity shown by the inhibition of release of radioactive mannose from $\text{Glc}_3([\text{}^3\text{H}]\text{Man})_9\text{GlcNAc}$ and $[\text{}^{14}\text{C}]\text{Man}_9\text{GlcNAc}$; no effect on the activity of the α -glucosidase was detected. For inhibition of glucosidase with bromoconduritol, see Fig. 3. Glycosylation *in vitro* was done as described (8). Pronase digestion of glycoproteins and treatment with endo H and α -mannosidase were as described (9). Virus quantitation was done by the plaque assay (13).

RESULTS

Effect of Bromoconduritol on Glycosylation in PR8-Infected Cells. The residue obtained after extracting virus-infected cells with organic solvents and water (8) contains the virus-specific glycoproteins. The virus used in this particular experiment was the influenza virus strain PR8, which has a high proportion of complex oligosaccharides (14). An electropherogram of this residue from chicken embryo cells infected with virus and labeled with $[\text{}^3\text{H}]\text{mannose}$ is shown in Fig. 1A (*Inset*). The two glycoproteins hemagglutinin and neuraminidase were detected. Digestion of this residue with Pronase gave rise to glycopeptides of the high-mannose and complex types. Subsequent treatment with endo H cleaved high-mannose oligosaccharides from the peptide to yield free oligosaccharides lacking the GlcNAc residue that was attached to asparagine. Complex glycopeptides are not cleaved by the enzyme. The elution pattern from a Bio-Gel P-4 column of glycopeptides and oligosaccharides obtained after endo H treatment of the Pronase digest is shown in Fig. 1A. The complex glycopeptides eluted before the marker $[\text{}^{14}\text{C}]\text{Glc}_3\text{Man}_9\text{GlcNAc}$ (arrow), and the high-mannose oligosaccharides eluted after this marker.

The results obtained from PR8-infected cells treated with the α -glucosidase inhibitor bromoconduritol are shown in Fig. 1B. The inhibitor (2 mM) was added to the cells 3.5 hr after infection (i.e., 30 min before addition of $[\text{}^3\text{H}]\text{mannose}$) and was present during the labeling period. The incorporation of $[\text{}^3\text{H}]\text{mannose}$ into the viral glycoproteins was inhibited slightly, but the same glycoproteins were detected in the same ratio (*Inset*). However, the analysis on Bio-Gel P-4 columns of the endo H-treated Pro-

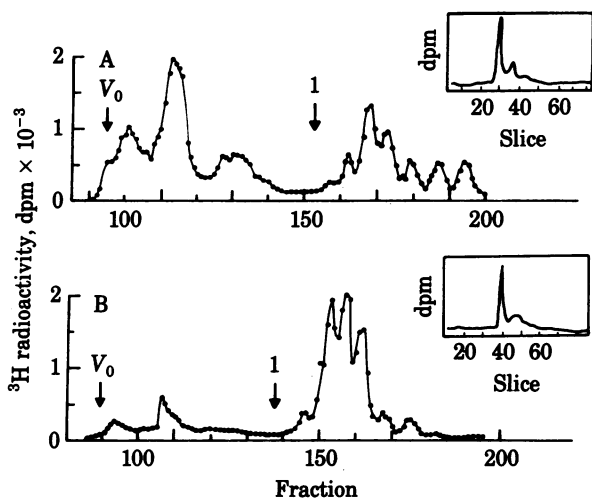


FIG. 1. Bio-Gel P-4 chromatography of $[\text{}^3\text{H}]\text{mannose}$ -labeled oligosaccharides and glycopeptides from nontreated (A) or bromoconduritol-treated cells (B). Cells infected with influenza virus PR8 were labeled with $[\text{}^3\text{H}]\text{mannose}$ 4–7 hr after infection in the absence (A) or presence (B) of 2.5 mM bromoconduritol (added 30 min prior to addition of the label). One portion of the lipid-free residue was subjected to electrophoresis (*Insets*) and another portion was digested with Pronase and then with endo H. Arrows: 1, elution position of the standard $\text{Glc}_3\text{Man}_9\text{GlcNAc}$; V_0 , void volume (bovine serum albumin).

nase digest showed that the formation of complex oligosaccharides was strongly inhibited. Also, a set of different high-mannose oligosaccharides was obtained (see below).

Bromoconduritol Inhibits the Formation of Complex Oligosaccharides Preferentially. Bromoconduritol does not inhibit uptake by the infected cells of radiolabeled mannose, fucose, and galactose. The incorporation of $[\text{}^3\text{H}]\text{mannose}$ into dolichol diphosphate-linked oligosaccharides, extracted from intact cells (8), was increased in the presence of bromoconduritol by a factor of 3. On the other hand, the *in vitro* formation of dolichol diphosphate-linked oligosaccharides by a microsomal preparation from chicken embryo cells (8) was not affected by bromoconduritol (results not shown).

The incorporation of ^{14}C -labeled amino acids into fowl plague viral proteins in the presence of inhibitor is shown in Fig. 2A. When labeling periods of 15 min were used, only a small decrease of incorporation into proteins was observed.

$[\text{}^3\text{H}]\text{Fucose}$ specifically labels the complex oligosaccharide chains of the influenza virus glycoproteins (14). As expected from the results shown in Fig. 1, the incorporation of $[\text{}^3\text{H}]\text{fucose}$ into viral glycoproteins was inhibited by bromoconduritol; however, the extent of inhibition was dependent on the time of addition of the inhibitor relative to that of the labeled compound (Fig. 2B). This was expected because fucose is incorporated into existing processed oligosaccharide chains. The incorporation of $[\text{}^3\text{H}]\text{mannose}$ into total oligosaccharides was decreased only slightly by high concentrations of bromoconduritol (Fig. 2A; see also the analysis of high-mannose oligosaccharides below). Therefore, the inhibition of formation of complex oligosaccharides by bromoconduritol cannot be caused by inhibition of protein synthesis, or by inhibition of formation of lipid-linked oligosaccharides, or by inhibition of transfer of the oligosaccharides to protein.

Inhibition of Glucose Trimming *in Vitro*. To show directly that the glucosidase inhibitor bromoconduritol inhibits the

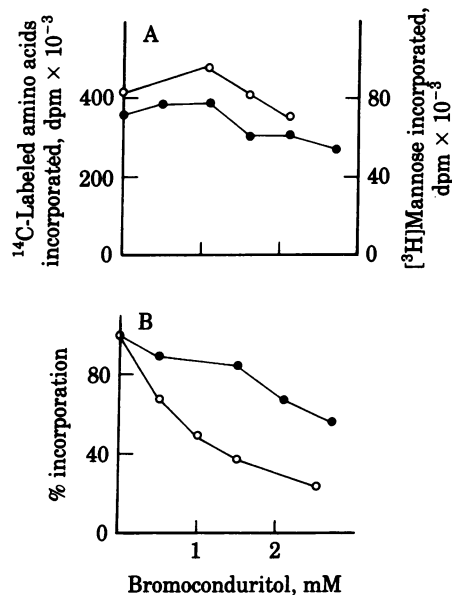


FIG. 2. Effect of bromoconduritol on the incorporation of $[\text{}^3\text{H}]\text{mannose}$ and ^{14}C -labeled amino acids (A) and of $[\text{}^3\text{H}]\text{fucose}$ (B) into viral glycoproteins. Virus-infected cells were treated with bromoconduritol 30 min before addition of the labeled sugars or 2 hr before addition of the isotope. The radioactivity in the lipid-free glycoprotein-containing fraction was determined. Labeling time was 15 min (^{14}C -labeled amino acids) or 60 min ($[\text{}^3\text{H}]\text{sugars}$). (A) ●, ^{14}C -Labeled amino acids; ○, $[\text{}^3\text{H}]\text{mannose}$. (B) ●, Cells were treated with bromoconduritol for 30 min; ○, cells were treated with bromoconduritol for 120 min.

trimming of glucose residues from the precursor oligosaccharide $\text{Glc}_3\text{Man}_9\text{GlcNAc}$, a microsomal preparation from rat liver was obtained (12) and incubated with this oligosaccharide labeled in the glucose residues. It is shown in Fig. 3 that glucose is removed from the oligosaccharide in a pH 6.8 sodium phosphate buffer. At pH 4.6 (in citrate phosphate buffer), no glucose release was detected after incubation for 90 min (not shown) whereas, in this buffer at pH 6.5, 47% of the glucose in the original oligosaccharide was released after 90 min as free glucose. This result indicates that the trimming of glucose residues was caused by the neutral glucosidases rather than by the lysosomal acid α -glucosidase.

The addition of bromoconduritol decreases the rate of glucose release (Fig. 3). Of the two isomers (bromoconduritol A and bromoconduritol B), bromoconduritol A was the stronger inhibitor (Fig. 3). This may be related to the fact that the half-life for decomposition of bromoconduritol A into conduritol B and F in water at pH 7.3 and 37°C (16 min) is higher than that of bromoconduritol B (2 min). Bromoconduritol A and B were each contaminated with about 5% of the other isomer, and we ascribe the smaller difference in the inhibitory effects of the higher concentration to this fact. Other derivatives, conduritol B and conduritol epoxide (up to 5 mM), had no effect on glucose release.

The analysis on Bio-Gel P-4 columns of the oligosaccharides after release of 40% or 60% of the glucose as free glucose showed that only the original material was present (Fig. 4A). Incubation of the microsomal enzyme preparation with $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ labeled in the mannose residues did not result in the release of significant amounts of free mannose, and analysis of the oligosaccharides (Fig. 4B) shows some $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ but mainly $\text{Man}_9\text{GlcNAc}$. Thus, under the particular conditions of the assay, the reaction catalyzed is the conversion of $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ to $\text{Man}_9\text{GlcNAc}$.

The analysis of the oligosaccharides obtained after glucosidase treatment of (^3H) $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ in the presence of 5 mM bromoconduritol is shown in Fig. 4C. Three major oligosaccharides are seen, differing from one another by one hexose unit. Because of the absence of mannosidase activity, the oligosaccharides are $\text{Glc}_3\text{Man}_9\text{GlcNAc}$, $\text{Glc}_2\text{Man}_9\text{GlcNAc}$, and $\text{GlcMan}_9\text{GlcNAc}$. After incubation of $\text{Glc}_3(^3\text{H})\text{Man}_9\text{GlcNAc}$ in the presence of 5 mM bromoconduritol, the pattern of Fig. 4D is obtained. It shows the presence of $\text{Glc}_3\text{Man}_9\text{GlcNAc}$, $\text{Glc}_2\text{Man}_9\text{GlcNAc}$, $\text{GlcMan}_9\text{GlcNAc}$, and $\text{Man}_9\text{GlcNAc}$ in the molar proportions 1.8:5.6:10.9:1.0. Clearly, in the presence of

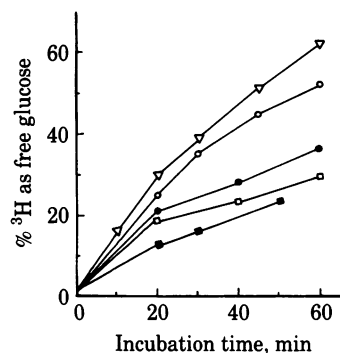


FIG. 3. Effect of bromoconduritol on the release of (^3H)glucose from (^3H) $\text{Glc}_3\text{Man}_9\text{GlcNAc}$. A rat liver microsomal enzyme preparation (12), previously incubated for 2 min with the indicated amount of bromoconduritol, was incubated with (^3H) $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ and, at the times indicated, the relative proportion of free (^3H)glucose was determined by paper chromatography as described in ref. 12. ∇ , No additions; \bullet and \blacksquare , 2.5 and 5 mM bromoconduritol A; \circ and \square , 2.5 and 5 mM bromoconduritol B.

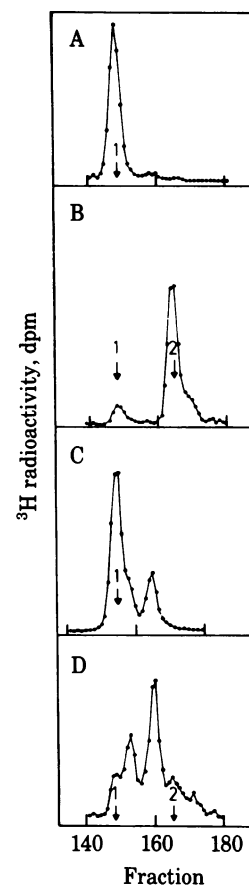


FIG. 4. Bio-Gel P-4 chromatography of oligosaccharides formed after incubating rat liver glucosidase with $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ in the absence (A and B) and presence (C and D) of bromoconduritol. $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ labeled in the glucose (A and C) or in the mannose (B and D) residues was incubated with rat liver glucosidase previously incubated with (C and D) or without (A and B) bromoconduritol (5 mM). After release of 60% of the glucose from $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ in the absence of inhibitor (A), the reactions were stopped by boiling the mixtures for 3 min. Internal ^{14}C -labeled standards were added before chromatography: 1, $\text{Glc}_3\text{Man}_9\text{GlcNAc}$; 2, $\text{Man}_9\text{GlcNAc}$.

bromoconduritol, the trimming of the innermost glucose residue is inhibited.

Inhibition of Glucose Trimming *in Vivo*. To detect glycosylated high-mannose oligosaccharides attached to viral glycoproteins, primary chicken embryo cells infected with fowl plague virus were labeled with (^3H)mannose or (^3H)galactose for 40 min and then chased for 2 hr. The inhibitor (2.4 mM) was added 90 min before the isotopes and was also present in the chase medium. The viral glycoproteins were isolated and digested with Pronase and then with endo H. The mixture of glycopeptides and oligosaccharides was separated on a Bio-Gel P-4 column (Fig. 5).

The formation of (^3H)mannose-labeled complex oligosaccharides (eluted between fractions 90 and 120) was inhibited 60% in this particular experiment in cells treated with bromoconduritol, whereas the formation of high-mannose oligosaccharides was increased 1.4-fold. Furthermore, the pattern of high-mannose oligosaccharides from inhibitor-treated cells was clearly different from that of untreated cells (Fig. 5 A and B). The fractions under peaks a, b, c, d, e, and f were pooled separately, and the oligosaccharides were analyzed by glucosidase and α -mannosidase treatment. After glucosidase treatment (using the rat liver enzyme preparation), the oligosaccharides were mixed with ^{14}C -labeled standards— $\text{Glc}_3\text{Man}_9\text{GlcNAc}$,

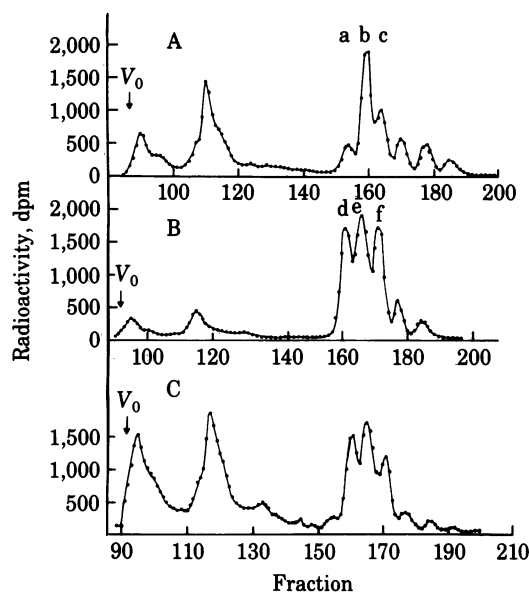


FIG. 5. Chromatography on Bio-Gel P-4 columns of oligosaccharides and glycopeptides from [^3H]mannose-labeled (A and B) or [^3H]galactose-labeled (C) virus-infected cells treated or not treated with bromoconduritol. Cells infected with fowl plague virus were incubated for 90 min with (B and C) or without (A) 2.4 mM bromoconduritol before the addition of [^3H]mannose or [^3H]galactose. After 40 min, the labeling medium was removed and the cells were incubated for 2 hr in unlabeled medium. The glycoprotein fraction was isolated and digested with Pronase and then with endo H. V_0 , elution position of bovine serum albumin. For peaks a-f, see Fig. 6 and the text.

$\text{Man}_9\text{GlcNAc}$, $\text{Man}_8\text{GlcNAc}$, and $\text{Man}_7\text{GlcNAc}$ —and then separated on Bio-Gel P-4 columns.

Oligosaccharide d, untreated, eluted at the position of $\text{GlcMan}_9\text{GlcNAc}$ and, after glucosidase treatment, was shifted one hexose unit on the column to coelute with $\text{Man}_9\text{GlcNAc}$ (Fig. 6A). Consistent with the identity of d as $\text{GlcMan}_9\text{GlcNAc}$ is the result that, after α -mannosidase treatment, no β - ManGlcNAc was detected on TLC of the digest (cf. ref. 9). Fig. 6A also shows that glucose removal by the glucosidase is almost quantitative. Oligosaccharide e, untreated, eluted slightly ahead of $\text{Man}_9\text{GlcNAc}$ and, after glucosidase treatment, a part was shifted one hexose unit to coelute with $\text{Man}_9\text{GlcNAc}$ (Fig. 6B). Thus, oligosaccharide e contains $\text{GlcMan}_8\text{GlcNAc}$. After α -mannosidase digestion, 2% of the radioactivity was found in β - ManGlcNAc , indicating the presence of some nonglycosylated species, possibly $\text{Man}_9\text{GlcNAc}$. Oligosaccharide f, untreated, eluted slightly ahead of the standard $\text{Man}_8\text{GlcNAc}$ and, after glucosidase treatment, a part was shifted one hexose unit to coelute with $\text{Man}_7\text{GlcNAc}$ (Fig. 6C). Thus, oligosaccharide f contains $\text{GlcMan}_7\text{GlcNAc}$. The fact that glucosidase treatment of oligosaccharides e and f does not go to completion is consistent with the result (15) that glucosidase activity is hampered by the removal of the peripheral mannose residues. Treatment of oligosaccharide f with α -mannosidase gives 4% of the radioactivity as β - ManGlcNAc , indicating the presence of some nonglycosylated oligosaccharide. Oligosaccharide a behaved as oligosaccharide d, and oligosaccharides b and c, coeluting with $\text{Man}_9\text{GlcNAc}$ and $\text{Man}_8\text{GlcNAc}$, respectively, had the same mobilities after glucosidase treatment.

Consistent with the presence of glucose in oligosaccharides a, d, e, and f is the fact that they can be labeled with [^3H]galactose (Fig. 5C). Incubation of these oligosaccharides, labeled in the glucose residues, with the rat liver glucosidase in the presence of bromoconduritol completely inhibited glu-

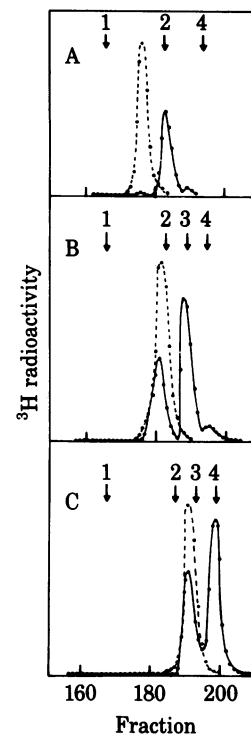


FIG. 6. Chromatography on Bio-Gel P-4 columns of oligosaccharides d (A), e (B), and f (C) before (---) and after (—) treatment with rat liver glucosidase. Oligosaccharides d, e, and f (Fig. 5B) were incubated with rat liver glucosidase for 120 min. The reaction was stopped by boiling the mixture for 3 min, and [^{14}C]labeled standards (1, $\text{Glc}_3\text{Man}_9\text{GlcNAc}$; 2, $\text{Man}_9\text{GlcNAc}$; 3, $\text{Man}_8\text{GlcNAc}$; 4, $\text{Man}_7\text{GlcNAc}$) were added.

cose release. Taken together, these results indicate that the mechanism of inhibition of bromoconduritol is the same in intact cells and in a rat liver enzyme preparation.

Effect of Bromoconduritol on Virus Formation. Three hours after infection, chicken embryo cells infected with fowl plague virus were treated with bromoconduritol (2.4 mM and 4.8 mM) or tunicamycin at $2\ \mu\text{g}/\text{ml}$ or not treated. One hour later, $50\ \mu\text{Ci}$ of [^{35}S]methionine was added. The virus was harvested 8 hr after infection and layered on a sucrose gradient. In a separate experiment, the infectivity was determined. Fig. 7 shows that incorporation of radioactivity into virus particles (fractions 5, 6, and 7) is inhibited 75% by 2.4 mM bromoconduritol (Fig. 7B) and completely by 4.8 mM bromoconduritol (Fig. 7C) and by tunicamycin (Fig. 7D). In parallel, a 90–98% inhibition of infectivity (measured as plaque-forming units per ml) was measured with 2.4 mM bromoconduritol. Apparently, the release of infectious virus is inhibited when the formation of complex oligosaccharides is inhibited. It remains to be established whether the lack of complex oligosaccharides *per se* or secondary effects caused by inhibition of processing (such as those resulting in increased proteolytic susceptibility of the viral glycoproteins) are responsible for the inhibition of formation of infectious virus.

DISCUSSION

The α -glucosidase inhibitor bromoconduritol inhibits trimming of glucose residues from the glycosylated protein-linked precursor oligosaccharide for high-mannose and complex oligosaccharide side chains of viral glycoproteins. Also, by using a microsomal glucosidase, it could be shown directly that bromoconduritol inhibits the deglycosylation of $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ to $\text{Man}_9\text{GlcNAc}$. There is no indication that bromoconduritol in-

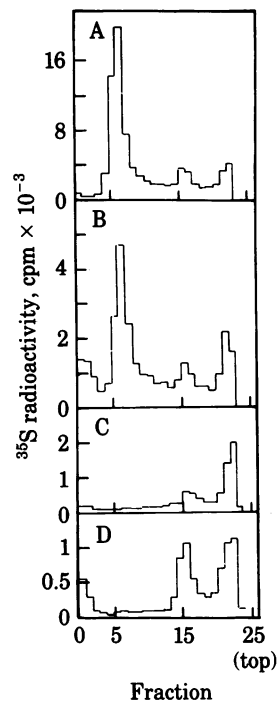


FIG. 7. Sucrose density gradient analysis of virus particles labeled with [^{35}S]methionine. Fowl plague virus-infected cells were labeled with [^{35}S]methionine from 4 to 8 hr after infection; 2.4 mM (B) or 4.8 mM (C) bromoconduritol or tunicamycin at 2 $\mu\text{g}/\text{ml}$ (D) was added 1 hr before addition of the isotope. (A) Control (no sugar analog or drug). (Note the different ordinate scales.) The virus was pelleted from the medium by centrifugation at $100,000 \times g$ for 1 hr, layered on a 10-ml sucrose gradient (15–40%) containing a cushion of 1 ml of 50% sucrose at the bottom, and centrifuged for 2 hr at 40,000 rpm (SW 41 rotor).

hibits the formation of the lipid-linked oligosaccharide or the transfer of the oligosaccharide from the lipid to the protein. Thus, the observed inhibition of formation of complex oligosaccharides in bromoconduritol-treated cells is probably caused by inhibition of glucose trimming.

Indeed, glucosylated high-mannose oligosaccharides were isolated from bromoconduritol-treated cells after endo H treatment and their compositions were determined to be $\text{GlcMan}_9\text{GlcNAc}$, $\text{GlcMan}_8\text{GlcNAc}$, and $\text{GlcMan}_7\text{GlcNAc}$. This result has three implications. (i) Mannose trimming can occur without complete removal of glucose. This phenomenon has been observed previously as a minor pathway (16). (ii) The glucosylated glycoprotein can leave the rough endoplasmic reticulum, because the $\alpha(1\rightarrow2)$ mannosidase is located in the Golgi complex (2). (iii) Bromoconduritol does not inhibit this mannosidase activity.

The results indicate that bromoconduritol does not inhibit the activity of glucosidase I (Glc_3 -glucosidase), because oligosaccharides containing three glucoses did not accumulate. However, if bromoconduritol were to affect only the catalytic activity of glucosidase II (Glc_2 -glucosidase), oligosaccharides containing

two glucose residues rather than one should be found. The active site of glucosidase II may contain at least two glucose-binding sites since nigerose is a competitive inhibitor of this enzyme (17). Possibly, therefore, bromoconduritol interferes with the active site of the enzyme, preventing binding of the oligosaccharide after release of the glucose group from $\text{Glc}_2\text{Man}_9\text{GlcNAc}$, rather than with the catalytic site of the enzyme. This proposal avoids postulating a third glucosidase.

In tunicamycin-treated fowl plague virus-infected chicken embryo cells, the hemagglutinin is not glycosylated and virus formation is blocked (11). In bromoconduritol-treated fowl plague virus-infected chicken embryo cells, the hemagglutinin is glycosylated, but with mainly high-mannose oligosaccharides, and yet the release of infectious virus is inhibited. This suggests a role for glycoprotein processing in virus formation in this particular system. This conclusion seems to be in accord with the results from a survey of 19 different influenza virus strains grown in chicken cells (14), in which it was shown that hemagglutinin molecules containing only high-mannose oligosaccharides do not occur in intact viruses.

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