THE POLYSACCHARIDES SYNTHESIZED BY STREPTOCOCUS SALIVARIUS AND STREPTOCOCUS BOVIS

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In a previous paper (1) it was reported that certain non-hemolytic streptococci from the human throat are capable of synthesizing large amounts of a carbohydrate from sucrose and raffinose. These organisms seem to be similar to those studied by Oerskov and Poulsen (2). When grown on the surface of agar containing 5 per cent sucrose or raffinose, large mucoid colonies are produced which are about the size and appearance of the colonies formed by most varieties of the genus Rhizobium. Upon detailed study these slime producers were found to be typical strains of Streptococcus salivarius.

This ability to produce very large mucoid colonies is possessed by no other recognized species of streptococcus, with the exception of occasional strains of Streptococcus bovis. From a large collection of Streptococcus bovis cultures only one strain was able to produce large mucoid colonies which were similar in appearance to those formed by Streptococcus salivarius; three other cultures synthesized a carbohydrate but the surface colonies on sucrose agar were tenacious and dry and were much smaller than the large, moist, soft, mucoid colonies produced by Streptococcus salivarius.

Oerskov (3) reported on the chemical properties of a carbohydrate synthesized by a streptococcus which he isolated from the mesenteric glands of a mouse. His findings indicate that the carbohydrate was a dextran. The present paper is a report on the chemical nature of the carbohydrates produced by strains of Streptococcus salivarius and Streptococcus bovis.
Synthesis of Polysaccharides

Purification

A representative slime-producing strain of *Streptococcus salivarius* was grown in a medium containing 1 per cent tryptone, 8 per cent sucrose, and 0.2 per cent K$_2$HPO$_4$. The acid produced was neutralized intermittently, as formed, with 1 M NaOH. After acid production ceased (about 5 days at 37°), the bacterial cells were removed by centrifugation. The polysaccharide was precipitated from the supernatant liquid medium by the slow addition of alcohol with constant and vigorous agitation, 2.5 volumes of 95 per cent alcohol being added to 1 volume of the medium. The polysaccharide precipitates as a white flocculent substance which tends to adhere to the walls of the container.

The polysaccharide was further purified by precipitating aqueous solutions with alcohol four successive times. The solution was then dissolved in warm water and decolorized with norit, after which it was dialyzed in a cellophane tube against running tap water overnight. The dialyzed solution was precipitated once more with alcohol and then dried overnight at 55° to a transparent glass. This glass was ground to a fine powder and dried to a constant weight at 130°, 1.5 mm. pressure. The polysaccharide is very soluble in water, forming a solution which is bluish in reflected light and yellowish in transmitted light. It precipitates completely in 65 per cent alcohol and is easily hydrolyzed by dilute acids.

The carbohydrate synthesized by *Streptococcus bovis* is quite different in its physical properties, being insoluble in water after precipitation with alcohol. A final concentration of 50 per cent alcohol is required for complete precipitation. The substance is partially soluble in 1 M NaOH, resulting in a turbid suspension; as noted later, it is hydrolyzed by acids with great difficulty. This polysaccharide, prepared and purified as was the *Streptococcus salivarius* carbohydrate, was dissolved in 1 M NaOH after each alcoholic precipitation, and decolorized and dialyzed in neutral suspension, the carbohydrate being reprecipitated and dried as described above.

EXPERIMENTAL

When the polysaccharide was dried to constant weight at 100°, 1.5 mm. pressure, only 92 per cent of the carbohydrate could be
accounted for after hydrolysis. When dried at 130° to constant weight, the polysaccharide lost about 6.3 per cent of its 100° weight, and between 120–125° the powder swelled to about 3 times its original volume. When the material was heated to 135°, a few drops of distillate were recovered which possessed a typical odor of alcohol and gave a strong iodoform test, thus indicating that on precipitation with alcohol the polysaccharide may form an alcoholate having 1 molecule of alcohol to 4 or 5 constituent monosaccharide molecules.

The unhydrolyzed Streptococcus salivarius polysaccharide does not reduce Fehling's solution. Because of the turbidity of the suspension the specific rotation could not be determined accurately, but it seems to have a definite negative rotation, $[\alpha]_{D}^{25} = \text{approximately } -45° (c = 0.1 \text{ per cent}).$

The polysaccharide can be hydrolyzed within 5 hours at 20° by 1 M HCl. When hydrolyzed at 70° for 8 minutes, it yielded 97.3 per cent reducing sugars estimated as fructose by the method of Shaffer and Somogyi (4). The Seliwanoff test (Roe (5)) indicated 95 per cent ketose sugars. The specific rotation after hydrolysis, $[\alpha]_{D}^{25} = -87.3° (c = 1.5 \text{ per cent})$, indicated 98.5 per cent fructose.

An osazone from the hydrolyzed sample formed within 2 minutes after it was immersed in the boiling water bath, thus indicating the presence of fructose. The purified osazone appeared microscopically as glucosazone and showed a melting point of 205°. A micro-Kjeldahl determination on the unhydrolyzed polysaccharide yielded a mere trace of nitrogen.

The evidence points to the conclusion that this soluble polysaccharide is a levan, and this was verified by less extensive studies on twenty-five other strains of Streptococcus salivarius, all of which synthesized large quantities of what appeared to be the same carbohydrate. However, a few of these cultures synthesized a second carbohydrate which was insoluble in water, as evidenced by a flocculent, gelatinous material which occupied about one-third of the volume of the liquid medium. This second carbohydrate is quite different from the levan, being very insoluble in water, but soluble in 1 M NaOH. 2 hours in a boiling water bath in the presence of 1 M HCl are insufficient for complete hydrolysis, but about 80 per cent of the carbohydrate was accounted for.
reducing sugars in the filtrate of the partially hydrolyzed sample. Optical rotation methods showed about the same amount, calculated as glucose. Though the data on this carbohydrate are incomplete, it is probably safe to conclude that it contains large amounts of glucose.

The carbohydrate synthesized by a few cultures of Streptococcus bovis seemed to be essentially composed of glucose. It does not possess the viscous properties of the insoluble carbohydrate from Streptococcus salivarius but it is also hydrolyzed with great difficulty. Due to the fact that a neutralized aqueous suspension is very turbid, accurate polarimetric determinations could not be made before hydrolysis. The specific rotation was \([\alpha]_D^{25} = \text{approximately } +180^\circ \) (\(c = 0.1\) per cent). A sample hydrolyzed 1 hour in 1 m HCl at 100° showed a specific rotation approaching that for glucose, \([\alpha]_D^{25} = +53.3^\circ \) (\(c = 1.0\) per cent). About 93 per cent of the original carbohydrate was accounted for as reducing sugars in the hydrolyzed sample. Practically no nitrogen was demonstrated by the micro-Kjeldahl method.

One culture of Streptococcus bovis was found which synthesized minute amounts of a levan from sucrose in liquid media. This levan seemed to be identical with that produced by Streptococcus salivarius. This culture, however, produced large amounts of the dextran in the same medium.

**DISCUSSION**

Harrison, Tarr, and Hibbert (6) reported that certain members of the genus Bacillus are able to synthesize a levan from sucrose and raffinose. They concluded that this polysaccharide could be synthesized only from sugars containing a terminal fructofuranose residue in their molecules. If this is the case, we should expect to obtain synthesis of the levan from inulin, but no slime is produced by Streptococcus salivarius from inulin though it is fermented with acid production (1).

Although immunological tests are not included, it should perhaps be noted that Oerskov (3) found the material studied by him to be serologically inactive and there is no reason at present for thinking that the polysaccharides here considered are related to the so called soluble specific substances.
SUMMARY

Many strains of *Streptococcus salivarius* synthesize a soluble levan in large quantities from sucrose and raffinose. A few of these strains synthesize an insoluble polysaccharide, having the properties of a dextran, in addition to the levan. The production of these polysaccharides can be demonstrated either on the surface of agar or in liquid media.

A few strains of *Streptococcus bovis* are able to synthesize an insoluble carbohydrate from sucrose and raffinose which seems to be a dextran.

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