Streptovirudins of Series I and II: Chemical and Biological Properties†

Alan D. Elbein,* John L. Occolowitz, Robert L. Hamill, and Klaus Eckardt

ABSTRACT: Streptovirudins are tunicamycin-related antibiotics that have been separated into two distinct series, referred to as series I and II. Streptovirudins of both series contain glucosamine, but those of series I show no ultraviolet light absorption and do not contain uracil [Eckardt, K., Wetzstein, H., Thrum, H., & Ihn, W. (1980) *J. Antibiot. 38*, 908–910]. Field desorption mass spectrometry showed that the molecular masses of the series I streptovirudins were homologously related (790–818), with molecular masses 2 daltons greater than those of the corresponding members of the series II. The differece in molecular masses between corresponding members of the series was found to be due to the replacement of uracil in series II by dihydrouracil in series I. This conclusion is a consequence of the observation of fragments at m/z 114 ($C_4H_6N_2O_2$, dihydrouracil) and m/z 112 ($C_4H_4N_2O_2$, uracil) in the field desorption spectra of the series I and II, respectively. Thus the molecular masses of the streptovirudins are as follows: A_1, 790; A_2, 788; B_1, 804; B_2, 802; C_1, 818; C_2, 816. Both the series I and series II streptovirudins inhibited the in vitro synthesis of dolichylpyrophosphoryl-GlcNAc by using a pig aorta solubilized GlcNAc-1-P transferase (50% inhibition at 5–10 ng/mL), but the series II compounds were somewhat more active. However, while the series II compounds inhibited the synthesis of dolichylphosphorylglucose to about the same extent as tunicamycin (50% inhibition at 0.5–1.5 μg/mL), the series I compounds were much less active (50% inhibition at 50–100 μg/mL). All of the streptovirudins exhibited the typical competitive type of inhibition with respect to the substrates UDP-GlcNAc and UDP-glucose. However, with respect to the other reaction components, dolichyl phosphate, Mn^{2+}, or Nonidet P-40, the inhibition was not competitive. In cultured MDCK cells, the various streptovirudins inhibited the incorporation of [^{3}H]mannose into the lipid-linked oligosaccharides and into protein. But in this case, some differences were observed in the activity of the different fractions.

The streptovirudins are a group of antibiotics that are produced by *Streptomyces griseofulvis* and are chemically and biologically related to the tunicamycin group of antibiotics (Eckardt et al., 1975; Thrum et al., 1975). Thus, streptovirudin, like tunicamycin, is produced as a complex containing a number of closely related compounds. Although the detailed chemical structure of the streptovirudins has not been reported, these compounds do contain glucosamine and uracil (Eckardt et al., 1975). The streptovirudins, like tunicamycin, are potent inhibitors of the UDP-GlcNAc-dolichyl-P:GlcNAc-1-P transferase that is involved in the synthesis of dolichylpyrophosphoryl-GlcNAc, and they also inhibit the formation of dolichylphosphorylglucose (Elbein et al., 1979).

Recently the streptovirudin complex was separated by gel filtration and reverse-phase high-performance liquid chromatography into individual components of the two series, designated series I and II. Three components of each series were isolated and designated A_1, B_1, and C_1 and A_2, B_2, and C_2. Each of the components of both series contained glucosamine, but while the series II compounds had uracil, those of series I did not contain uracil and were not ultraviolet light absorbing. Instead, the series I compounds had an unidentified ninhydrin-positive spot not seen in the series II components (Eckardt et al., 1980).

In this paper, we show that each of the series I components differs from its corresponding member of series II by a mass of 2 (i.e., A_1 = 790, A_2 = 788; B_1 = 804, B_2 = 802; C_1 = 818, C_2 = 816). This and other mass spectral data indicate that the series I compounds contain dihydrouracil rather than uracil. This would explain both the absence of ultraviolet light absorption and the retention of biological activity. Both the series I and series II compounds were potent inhibitors of the formation of dolichylpyrophosphoryl-GlcNAc. However, while the series II compounds also inhibited the formation of dolichylphosphorylglucose to about the same extent as tunicamycin, the series I compounds showed very low activity toward this enzyme. The inhibition by the streptovirudins was shown to be of a competitive nature with respect to the substrates, UDP-GlcNAc and UDP-glucose, but was not competitive with the other reaction components, dolichyl-P, Mn^{2+}, and Nonidet P-40.

Experimental Procedures

Materials. UDP-[^{3}H]GlcNAc (6.6 Ci/mmol), UDP-[^{3}H]glucose (5 Ci/mmol), and GDP-[^{14}C]mannose (200 μCi/mmol) were obtained from New England Nuclear, and [2-^{3}H]mannose (10 Ci/mmol) and [^{3}H]leucine (15 Ci/mmol) were from Amersham Co. Dolichyl phosphate, unlabeled sugar nucleotides, and other biochemicals were from Sigma Chemical Co. or from Calbiochem. All other chemicals were from reliable chemical sources and were of the best grade available.

Mass Spectrometry and Nuclear Magnetic Resonance Determinations. Field desorption (FD) spectra were determined on a Varian-MAT 731 mass spectrometer using carbon dendrite emitters grown on 10 μM tungsten wires. Elemental compositions were determined from the results obtained by field ionization (FI)/FD peak matching at a resolution of 4000. Calibration for the peak matching experiments was performed in the FI/FD mode with xylene and uracil as mass standards. The streptovirudins field-desorbed at emitter currents of approximately 18 mA at an ion source temperature of 120 °C. The ^{1}H NMR spectra were obtained on a Varian HA1000 instrument with the samples in pyridine-d_5.

Preparation and Assay of Enzyme Fractions. The intimal layer from fresh pig aorta was homogenized, and a particulate

† From the Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78284 (A.D.E.), Lilly Research Laboratories, Indianapolis, Indiana 46285 (J.L.O. and R.L.H.), and Zentralinstitut für Mikrobiologie und Experimentalle Therapie, 69 Jena, German Democratic Republic (K.E.). *Received October 29, 1980.* Supported by grants from the National Heart, Blood and Lung Institute (HL 17783) and the National Institute of Arthritis and Metabolic Diseases (AM 21800).

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enzyme fraction was isolated as described previously (Chambers & Elbein, 1975). This particulate fraction was solubilized by mixing it with 0.5% Nonidet P-40 (NP-40) and allowing it to stir in an ice bath for several minutes. The mixture was centrifuged at 100000*g* for 45 min, and the supernatant liquid was used as the soluble enzyme preparation (Heifetz & Elbein, 1977).

A typical assay mixture with the particulate enzyme contained the following components in a final volume of 0.5 mL: 10 μmol of tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.5, 2.5 μmol of $MnCl_2$, 100000 cpm of labeled sugar nucleotide (UDP-[^{3}H]GlcNAc, UDP-[^{3}H]glucose, or GDP-[^{14}C]mannose), various concentrations of the streptovirudin fractions as shown in the figures, and 100 μL of particulate enzyme (1–2 mg of protein). Incubations were done at 37 °C for GlcNAc and mannose incorporation and at room temperature for glucose incorporation. In some experiments, the concentration of UDP-glucose was varied in order to study the nature of the streptovirudin inhibition. For these experiments, labeled UDP-glucose was mixed with varying concentrations of unlabeled UDP-glucose, and appropriate amounts of the mixtures were used.

For assays with the soluble enzyme, it was necessary to add dolichyl-P as a glycosyl acceptor. In these experiments, 5 μg of dolichyl-P dissolved in 10 μL of chloroform was usually added to each tube, and the solvent was removed with a stream of air. One hundred microliters of 1% NP-40 was then added to suspend the lipid, and the tubes were mixed vigorously. The other reaction components were added in the following order with mixing between each addition: 10 μmol of Tris buffer, pH 7.5, 2.5 μmol of $MnCl_2$, the appropriate concentration of antibiotic, 100000 cpm of sugar nucleotide, and 100 μL of soluble enzyme (0.5–1 mg of protein). In some experiments, the concentrations of the various reaction components (UDP-GlcNAc, dolichyl-P, $MnCl_2$, or NP-40) were varied in order to determine the nature of the streptovirudin inhibition.

Incubations were done at room temperature or at 37 °C for the times indicated, and reactions were terminated by the addition of 2 mL of $CHCl_3/CH_3OH$ (1:1) and 0.5 mL of water. After thorough mixing, the phases were separated by centrifugation, and the dolichyl-linked monosaccharides were isolated as previously described (Chambers & Elbein, 1975; Heifetz & Elbein, 1977). The radioactive contents of these lipids were determined by scintillation counting as a measure of their synthesis. A number of control experiments were done in which antibiotic was added at the end of the incubations to be certain that it had no effect on the extraction of the various lipids. Another series of controls involved the addition of alkali to incubation mixtures, since the streptovirudin is dissolved in dilute alkali (0.01 N), to be certain that this did not cause any inhibition. All of these controls showed the same level of enzymatic activity as those incubations that did not contain antibiotic.

Effect of Streptovirudin on MDCK Cells. The effect of streptovirudin on the synthesis of influenza viral glycoproteins was studied in a stable canine kidney cell line (MDCK cells). Cells were grown in Eagle basal medium supplemented with 10% fetal calf serum, 0.03% glutamine, and 20 μg of neomycin/mL. Forty-eight hours before the start of an experiment, the cells were plated into a 6-well Linbro dish (Linbro Scientific Co.) in the above medium and incubated at 34 °C in air containing 5% CO_2. The cells reached confluency in 48 h, at which time each well contained about 5.5×10^6 cells. Cell viability was determined by trypan dye exclusion. The confluent monolayers were infected with the NWS strain of influenza virus as previously described (Sanford et al., 1978). After incubation for 1 h to allow the virus to penetrate, the monolayers were placed in Eagle basal medium with 2% fetal calf serum, and various amounts of the streptovirudin fractions were added. Cells and antibiotic were incubated for 1 h in order to let the antibiotic take effect, and then 2 μCi of [^{3}H]mannose or [^{3}H]leucine was added. After incubation with the radioisotopes for 3 h, the monolayers were washed several times with phosphate-buffered saline solution. The cell monolayers were dislodged from the plates by digestion with trypsin–EDTA, and the cells were extracted with $CHCl_3/CH_3OH$ to obtain lipid-linked monosaccharides, lipid-linked oligosaccharides, and glycoproteins (Pan et al., 1979). The radioactivity in these various materials was determined by scintillation counting.

Table I: Field Desorption Mass Spectrometry and Nuclear Magnetic Resonance Spectrometry of the Streptovirudin Components

streptovirudin component	molecular[a] mass (FDMS)	prominent mass fragment (for identification of the base)	fatty acid[b] type (NMR)
A_1	790	114	I
B_1	804	114	N
C_1	818	114	I
A_2	788	112	I
B_2	802	112	N
C_2	816	112	I

[a] The molecule is cationized during the FD process and is observed at (M + Na), e.g., for A_1 at m/z 813. [b] I, iso fatty acid [δ 0.87 (doublet) indicated isopropyl group]; N, normal fatty acid.

Results

Chemistry of the Streptovirudins. The individual streptovirudins of series I (A_1, B_1, and C_1) as well as those of series II (A_2, B_2, and C_2) were analyzed by field desorption mass spectrometry to determine their molecular weights. These results are presented in Table I. The molecule is cationized during the FD process and is observed at (M + Na), e.g., for A_1 at m/z 813. It can be seen that B_1 (M_r 804) differed from A_1 (M_r 790) by 14 mass units, while C_1 (M_r 818) was 14 mass units higher than B_1. These results suggested the presence of an additional methylene group in going from A_1 to B_1 to C_1. On the basis of similar studies with tunicamycin (Ito et al., 1980), it seems likely that A_1, B_1, and C_1 differ from each other in the length of the fatty acid chain; i.e., B_1 would have a fatty acid containing one carbon more than that of A_1, while C_1 would have a fatty acid one carbon longer than that of B_1. A similar situation applies to the components of series II. That is B_2 (M_r 802) is 14 mass units higher than A_2 (M_r 788), while C_2 (M_r 816) is 14 mass units higher than B_2. NMR studies (Table I) indicated the nature of the fatty acid moieties (Ito et al., 1980). Component C_2 is identical with tunicamycin A_1 (Keenan et al., 1981).

Table I also shows a comparison of the molecular weights of series I components to those of the corresponding series II components. In this case, it can be seen that the corresponding members (i.e., A_1–A_2, B_1–B_2, and C_1–C_2) differed from each other by a mass of 2 daltons. Since components of series I do not show any ultraviolet light absorption, it seemed likely that this increase in mass could be due to reduction of uracil to dihydrouracil. This hypothesis was confirmed by the finding to a fragment at m/z 114 in each of the series I components while the series II members had a fragment at m/z 112. The fragment at m/z 112 ($C_4H_4N_2O_2$) is indicative of uracil, while

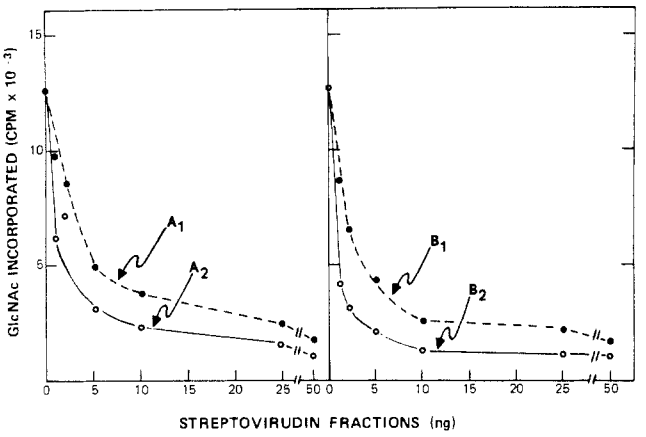

FIGURE 1: Effect of streptovirudin components on the incorporation of GlcNAc from UDP-[^{3}H]GlcNAc into dolichylpyrophosphoryl-GlcNAc. Incubations were as described in the text with 100 μL of soluble enzyme (0.5–1 mg of protein) and 5 μg of dolichyl-P. Various concentrations of the streptovirudin component were added as indicated, before the addition of enzyme. After an incubation of 10 min, the GlcNAc-lipids were isolated as described.

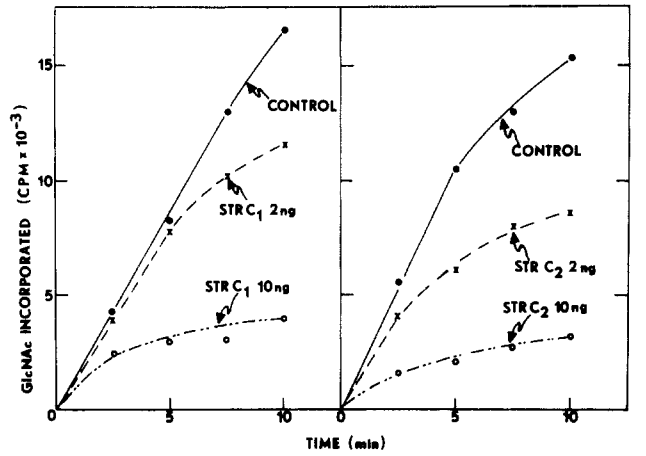

FIGURE 2: Time–course incorporation of GlcNAc from UDP-[^{3}H]-GlcNAc into dolichylpyrophosphoryl-GlcNAc in the presence of streptovirudins C_1 and C_2. Assay mixtures with the soluble enzyme were as described in the text and contained the indicated amounts of antibiotics. At the times indicated, GlcNAc-lipids were isolated as described.

that at m/z 114 ($C_4H_6N_2O_2$) represents dihydrouracil.

Streptovirudin Inhibition of Dolichylpyrophosphoryl-GlcNAc Formation. The effect of the various streptovirudin components on the synthesis of dolichylpyrophosphoryl-GlcNAc was studied by using the solubilized GlcNAc-1-P transferase. Figure 1 compares the inhibitory activity of A_1 and B_1 to that of A_2 and B_2 in terms of GlcNAc incorporation. It can be seen that the series II components were somewhat more inhibitory than those of series I, but in general, 50% inhibition required 5–10 ng of antibiotic/mL. Similar results were observed with respect to C_1 and C_2. A time–course study was done with the GlcNAc-1-P transferase as shown in Figure 2. As observed for the other series II components, C_2 was somewhat more active than C_1, but both showed good inhibition at about 10–20 ng/mL. In these experiments, antibiotic was not preincubated with enzyme, so there was some lag in the inhibition.

Streptovirudin Inhibition of Dolichylphosphorylglucose Formation. The streptovirudin components were tested to determine whether they inhibited the formation of dolichylphosphorylglucose using the particulate enzyme preparation. Figure 3 shows that the series II components (A_2 and B_2) inhibited this reaction, with 50% inhibition requiring 0.5–1 μg of antibiotic/mL. C_2 also inhibited glucose incorporation

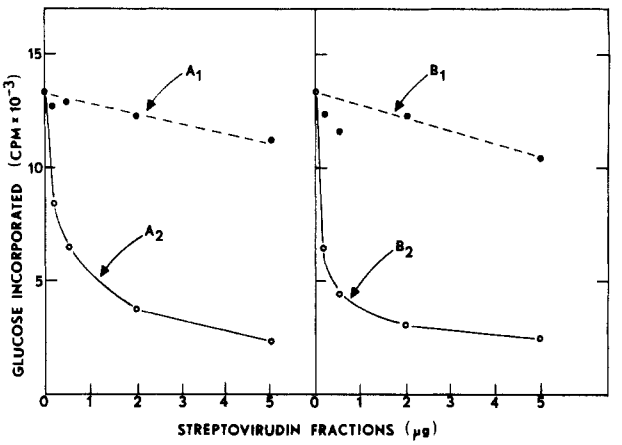

FIGURE 3: Effect of the streptovirudin components on the incorporation of glucose frm UDP-[^{3}H]glucose into dolichylphosphorylglucose. Incubations with the particulate enzyme (100 μL, 1–2 mg of protein) were as described in the text. Various concentrations of streptovirudin were added before the addition of enzyme. After incubation at room temperature for 10 min, dolichylphosphorylglucose was isolated as described.

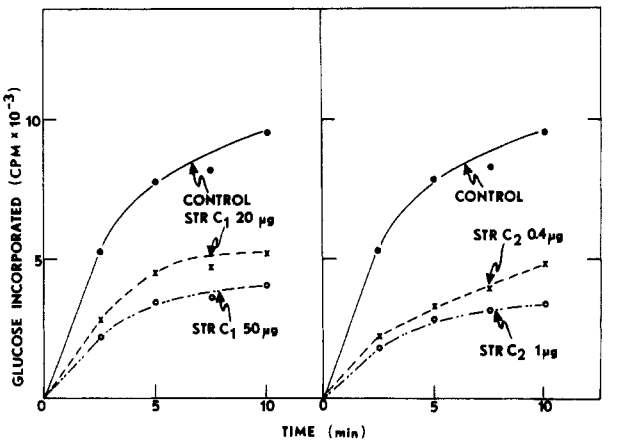

FIGURE 4: Time–course incorporation of glucose from UDP-[^{3}H]-glucose into dolichylphosphorylglucose in the presence of streptovirudins C_1 and C_2. Assay mixtures with particulate enzyme were as described in the text and contained the indicated amounts of antibiotic. The glucose-lipid (Dol-P-Glc) was isolated as described.

to the same extent. However, A_1 and B_1 (and also C_1) were not effective inhibitors and showed only slight inhibition even at 5–10 μg/mL. Figure 4 shows a time–course study on glucose incorporation in the presence of different amounts of streptovirudin C_1 or C_2. It can be seen that C_2 inhibited 50% or more at about 0.4 μg/mL but C_1 was much less effective and required 50 μg or more for the same degree of inhibition. Similar results were observed with the A_1–A_2 and B_1–B_2 components.

Effect of Sugar Nucleotide Concentration on the Streptovirudin Inhibition. In order to determine whether the streptovirudin inhibition was of a competitive nature, we examined the effect of concentration of UDP-GlcNAc and UDP-glucose on the inhibition. Figure 5 shows the effect of increasing concentrations of UDP-GlcNAc on the inhibition by B_1 (Figure 5A) and B_2 (Figure 5B). In this experiment, the soluble GlcNAc-1-P transferase was used, and concentrations of Mn^{2+} and dolichyl-P were optimum. In both cases, the data plotted by the method of Lineweaver and Burk (using the least-squares method to fit the lines) show that the streptovirudin inhibition is of a competitive nature and can be reversed by high concentrations of UDP-GlcNAc. The same results were obtained with respect to streptovirudins C_1 and C_2. The K_m for UDP-NAc was calculated to be between 2×10^{-7} and

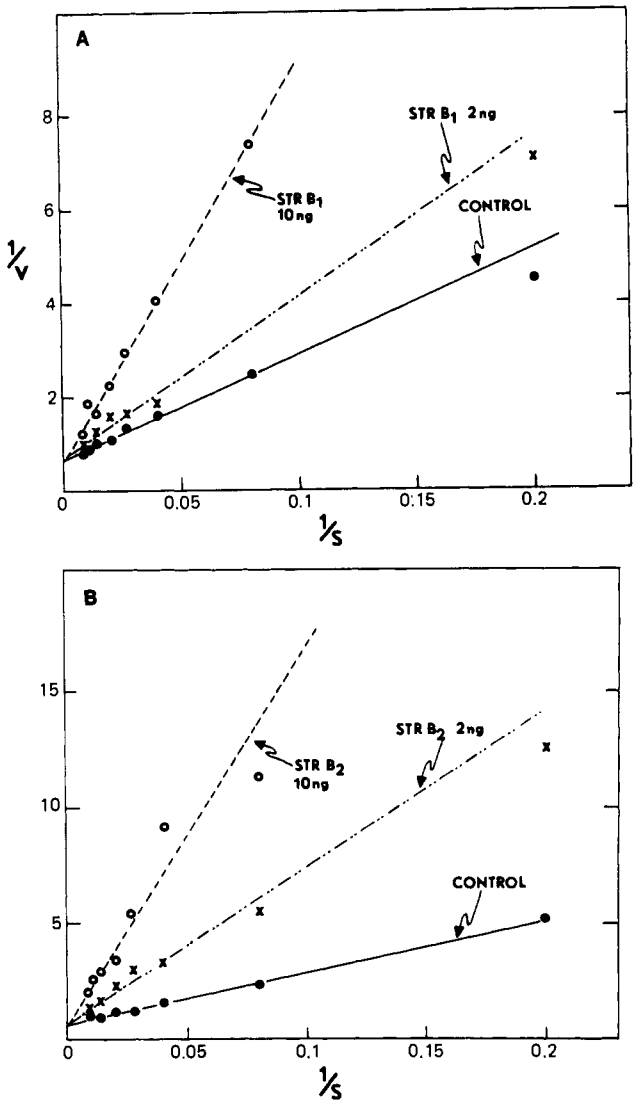

FIGURE 5: Effect of UDP-GlcNAc concentration on the inhibition by streptovirudins B_1 and B_2. Assay mixtures with the soluble enzyme were as described except that the concentration of UDP-GlcNAc was varied as indicated. (A) shows the effect of streptovirudin B_1, while (B) shows the effect of B_2. Incubations were for 10 min, and GlcNAc-lipids were isolated as described. Data was plotted according to Lineweaver and Burk.

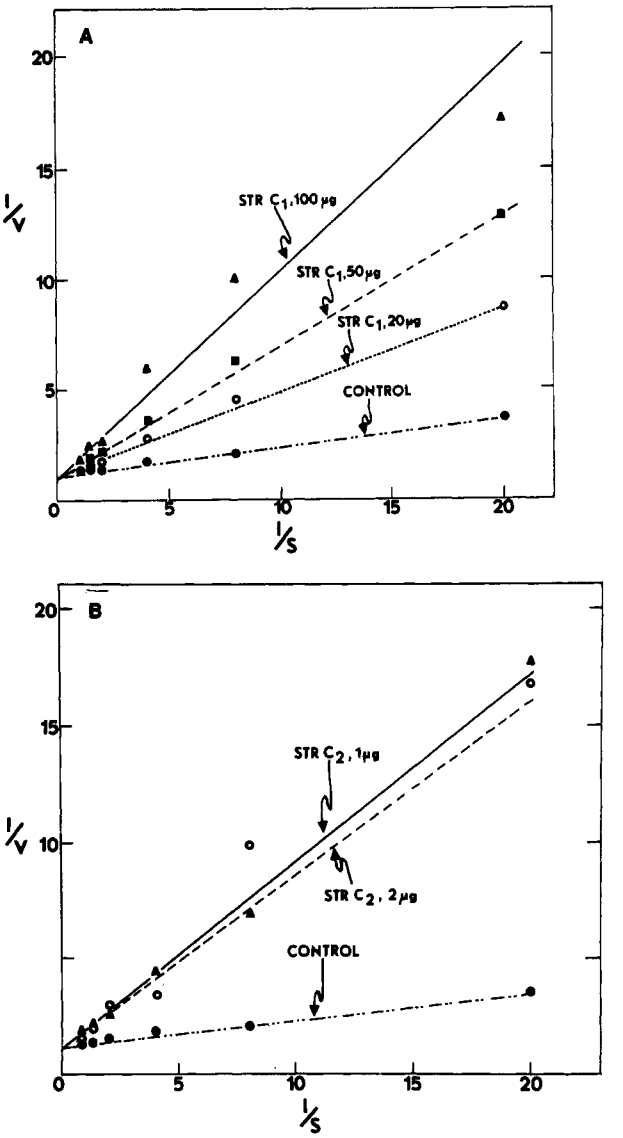

FIGURE 6: Effect of UDP-glucose concentration on the inhibition by streptovirudins C_1 and C_2. Assay mixtures with the particulate enzyme were as described in the text except that the concentration of UDP-glucose was varied as indicated. (A) shows the results with streptovirudin C_1, and (B) shows the results with C_2. After an incubation of 10 min at room temperature, the Dol-P-Glc was isolated as described. Data were plotted by the method of Lineweaver and Burk.

5×10^{-7} M, and the K_i for the streptovirudins (B_1, B_2, C_1, and C_2) was about (1–3) $\times 10^{-8}$ M.

The concentration of UDP-glucose was also varied to determine what effect this would have on the streptovirudin inhibition. Figure 6 presents the results with respect to C_1 (Figure 6A) and C_2 (Figure 6B). In these experiments, the particulate enzyme was used, and the incubations were done at room temperature because the glucosyl transferase is not too stable. The results of these experiments are plotted by the method of Lineweaver and Burk (using the least-squares method) and clearly show that both C_1 and C_2 are competitive inhibitors with respect to the concentration of UDP-glucose. The K_m for UDP-glucose was found to be about 1×10^{-7} M, while the K_i for streptovirudin C_2 was calculated to be 1×10^{-6} M and that for streptovirudin C_1 was 3×10^{-4} M.

Effect of Other Reaction Components on Streptovirudin Inhibition. The synthesis of dolichylpyrophosphoryl-GlcNAc by the soluble enzyme requires the addition of dolichyl-P as a glycosyl acceptor as well as the cation Mn^{2+} and the detergent NP-40. Therefore it was of interest to examine the effect of increasing concentrations of these compounds on the streptovirudin inhibition. Figure 7 shows the effect of increasing concentrations of dolichyl-P on the inhibition of GlcNAc incorporation by B_1 and B_2. In the control incubations (i.e., without antibiotic), dolichyl-P was optimum at about 5 µg, and these curves showed typical saturation kinetics. Streptovirudin B_2 almost completely inhibited GlcNAc incorporation at 10 ng/incubation mixture, while at 2 ng it inhibited more than 70%. Nevertheless, as shown by the curves in Figure 7B and also as demonstrated by Lineweaver–Burk plots of these data (not shown), the inhibition was not competitive. A similar situation was seen with respect to inhibition by B_1 (Figure 7A), but in this case, less inhibition occurred at 2 and 10 ng of the antibiotic. Similar results were observed when streptovirudin C_1 or C_2 was used.

The effect of Mn^{2+} concentration was also examined, as shown in Figure 8. In this experiment, the soluble GlcNAc-1-P transferase was used, and streptovirudins C_1 and C_2 were tested. In the control experiments (without antibiotic) as well as in those with antibiotic, Mn^{2+} concentration was optimum at about 2 or 3 mM, and the activity decreased slightly beyond this concentration. However, as shown in the

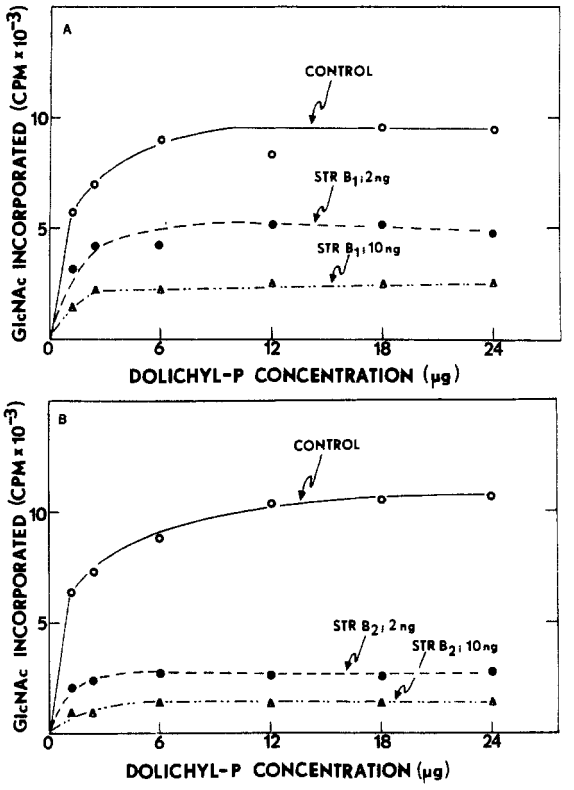

FIGURE 7: Effect of concentration of dolichyl-P on the inhibition of GlcNAc incorporation by streptovirudins B_1 and B_2. Assay mixtures with the soluble enzyme were as described in the text except that the concentration of dolichyl-P was varied as indicated. (A) Results with streptovirudin B_1; (B) with B_2. After an incubation of 10 min, GlcNAc-lipids were isolated as described.

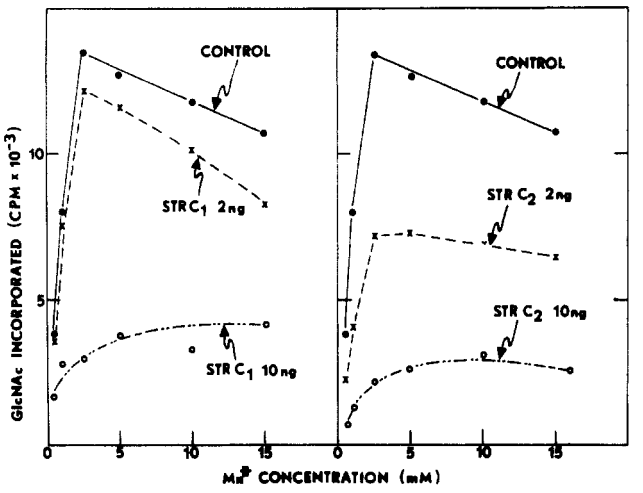

FIGURE 8: Effect of Mn^{2+} concentration on the inhibition of GlcNAc incorporation by streptovirudins C_1 and C_2. Assay mixtures with the soluble enzyme were as described except the concentration of Mn^{2+} was varied as shown. (A) Results using streptovirudin C_1; (B) results using C_2. GlcNAc-lipid was isolated as described.

figure, and as indicated by Lineweaver–Burk plots (not shown), the inhibition was not competitive.

Since these incubations with soluble enzyme require detergent (NP-40) to solubilize the dolichyl-P and since streptovirudin is a lipophilic molecule, we tested the effect of NP-40 concentration on the streptovirudin inhibition (Figure 9). Figure 9A shows the effect of increasing concentrations of NP-40 on the inhibition of dolichylpyrophosphoryl-GlcNAc by streptovirudin C_1, while Figure 9B shows the results by using C_2. It can be seen that the optimum concentration of NP-40 was about 0.2% and that inhibition occurred above this

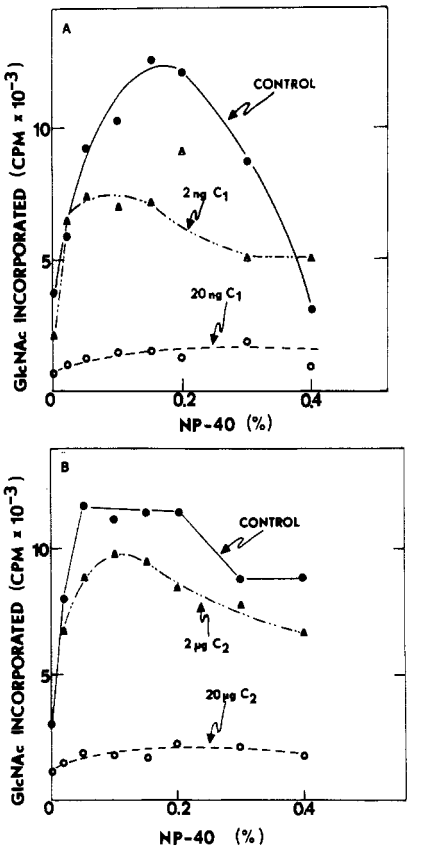

FIGURE 9: Effect of concentration of NP-40 on the inhibition of GlcNAc incorporation by streptovirudins C_1 and C_2. Incubation mixtures with soluble enzyme were as described except that the concentration of NP-40 was varied as indicated. (A) Results with streptovirudin C_1; (B) results with C_2. GlcNAc-lipid was isolated as described.

concentration. GlcNAc incorporation was inhibited by both streptovirudins C_1 and C_2, and this inhibition could not be overcome by increasing the concentration of the detergent. The extent of streptovirudin inhibition was of the same order of magnitude as seen in other experiments.

Effect of Streptovirudin Components on Protein Glycosylation and Protein Synthesis in MDCK Cells. For determination of whether the series I compounds were as effective as the series II compounds on cells in culture, these antibiotics were tested on MDCK cells to determine their effect on the incorporation of [^{3}H]mannose into lipid-linked saccharides and glycoproteins and on the incorporation of [^{3}H]leucine into protein. Influenza virus infected MDCK cells were used so that the synthesis of viral glycoproteins could be examined, and the infected cells were preincubated for 1 h with various concentrations of the streptovirudin components. The radioisotopes (i.e., mannose or leucine) were then added; after incubation for 3 h, lipid-linked saccharides and glycoproteins were isolated, and their radioactive content was measured. The results of this experiment are presented in Table II. As expected, none of the streptovirudins showed any inhibition of mannose incorporation into dolichylphosphorylmannose, and, in fact, a slight stimulation of this reaction was observed. This may be due to the accumulation of this lipid as a result of the inhibition of lipid-linked oligosaccharides (LLO). As shown in the table, streptovirudins B and C inhibited the incorporation of mannose into both LLO and glycoprotein in these MDCK cells. While B_2 appeared to be slightly more effective than B_1, C_1 was somewhat more inhibitory than C_2. A_2 also inhibited to some extent, but A_1 was inactive even at 10 μg/mL. These differences may be due to the rate of uptake

Table II: Effect of Streptovirudin Fractions on Incorporation of [^{3}H]Mannose and [^{14}C]Leucine by MDCK Cells

fractions (μg)		[^{3}H]mannose (cpm) into Dol-P-Man	LLO	protein	[^{14}C]leucine (cpm) into protein
A_1:	0.05	1698	1807	15 152	10 249
	0.5	1756	1779	13 977	10 668
	2.0	1555	1629	17 999	10 800
	10.0	1583	1178	12 837	10 630
A_2:	0.05	1749	1673	14 108	9 741
	0.5	1514	1540	12 086	9 514
	2.0	1711	970	11 727	10 540
	10.0	1900	225	6 217	10 273
B_1:	0.05	1511	2088	12 549	9 424
	0.5	1504	1350	11 688	9 476
	2.0	1672	1114	12 302	9 870
	10.0	1452	395	7 598	8 775
B_2:	0.05	1571	1552	11 863	8.452
	0.5	1551	823	8 957	8 840
	2.0	1765	365	6 987	10 020
	10.0	1546	116	3 434	8 070
C_1:	0.05	1685	1622	13 765	9 507
	0.5	1477	1165	11 640	9 600
	2.0	1460	431	7 360	8 620
	10.0	1676	133	4 032	9 020
C_2:	2.0	1436	1287	10 351	8 463
	10.0	1678	691	7 718	8 890
control		1412	1503	11 150	9 650

of the streptovirudins by the cells since these experiments were all of fairly short duration. None of the streptovirudin components inhibited the incorporation of leucine into protein, even at 10 μg/mL, indicating that protein synthesis was unaffected at these levels of antibiotic. It should be pointed out, however, that none of the streptovirudins, even at 10 μg/mL, completely inhibited mannose incorporation into protein. Thus, at higher levels of antibiotic where protein glycosylation is completely inhibited, one might see some effect on protein synthesis. It has been suggested that protein synthesis and protein glycosylation are linked in a regulatory fashion such that when glycosylation is inhibited, the formation of the protein portion of the molecule is also blocked (Schwaiger & Tanner, 1978). The experiments shown in Table II were repeated several times with the MDCK cells. Although the amount of radioactivity incorporated into product from mannose or leucine varied from one cell culture to another, the results with the antibiotics were virtually the same as those shown in the table.

Effect of Phospholipids on the Streptovirudin Inhibition. Kuo & Lampen (1976) previously reported that phospholipids such as phosphatidylcholine, when added to yeast protoplasts at the same time as tunicamycin, blocked the inhibition by this antibiotic. In order to determine whether phospholipids could reverse or block the inhibition by streptovirudin, we added various concentrations of several phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidyldimethylethanolamine) to incubation mixtures containing soluble GlcNAc-1-P transferase, UDP-[^{3}H]GlcNAc, streptovirudin C_1 or C_2, and the other reaction components. Table III shows the incorporation of GlcNAc into dolichylpyrophosphoryl-GlcNAc in these various incubations. With the exception of phosphatidyldimethylethanolamine which perhaps showed some protection at low levels of streptovirudin, none of the other phospholipids were able to block the streptovirudin inhibition. Some of the phospholipids did stimulate the GlcNAc-1-P transferase, as has been observed previously (Heifetz et al., 1979). But this stimulation was largely abolished in the presence of antibiotic. Thus phospholipids do not appear to have any effect in the in vitro system. We also tested the effect of phospholipids on streptovirudin inhibition in MDCK cells by adding various concentrations of phospholipid to the medium at the same time as the antibiotic (data not shown). In this experiment also there was no reversal of inhibition observed with any of these compounds.

Table III: Effect of Phospholipids on the Streptovirudin Inhibition of GlcNAc-1-P Transferase

additions to the incubation	GlcNAc incorporated (cpm) after streptovirudin addition: none	2 ng of C_1	20 ng of C_1	2 ng of C_2	20 ng of C_2
none	12 606	4730	1229	6473	1565
phosphatidylcholine					
250 μg	10 035	4215	2105	5490	2191
1.5 mg	8 745	4221	1547	5876	4695
phosphatidyl-ethanolamine					
22 μg	16 596	7376	1530	7908	2414
110 μg	16 227	6375	1210	6676	2673
phosphatidyldimethyl-ethanolamine					
100 μg	17 682	9886	1547	9275	3644
500 μg	21 037	7747	1538	8079	2173
cerebrosides					
100 μg	16 058	7480	1538	8079	2173
500 μg	14 706	4987	1414	6813	2415

Discussion

The streptovirudins are an interesting group of antibiotics that have been shown to have inhibitory activity against Gram-positive bacteria, mycobacteria, and various RNA and DNA viruses (Thrum et al., 1975). These antibiotics have been found to be chemically related to tunicamycin (Takatsuki et al., 1971), mycospocidin (Tkacz & Wong, 1978), antibiotic 24010 (Mizuno et al., 1971), and antibiotic MM 19290 (Kenig & Reading, 1979). Streptovirudin and antibiotic 24010 were shown to have the same mechanism of action as tunicamycin in inhibiting the in vitro transfer of GlcNAc from UDP-[^{3}H]GlcNAc to dolichyl phosphate, thereby preventing the formation of dolichylpyrophosphoryl-GlcNAc. These antibiotics also inhibited the formation of dolichylphosphoryl-glucose, but this inhibition required much higher concentrations of antibiotic (Elbein et al., 1979).

By use of gel filtration and reverse-phase high-performance liquid chromatography, the streptovirudin complex was separated into two series of compounds: series I compounds were reported to lack uracil while the series II compounds contained uracil (Eckardt et al., 1980). Both the series I and series II compounds had approximately equal inhibitory activity on the GlcNAc-1-P transferase, inhibiting this enzyme 50% at 2–10 ng/incubation. This level of activity is similar to that previously reported for tunicamycin (Heifetz et al., 1979). However, while the series II components (B_2 and C_2) were similar to tunicamycin in their inhibition of dolichylphosphorylglucose formation (50% inhibition at 0.5–1.5 μg/mL), the series I components showed very low activity on this reaction (50% inhibition at 50–100 μg/mL). These results suggest that the presence of uracil in the molecule is much more critical for glucose inhibition than for inhibition of GlcNAc incorporation. The evidence that the series I compounds contain dihydrouracil rather than uracil is based on mass spectroscopy which showed that each of the series I components had a molecular mass 2 daltons higher than that of its corresponding series II analogue. Furthermore, the series II compounds had a peak at m/z 112, indicative of uracil, while the series I components had a peak at m/z 114, indicative of dihydrouracil. It is not clear whether the series I streptovirudins are precursors or products of the series II components,

but dihydrouracil is usually formed by reduction of uracil by NADPH during catabolism.

Initially it was thought that the inhibition by tunicamycin was due to the fact that this antibiotic resembled the substrate UDP-GlcNAc and was therefore a competitive inhibitor of the UDP-GlcNAc-dolichyl-P:GlcNAc-1-P transferase. However, several studies with the solubilized GlcNAc-1-P transferase failed to demonstrate a reversal of inhibition with increasing concentrations of UDP-GlcNAc (Heifetz et al., 1979; Keller et al., 1979). Presumably, the reason for this difficulty is that tunicamycin has a very high affinity for this enzyme so that the K_i is in the order of 10^{-10} M. However, as shown in the present study, increasing concentrations of either of the sugar nucleotide substrates, UDP-GlcNAc or UDP-glucose, are able to overcome the inhibition by streptovirudins of series I or series II. Thus the inhibition by this antibiotic is clearly of the competitive type with respect to UDP-GlcNAc and UDP-glucose. However, when inhibition was studied as a function of the concentration of the other reaction components, dolichyl-P, Mn^{2+}, or NP-40, the inhibition was not competitive.

It is interesting to note that tunicamycins and streptovirudins (and perhaps mycospocidin, antibiotic 24010, and antibiotic MM 19290) are produced as a complex of closely related compounds that differ in the fatty acid moiety. This has been clearly shown for the tunicamycins (Ito et al., 1980), and the data presented here on the molecular masses and NMR data on the streptovirudins of series I and II also indicate that this is the case for these compounds. Indeed streptovirudin C_2 is identical with tunicamycin A_1 (Keenan et al., 1981). Thus, these components (i.e., A_1, B_1, and C_1 or A_2, B_2, and C_2) differed from each other by a molecular mass of 14, indicating the presence of one additional methylene group from A_1 to B_1 to C_1. These data also correlate with the migration of these compounds on reverse-phase high-performance liquid chromatography since A_1 elutes earliest from a Biosil ODS column while C_1 elutes last. The elution from this column is related to the hydrophobicity so that compounds with longer fatty acids should be retained longer.

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