SPECTROPHOTOMETRIC DETERMINATION OF A BACTERIAL LIPOPOLYSACCHARIDE FROM *Escherichia coli* 675

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A method has been developed for the quantitative spectrophotometric determination of the lipopolysaccharides of *E. coli* 675. The maximum absorption of the lipopolysaccharides from *E. coli* 675 (259 nm) coincides with the maximum absorption (259 nm) of a number of polysaccharides from other Gram-negative bacteria, which can be used to determine lipopolysaccharides in various media.

The study of the possibility of using modern physicochemical methods for the analysis of lipopolysaccharides (LPSs) of plant and, particularly, of bacterial origin (LPSs of bacterial origin possess pyrogenic properties) is of considerable interest.

The quantitative determination of the free LPSs of bacterial origin has been developed with the use of a colorimetric method based on the specific shift of the absorption maximum of a carbocyanine dye on its formation of aggregates with the LPSs [1].

There is a report of the use of spectrophotometry in the UV region (at λ_{max} 259 nm) for the quantitative determination of a number of LPSs of bacterial origin (*Salmonella abortus equi*, *Salmonella enteritidis*, etc.) [2].

In order to study the possibility of the spectrophotometric determination in the UV region of LPSs isolated by aqueous phenolic extraction by O. Westphal's method from *E. coli* 675 (not studied by Karamian [2]) we have recorded the absorption spectra of aqueous solutions of this LPS (Fig. 1). As can be seen from Fig. 1, the spectrum has an absorption maximum at λ_{max} 259 nm.

We considered the dependence of the intensity of absorption on the concentration at λ_{max} 259 nm, for whichwe prepared (accurately!) aqueous solutions of the LPS with concentrations of 0.002, 0.0025, 0.0030, 0.0035, 0.0040, 0.0045, 0.0050, 0.0055, and 0.0060%.

The results of the determinations treated by the method of mathematical statistics are given below:

Concentration, %	D	$E^{1\%}_{1cm}$	Metrological characteristics
0,0020	0,265	132,5	—
0,0025	0,330	132,0	X=131,5
0,0030	0,401	133,7	S=1,7832
0,0035	0,462	132,0	S_x=0,5944
0,0040	0,528	132,0	ε=11,3445
0,0045	0,585	130,0	A=±1,02%
0,0050	0,643	128,6	
0,0055	0,711	129,3	
0,0060	0,782	133,3	

As we see, the error in the specific absorption index found is insignificant, which permits the conclusion that the Lambert–Beer law is observed for solutions of the LPS from *E. coli* 675 in water in the range of concentrations from 0.002 to 0.006% at λ_{max} 259 nm.

On the basis of the results obtained it is possible to determine the concentration of a preparation from the specific absorption index, the amount of the substance being calculated from the formula (%)

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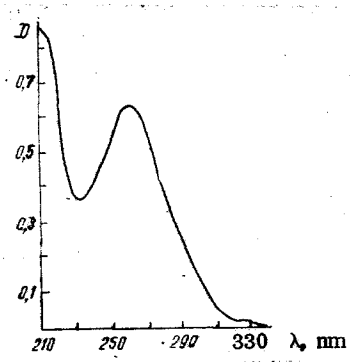

Fig. 1. UV absorption spectrum of the lipopolysaccharide from *Escherichia coli* 675 in water.

$$c = \frac{D \cdot V}{E \cdot l \cdot 100},$$

where D is the optical density; E, specific absorption index; V, volume of solution being analyzed; and l, thickness of the working layer of the cell.

The results of the spectrometric determination of the LPS from *E. coli* 675 were as follows:

Taken, g	D	Found		Metrological characteristics
		g	%	
0,00207	0,275	0,00200	96,62	$X=99,08$
0,00302	0,391	0,00297	98,34	$S=1,6061$
0,00401	0,526	0,00400	99,75	$S_x=0,7182$
0,00493	0,650	0,00494	100,20	$b=1,8465$
0,00574	0,759	0,00577	100,50	$A=\pm1,86\%$

The error of the determination does not exceed ±2%.

The sensitivity of the proposed method can be considerably increased; for example, by using a cell with a thickness of the working layer of 5-10 cm (in place of the cells which we used with a 1-cm thickness of the working layer) it is increased five- to tenfold, respectively. The determination of small amounts of bacterial LPSs in liquids (for example, in their detection as pyrogenic substances in distilled water and other solutions for injection) is possible after their preliminary concentration, for example, by the evaporation of the liquid under investigation, since on evaporation bacterial lipopolysaccharides do not pass into the vapor and, moreover, a characteristic feature of LPSs of bacterial origin is their great thermal stability, and they therefore remain unchanged during the evaporation process.

EXPERIMENTAL

About 0.002-0.006 g (accurately weighed) of LPS was placed in a 100-ml measuring flask and dissolved in water, and the solution was made up to the mark with water. The optical density was measured on a Hitachi spectrophotometer using quartz cells with a thickness of the working layer of 1 cm. Water was used as the comparison solution.

In order to exclude a possible contamination of the solutions of LPSs of bacterial origin under investigation, the glassware used in the determination (flasks, pipettes) was treated with chromic acid mixture and was sterilized in a drying chest at a temperature of 180°C for two hours. All the solutions were prepared in apyrogenic distilled water under aseptic conditions.

SUMMARY

1. It has been established that the absorption spectrum in the UV region of an aqueous solution of the LPS from *E. coli* 675 has an absorption maximum at λ_{max} 259 nm.

2. The absorption maximum of the LPS from *E. coli* 675 (259 nm) coincides with the absorption maxima (259 nm) of a number of LPS from other Gram-negative bacteria, which can be used for detecting LPSs of bacterial origin in various media.

3. It has been shown that, within the range of concentrations of 0.002-0.006%, aqueous solutions of LPSs obey the Bouguer–Lambert–Beer law, on the basis of which a method has been developed for the quantitative spectrophotometric determination of the LPS from *E. coli* 675.

LITERATURE CITED

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ACCUMULATION OF POLYSACCHARIDES UNDER THE INFLUENCE OF CHLOROCHOLINE CHLORIDE IN *Aronia melanocarpa*

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Water-soluble polysaccharides (WSPSs) have been isolated from the fruit and leaves of *Aronia melanocarpa* Elliot (black chokeberry) plants treated with chlorocholine chloride in various concentrations. It has been established that they are composed of galacturonic acid and the neutral monosaccharides galactose, glucose, arabinose, xylose, and rhamnose. In addition, another two unidentified spots of monosaccharides or their derivatives were found in the polysaccharides of the fruit, and one in those of the leaves. The ratios of the neutral monosaccharides in the WSPSs have been determined. Accumulation of the polysaccharides under the influence of various concentrations of chlorocholine chloride has been studied. It has been established that the retardant used (chlorocholine chloride) increases the accumulation of polysaccharides in the fruit by a factor of 1.5-2, but no appreciable accumulation of them was observed in the leaves.

At the present time, for regulating processes of growth and development and of the biosynthesis of biologically active substances (BASs) in plants wide use is made of synthetic bioregulators. Among them a special place is occupied by retardants [1, 2] which can exert a definite influence on the biosynthesis of BASs. There is no information in the literature on the influence of retardants — in particular, chlorocholine chloride (TUR) — on the accumulation of BASs, including polysaccharides, in *Aronia melanocarpa* Elliot (black chokeberry).

We have established previously [3] that a considerable amount of polysaccharides accumulates in the green fruit (8.52%) and in the leaves of the chokeberry in the green-fruit and fruit-fall phases (7.51 and 7.94%, respectively). The amount of polysaccharides in the ripe fruit falls to 2.37%, but by using trace elements the yield can be increased to 5% [4].

We have studied the accumulation and composition of the water-soluble polysaccharides (WSPSs) under the influence of various concentrations of chlorocholine chloride in the fruit and leaves of the black chokeberry.

The plants were treated in 1977 and 1978 in the morning hours a) with a 0.3%, and b) with a 0.6% solution, and c) twice with a 0.3% solution (0.3% × 2) of chlorocholine chloride as active substance. The first treatment was carried out at the beginning of the period of intensive growth of the shoots, and the second and third at intervals of 12-15 days after

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