OLIGOSTATINS*, NEW ANTIBIOTICS WITH AMYLASE INHIBITORY ACTIVITY

I. PRODUCTION, ISOLATION AND CHARACTERIZATION

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Oligostatins C, D and E, three new antibiotics were found in the culture filtrate of *Streptomyces myxogenes* nov. sp. SF-1130. Their spectral and chemical properties suggested that oligostatins were basic oligosaccharide antibiotics. They exhibited not only antibacterial activity but also strong amylase inhibitory activity.

In the course of screening study of new antibiotics, a new antibiotic complex of oligosaccharide nature was found in the culture filtrate of a *Streptomyces* strain. In the present paper, fermentation, isolation and characterization of oligostatins C, D and E are described. Biological characterization of a producing organism has already been reported[1].

Fermentation and Isolation of Oligostatins

The antibacterial activity of the culture broth was determined by a paper disc method using *Escherichia coli* K12R containing 0.125% maltotriose. A medium containing 5.0% maltose syrup, 2.5% soybean meal, 1.0% wheat germ and 0.25% NaCl (pH 7.0 before sterilization) was used for antibiotic production in a 300-liter tank. Fermentation was carried out at 28°C for 66 hours.

Broth filtrate (140 liters) was passed through a column of Dowex 50W-X2 (H^+, 1.4 liters) and washed with water (7 liters) in order to remove neutral oligosaccharides such as maltotriose or -pentaose which were co-produced. Oligostatins were eluted from a column by 0.1 N NH_4OH (1.2 liters). Biologically active fractions were concentrated (0.2 liters) and chromatographed on charcoal (0.7 liters). After washing with water, oligostatins were eluted by 35% ethanol (4 liters). The crude oligostatin complex showed several spots on silica gel TLC (ethyl acetate - methanol - water, 5: 3: 2) or PPC (pyridine - ethyl acetate - water, 10: 4: 3, descending) and each component was designated oligostatins A, B, C so on from the order of decreasing Rf value. HPLC analysis of oligostatin complex is shown in Fig. 1. Oligostatin C was relatively a major component. Each was purified by successive chromatography over AG50W-X2 (pyridine - formic acid, pH 3.1, 0.1 M) followed by Bio-Gel P-2 chromatography as shown in Chart 1. Concentration was carefully carried out under reduced pressure below 35°C and followed by lyophilized to yield a powder, because disintegration was sometimes observed after vacuum concentration.

* Formerly designated SF-1130 X.

Fig. 1. HPLC analysis of oligostatin complex.
Column : PNH_2-10/S (Shimadzu, 4.0 mm × 250 mm)
Mobile phase: CH_3CN-H_2O, 60: 40
Flow rate : 1.0 ml/minute: room temperature
Detector : RI, ×4

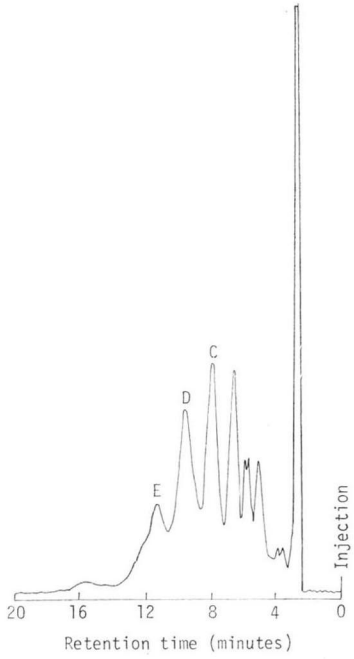
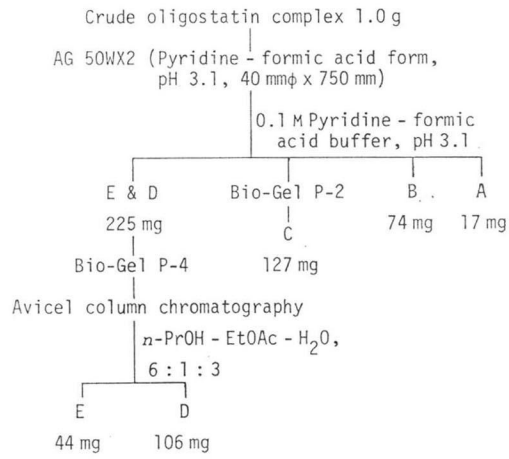


Chart 1. Separation of oligostatin complex.



Biological Activity of Oligostatins

1) Antimicrobial activity

Antimicrobial spectra of oligostatins C, D and E are shown in Table 1. The bioactivity was limited to Gram-negative bacteria and markedly enhanced in presence of maltodextrin, such as maltotriose. Among three components, oligostatin E was most active.

2) Amylase inhibitory activity

Oligostatins showed strong inhibitory activity against some selected amylases as shown in Table 2. Of particular interest was that the inhibitory activity was more effective to α-amylase of animal origin and less effective to those of microbial origin. Activity against glucoamylase was relatively strong.

They were inactive against α-glucosidase and invertase. It was also interesting that α-amylase in-

Table 1. Antibacterial spectrum of oligostatins.

Organisms	Inhibitory diameter* (paper disc, mm)		
	C	D	E
Escherichia coli IAM 1239	10.8 (0)**	14.8 (0)**	15.9 (0)**
Escherichia coli K12 R	14.4 (14.4)	18.8 (13.0)	19.5 (11.4)
Escherichia coli M-8032	11.6 (0)	23.6 (14.5)	23.2 (14.2)
Shigella sonnei	12.3 (0)	19.3 (13.0)	20.0 (13.5)
Proteus vulgaris	0 (0)	15.4 (t)	15.9 (t)
Salmonella typhi	20.6 (0)	19.0 (13.4)	20.1 (13.7)
Klebsiella pneumoniae	13.4 (16.0)	13.2 (13.2)	15.1 (13.0)
Bacillus subtilis	0 (0)	0 (0)	0 (0)
Micrococcus luteus	0 (0)	0 (0)	0 (0)
Staphylococcus aureus	0 (0)	0 (0)	0 (0)

* 2000 mcg/ml of oligostatins with 1.25 mg/ml of maltotriose.
** Without maltotriose.

Table 2. Amylase inhibitory activity of oligostatins.

Enzyme	Origin	I_{50}* (mcg/ml)		
		Oligostatin C	Oligostatin D	Oligostatin E
α-Amylase	*Bacillus subtilis* (liquef.)	>200	200	170
〃	*Aspergillus oryzae*	>200	72.0	7.0
〃	Porcine pancreas	2.9	0.09	0.12
〃	Human saliva	7.8	0.16	0.18
〃	Meilase	1.5	0.32	0.72
β-Amylase	Sweet potato	>200	>200	>200
Glucoamylase	*Rhizopus niveus*	0.03	0.05	1.5
α-Glucosidase	Brewers yeast	>200	>200	>200
Invertase	*Saccharomyces cerevisiae*	>200	>200	>200

* I_{50}: 50% inhibitory concentration

hibitory activity was very sensitive to the number of glucose residues in oligostatin, and among the three, oligostatin E, possessing five glucose residues was most active in general. On the other hand, oligostatin C, the smallest among the three, was the most active one against glucoamylase in contrast to its weak α-amylase inhibitory activity. Detailed structure-activity relationship of oligostatins will be discussed elsewhere.

Physico-chemical Properties of Oligostatins

Physico-chemical properties of oligostatins are shown in Table 3. Oligostatins were obtained as basic, water-soluble powders. As a representative, IR and PMR spectra of oligostatin C are shown in Figs. 2 and 3 as well as CMR spectrum in Fig. 4. Other components showed almost the same spectra as those of oligostatin C. The IR, PMR and CMR spectra as well as color reactions strongly suggested that oligostatins were of oligosaccharide nature. The PMR spectrum showed CH_3–CH– signal at 1.38 ppm in D_2O from external TMS, –C*H*–OH signals at around 3.5~4.0 ppm and –O–C*H*–O– signals at around 5.2~5.4 ppm, but no olefinic proton was observed. Physico-chemical properties of oligo-

Table 3. Physico-chemical properties of oligostatins.

	Oligostatin C	Oligostatin D	Oligostatin E
Appearance	Colorless powder	Colorless powder	Colorless powder
m.p.	183°C (dec.)	190°C (dec.)	195°C (dec.)
UV	End absorption	End absorption	End absorption
$[\alpha]_D^{23}$	+154° (*c* 1.0, H_2O)	+155° (*c* 1.0, H_2O)	+166° (*c* 1.0, H_2O)
Microanalysis	C, 43.31; H, 5.88; N, 1.71	C, 43.84; H, 6.41; N, 1.08	C, 43.65; H, 6.55; N, 1.05
M.w.	825	987	1149
TLC (Rf value)*	0.25	0.18	0.13
HPLC**	8.0 minutes	9.8 minutes	11.8 minutes
Glucose	3	4	5
Color reactions			
$AgNO_3$-NaOH	+	+	+
Red-tetrazolium	+	+	+
SAKAGUCHI	–	–	–

* Silica gel 60 (Merck), AcOEt-MeOH-H_2O (5:3:2), H_2SO_4

** PNH_2-10/S2504 (i.d. 4.0 mm×25 cm), CH_3CN-H_2O (60:40), 1.0 ml/minute, ~15 kg/cm², RI

Fig. 2. IR Spectrum of oligostatin C (KBr).

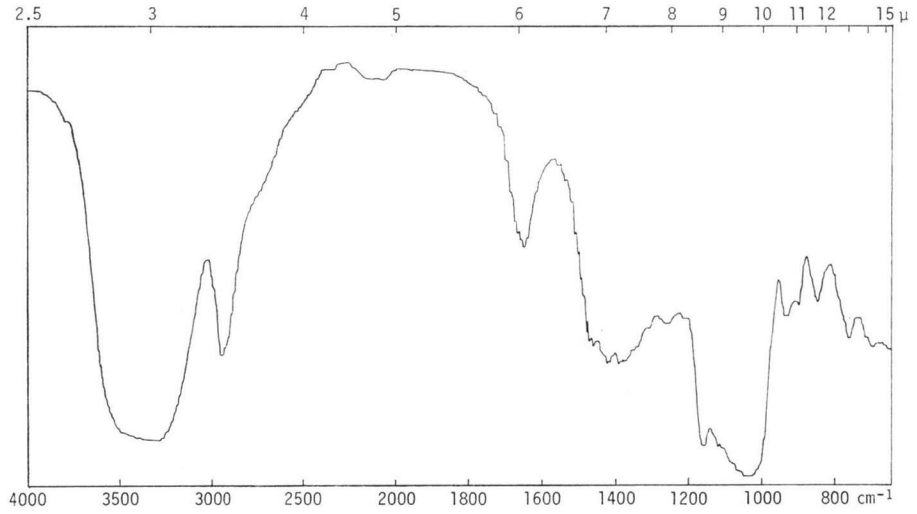


Fig. 3. PMR Spectrum of oligostatin C (100 MHz, D_2O).

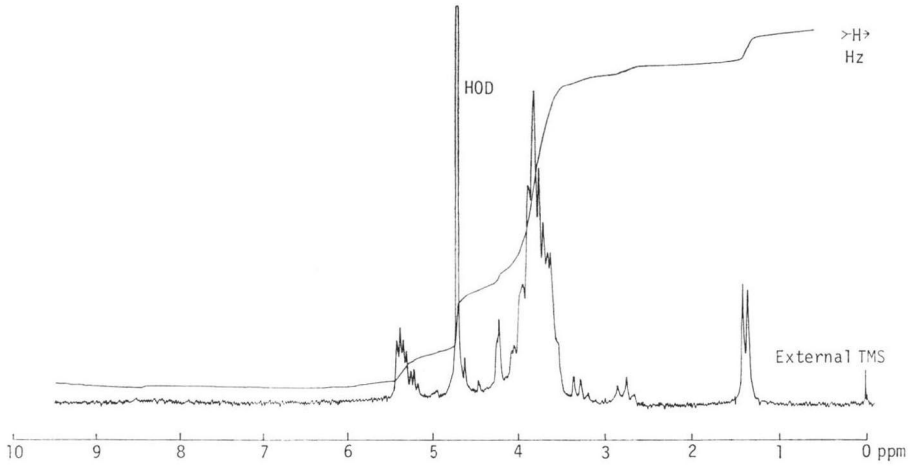
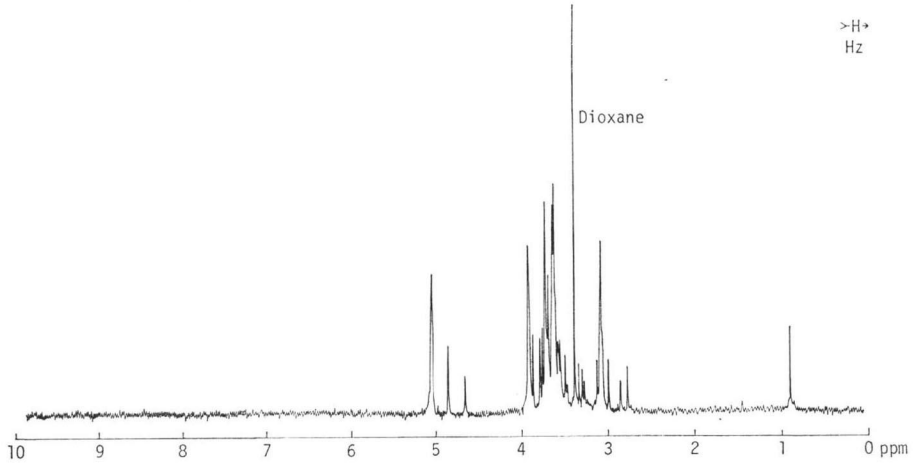


Fig. 4. CMR Spectrum of oligostatin C (25.16 MHz, D_2O).



statins suggested that these components might be glucose homologues possessing the same active center in common.

These physico-chemical properties and amylase inhibitory activity described above would make oligostatins similar to BAYe 5421[2], S-AI[3], TAI-I, -II (adiposin-I, -II)[4] and trestatins[5]. However, oligostatins could readily be differentiated from any of known amylase inhibitors since oligostatins possessed no double bond.

Acknowledgement

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