STREPTOVIRUDIN AND TUNICAMYCIN, TWO INHIBITORS OF GLYCOLIPID SYNTHESIS
DIFFERENTIATION BY USE OF GEL CHROMATOGRAPHY, H.P.L.C. AND HYDROLYSIS

Sir:

Recently, ELBEIN *et al.*[1] have shown that streptovirudin acts analogously to tunicamycin in that it blocks the formation of N-acetylglucosaminyl-pyrophosphoryl-dolichol in cell-free extracts of pig aorta and inhibits incorporation of glucose into glucosyl-phosphoryl-dolichol and into lipid-linked oligosaccharides. The antibiotic tunicamycin was proved to be an useful inhibitor of the synthesis of N-acetylglucosaminyl lipids in prokaryotes, eukaryotes and viruses.

Tunicamycin has been described as a mixture of homologous components. The structures of the components A, B, C, and D were determined by investigation of several degradation products obtained from the tunicamycin complex.[2] Recently, experiments described by MAHONEY and DUKSIN[3] and KENIG and READING[4] have proved that tunicamycin is composed of more than four components. But there are no data yet available about the physico-chemical properties which could be useful for differentiation or identification. Streptovirudin was isolated from another *Streptomyces* strain and was found to be a mixture of eight components which have been separated and characterized as pure substances.[5] In order to clarify whether or not individual tunicamycin components are identical with those of streptovirudin we used gel chromatography, h.p.l.c., and hydrolysis for direct comparisons.

Experimental

Gel chromatography: Sephadex LH-20 (Pharmacia, Uppsala, Sweden) was swelled in MeOH - H_2O (1.5: 8.5) and packed in a glass column (2.1 × 80 cm). Samples (6 mg of streptovirudin and 4.5 mg of tunicamycin,* resp.) dissolved in 1.0 ml of MeOH and 6.0 ml of hot water were applied at the top of the gel. Elution was with MeOH - H_2O (1.5: 8.5) at a rate of 60 ml/hour. The effluent was collected in 6.0 ml volumes. The fractions were tested against *Bacillus subtilis* using the agar plate test method.

H.p.l.c.: (Apparatus constructed in our institute). Samples were dissolved in MeOH. Column size 25 × 4 mm, packed with Separon AE (C_{18}-phase Lachema), flow rate 1.5 ml/min. UV Detection: 254 nm. Solvent: Acetonitrile - H_2O (1: 2). Peaks were identified by direct comparison with pure streptovirudin components.

Hydrolysis: Streptovirudin complex and individual components were hydrolyzed with 6 N HCl, 20 hours, 105°C. Glucosamine was detected on t.l.c. plates (Polygram Cel 300/UV_{254}, Machery Nagel + Co.; *i*-propanol - HCOOH (98%) - H_2O (40: 2: 10) and by use of an automatic amino acid analyzer. Uracil: Systems *i*-propanol - 2 N HCl (65: 35); *n*-butanol - H_2O (86: 14) (NH_3 saturated); acetone - *n*-butanol - H_2O (8: 1: 1), and H_2O).

Isolation of uracil: Sephadex G-10 was swelled in H_2O. The acidic, filtered hydrolysate was applied to the top of the gel. Elution was with water. The uracil-containing fractions were evaporated and identified by mass spectrometry.

Results and Discussion

The results of chromatographic experiments are expressed in representative elution profiles. The elution positions were highly reproducible. Gel chromatography of streptovirudins gave clear-cut separated peaks of streptovirudins A_1, A_2, B_1, B_2, C_1, C_2, D_1, and D_2 (Fig. 1). Chromatography of tunicamycin under the same conditions showed that streptovirudins of series I (A_1, B_1, C_1, and D_1) are not present in authentic tunicamycin. Streptovirudins B_2, C_2, and D_2 are obviously identical with tunicamycin components. Two shoulders near C_2 and D_2 as well as the main peak with two shoulders (fractions 268 ~ 365) in Fig. 1 indicate additional tunicamycin components different from streptovirudins.

H.p.l.c. separation (Fig. 2) of streptovirudin gave the four peaks, A_2, B_2, C_2, and D_2. (Streptovirudins of series I have no absorption at 254 nm and were therefore not recorded in these h.p.l.c. experiments). When tunicamycin was chromatographed under the same conditions six peaks (1 ~ 6, Fig. 2) were observed. Peaks 2, 3, and 4 had retention times identical to those of streptovirudins B_2, C_2, and D_2 supporting the re-

* Tunicamycin (Lot 361-26E-239-A) was a gift generously provided by Dr. R. L. HAMILL, Eli Lilly and Company.

Fig. 1. Elution profiles on Sephadex LH-20 of streptovirudin and tunicamycin.

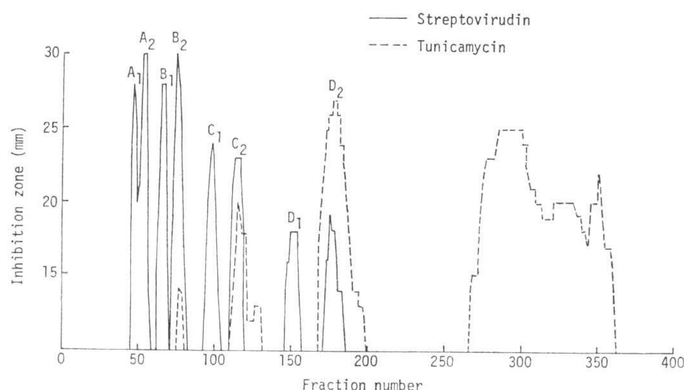
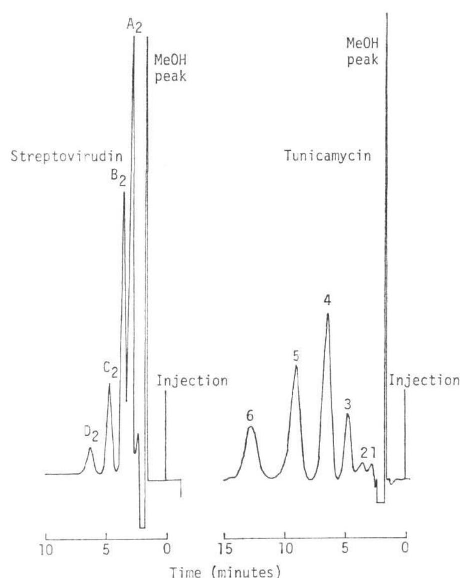


Fig. 2. H.p.l.c. of streptovirudin and tunicamycin.



sults obtained by gel chromatography. Peak 1 may possibly be due to trace amounts of A_2 in tunicamycin which could not be detected by gel chromatography. Peak 5 corresponds to fractions 268～365.

Glucosamine and uracil which are both moieties of the tunicamycin structures were detected in hydrolysates of streptovirudins as follows:

	A_1	A_2	B_1	B_2	C_1	C_2	D_1	D_2
Glucosamine	+	+	+	+	+	+	+	+
Uracil	−	+	−	+	−	+	−	+

The results obtained support our earlier suggestion that tunicamycin and streptovirudin are related but not identical antibiotic complexes. The main difference is that tunicamycin does not contain streptovirudins of series I which lack an uracil moiety in their molecules. The question still remaining is whether all streptovirudins are able to inhibit glycolipid formation or only streptovirudins of series II.

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