CHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITY OF LIPOPOLYSACCHARIDES FROM SOME STRAINS OF ACTINOMYCETES AND Escherichia coli

N. S. Gryaznova, L. V. Gubernatorova, and Yu. O. Sazykin

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Polysaccharides from Gram-negative bacteria and their complexes with lipids and proteins are known as high molecular weight compounds stimulating the defensive reactions of the host during bacterial and virus infections, including during the infectious complications of radiation sickness. The use of polysaccharides as antitumor agents in experiments on animals has also been described. One of the most effective preparations isolated from the Gram-negative bacteria is prodigiosan, obtained by Ermol'eva and co-workers, and widely used in various branches of chemotherapy [4].

Polysaccharide complexes from Gram-positive bacteria have been investigated less thoroughly, and practically no attempt has been made to study the polysaccharides of the actinomycetes. The authors of the few communications on this subject have mainly been concerned with establishing taxonomic relationships between individual species of actinomycetes and with studying the chemistry of the cell wall [2,6,7,9].

In the present investigation, the authors studied the lipopolysaccharides of actinomycetes producing antibiotics widely used in medicine, and also Escherichia coli No. 9637, the strain used as the source of the enzyme necessary for producing 6-aminopenicillanic acid.

EXPERIMENTAL METHOD

The microorganisms were grown in flasks on shakers and in a fermenter in various media, including some optimal for the production of other biologically active preparations (penicillinamidase in the case of E. coli and antibiotics in the case of the actinomycetes).

The polysaccharide complexes were isolated by extraction with aqueous phenol and then purified by the methods of Tauber and Russell [13] and of Tsyganov and co-workers [9] with slight modifications. The second method was more suitable for isolation of the actinomycete preparations, and it provided a relatively high yield of lipopolysaccharides.

The following characteristics of the preparations were determined: the content of reducing substances (by the Hagedorn–Jensen method [1]); the content of glucosamine (the method of Elson and Morgan [7]); the protein content (by Lowry's method [10]); the total content of nucleic acids (spectrophotometrically by Spirin's method [8]); the content of lipids (the lipid part of the complex was characterized by the content of fatty acids, determined by the spectrophotometric method of Snyder and Stephens, in Tauber's new modification [11, 12]).

The biological characteristics of the preparations were determined by the study of their protective action in experiments on mice (weight 16-18 g) with experimental septicemia caused by pathogenic strains of E. coli and Staphylococcus aureus. Each group consisted of seven animals (the experiment was repeated three times). The preparations isolated from E. coli No. 9637 were also tested in infectious complications of radiation sickness. Irradiation was given on a type RUM-3 apparatus (voltage 180 kV, current 15 mA, skin-focus distance 40 cm, filters Cu 0.5 mm and Al 1 mm, dose rate 25 R/min, total dose 650 R/mouse).

The preparations were injected in physiological salines (in doses of 0.5 ml) intraperitoneally, 24 h before infection. The statistical significance of the results obtained was determined by Student's method. LD_{50} was calculated by Kärber's method.

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Table 1. Chemical Characteristics of Preparations of Lipopolysaccharides from Actinomycetes (Mycelium on 5th-6th day of fermentation)

Producer of polysaccharide	Reducing sugars	Fatty acids	Protein	Nucleic acids
	in %			
Act. streptomycini	25,64	30,90	4,70	5,10
Act. kanamycini	24,50	24,00	4,00	1,13
Act. orientalis	69,50	2,00	1,19	9,50
Act. rimosus LS-T hybrid	41,10	13,37	6,32	0,97
Act. rimosus	42,00	16,90	5,84	0,72
Act. aureofaciens № 2201	54,10	13,95	2,93	2,92
Act. aureofaciens № 2201	43,70	15,00	2,12	3,60
Act. aureofaciens № 2201	54,30	13,85	2,65	2,70
Act. aureofaciens	72,80	12,60	0,60	1,54
Act. aureofaciens	68,60	12,32	0,50	0,96

Table 2. Protective Action of Preparations from Actinomycetes on Mice Infected with S. aureus and E. coli

Producer of polysaccharide	Staph. aureus			E. coli	
	dose (μg per mouse)	survival rate in control	survival rate in expt.	survival rate in control	survival rate in expt.
	in %				
Act. orientalis	0	28,6		14,3	
	10		62		28,6
	100		86		57,0
	500		91		71,5
Act. rimosus LS-T hybrid	0	14,3		19	
	10		48		57,0
	100		52,5		86,0
	500		76,3		91,6
Act. aureofaciens (TTs)	0	14,3		0	
	10		62		14,3
	100		81		33,3
	500		81		86,0
Act. aureofaciens № 2201 (KhTTs)	0	9,5		14,3	
	10		43		43,0
	100		67		47,5
	500		77		100,0
Act. aureofaciens № 2201 (TTs)	0	9,5		19	
	10		33		38
	100		57		67,0
	500		81		86,0
Act. kanamycini	0			14,3	
	10				47,5
	100				52,5
	500				95,3

EXPERIMENTAL RESULTS

A lipopolysaccharide complex was isolated from E. coli No. 9637 by Tauber's method (the culture was grown in meat-peptone broth, pH 7.3, at 36° for 6 h). Its chemical characteristics were: the complex contained 20% of reducing substances and 32% of fatty acids, but only 5.18% of protein and slight traces (1.82%) of nucleic acids. According to data in the literature [3], the content of reducing sugars in prodigiosan is 30-40%, while the antigens from cells of Salmonella paratyphi B contain from 23 to 40% of reducing sugars [5].

The preparation had a protective action against infections caused by E. coli and S. aureus: after injection of 0.05 μg of the preparation per mouse, the survival rate of the animals reached 70-80%, whereas all the control animals died; in doses of 1-10 μg per mouse it enabled all the animals to survive the infection.

The effect of the isolated preparation on the rate of survival of the irradiated mice was also studied. The animals were irradiated with x-rays in a dose of 650 R. Twenty-four hours before irradiation the preparation was injected into the mice in a dose of between 10 and 100 μg per animal. Each group consisted of 20 animals. By the 16th day after irradiation the number of surviving animals in the experimental series receiving a dose of 10 μg per animal was 70%, and in the series receiving 100 μg it was 80%, compared with only 25% in the controls.

The toxicity of the preparation was determined in mice by intraperitoneal and intravenous injection. LD_{50} (determined by Kärber's method) by intraperitoneal injection was 370 μg per mouse and by intravenous injection 224 μg per mouse.

The chemical and biological data shown above thus demonstrated that the strain of E. coli (No. 9637) used in the experiments contains a biologically active complex, and that a preparation from a Gram-negative microorganism was available for direct comparison of its biological properties with the biological properties of the actinomycete preparations.

Ten polysaccharide preparations were isolated by Tsyganov's method from actinomycetes producing streptomycin, kanamycin, vancomycin, chlortetracycline, oxytetracycline, and tetracycline. Data for the composition of the isolated complexes (the percentages of the main groups of substances present) are given in Table 1.

As Table 1 shows, the isolated preparations possessed a high content of polysaccharide component (up to 70% of reducing sugars). The fatty acid content also was high – from 12 to 30% (except the prepara-

tion from Actinomyces orientalis). The protein content (1-6%) and the nucleic acid content (except the preparation from A. orientalis) were relatively low.

The characteristics of the protective properties of some of the actinomycete preparations are given in Table 2. According to the data of Table 2, the preparations from the actinomycetes had a protective action in higher concentrations than the preparation from E. coli. The highest activity was exhibited by the lipopolysaccharides isolated from Actinomyces aureofaciens.

The lipopolysaccharides from the actinomycetes possessed low toxicity: intraperitoneal injection of preparations from A. orientalis, A. aureofaciens, and A. rimosus in doses of up to 7000 μg per mouse into the animals did not cause death. After intravenous injection of the preparation from A. aureofaciens in a dose of 6000 μg per mouse,all the animals survived; while if the dose was 8000 μg per mouse, 1 of the 5 animals died; and if 16,000 μg per mouse, 4 of the 5 animals died. The preparation from A. orientalis did not cause death of the animals when injected intravenously in a dose of 16,000 μg per mouse. The investigated lipopolysaccharide complexes from the actinomycetes were thus characterized, from the preliminary evidence, by extremely low toxicity.

The results obtained indicate the desirability of further study of the polysaccharides and of their complexes from various species of actinomycetes.

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