Transglucosidase Activity of Rumen Strains of *Streptococcus bovis*: Structure of the Dextran Produced from Sucrose

By R. W. BAILEY

Plant Chemistry Division, D.S.I.R., Palmerston North, New Zealand

(Received 21 May 1958)

Many extensive studies have been made on the glucose polymers, known as dextrans, which are produced from sucrose by the transglucosidase activity of species in the genus *Leuconostoc*. Less work has been described, however, on similar polysaccharides produced from sucrose by streptococcal species. Niven, Smiley & Sherman (1941) reported that various strains of *Streptococcus bovis*, of unrecorded origin, produced from sucrose a polysaccharide which yielded glucose on hydrolysis and which was therefore assumed to be dextran. Hehre & Neil (1946) showed, mainly by serological tests, that many strains of '*viridans*' streptococci isolated from the blood of patients with subacute bacterial endocarditis could convert sucrose into a polysaccharide indistinguishable from *Leuconostoc* dextran. *S. bovis* is itself in many respects a '*viridans*' streptococcus (Topley & Wilson, 1946). Jeanes *et al.* (1954) measured, by periodate oxidation, the amounts of main-chain linkages (α -1:6) and branching linkages (α -1:3 and α -1:4) in the dextrans produced by two strains of *Streptococcus viridans*. These two dextrans were reported to contain 11 and 31% of α -1:3, and 4 and 2% of α -1:4-glucosidic linkages respectively. In contrast with these results Barker, Pautard, Siddiqui & Stacey (1955*b*) reported that the dextran produced from sucrose by an unidentified *Streptococcus*, isolated from ropey elderberry wine, contained only α -1:6-glucosidic linkages and was essentially unbranched.

Recently Dain, Neal & Seely (1956) reported that with most strains of *S. bovis* grown on sucrose-agar mucoid colonies containing a glucose polymer were produced only if carbon dioxide was present. The effect of carbon dioxide on the production of this polysaccharide, in liquid sucrose media, by several strains of rumen *S. bovis* has been investigated in detail by Bailey & Oxford (1958). They found that most of the strains failed to produce dextran in such a medium unless carbon dioxide or HCO_3^- ion was present. The polysaccharide appeared to be a dextran containing few, if any, branching linkages. In view of the lack of information on *Streptococcus* dextrans it was decided to investigate, in more detail, the structure of the dextrans produced by the *S. bovis* strains used in

the above work. This paper reports the results obtained in a study of the dextrans produced by six strains of rumen *S. bovis* from sucrose at 37° in the presence of carbon dioxide. The effect of incubation temperature and of Mg^{2+} ions in the medium on dextran structure is also briefly described.

EXPERIMENTAL

Streptococcus bovis strains and cultures. Six strains were used comprising two (1010Y and 18M2) supplied as freeze-dried cultures by Dr P. N. Hobson and four (A1, A2, I and 293C) freshly isolated from cows at this Laboratory. Full details of all of these strains and of the sucrose (8%, w/v) medium used for the production of dextran are given by Bailey & Oxford (1958). Cultures (100 ml.) were incubated anaerobically for 48 hr. at 37°, CO_2 being provided either as 100% CO_2 gas atmosphere or as HCO_3^- ion (0.01 M).

Isolation and purification of dextran. Crude dextran was precipitated from the cell-free culture fluid with ethanol (2 vol.). Purification of the precipitated dextran was carried out by the usual methods (Barker, Bourne, Bruce, Neely & Stacey, 1954*a*; Bailey & Oxford, 1958). The purified dextran was freeze-dried and finally dried *in vacuo* at 60° over P_2O_5 .

Acid hydrolyses. Dextran (20 mg.) in 1.5N- H_2SO_4 (10 ml.) was heated for 9 hr. at 100°, neutralized (3N-NaOH) and diluted to 100 ml. with water. Reducing sugars in the hydrolysate were measured by the cuprimetric method of Shaffer & Hartmann (1921) and the results, corrected by the factor of Pirt & Whelan (1951), reported as glucose. For paper-chromatographic analyses the acid hydrolysate was neutralized with solid BaCO_3 , filtered and concentrated to 0.2 ml. Partial acid hydrolyses were carried out by heating the dextran-acid mixture (above) at 100° for 2 hr.

Paper-chromatographic analyses. Chromatograms were prepared and developed by the usual techniques with a solvent consisting of the top layer of either: (a) butanol-1-ethanol-water-aq. NH_3 soln., density 0.91 (49:10:40:1, by vol.; Barker *et al.* 1954*a*) or (b) ethyl acetate-water-pyridine (2:2:1, by vol.; Jermyn & Isherwood, 1949). Sugars were detected with AgNO_3 (Trevelyan, Procter & Harrison, 1950), aniline hydrogen phthalate (Partridge, 1949) and benzylamine-ninhydrin (Bayly & Bourne, 1953).

Periodate oxidations. Dextran (100 mg.) dissolved in water (250 ml.) containing 5 ml. of 0.3M-sodium metaperiodate was incubated in the dark at 28°. Periodate consumption and formic acid production were measured at

24 hr. intervals by the methods described by Jeanes & Wilham (1950). After 120 hr. the oxidized dextran solution (80 ml.) was treated with ethylene glycol (1 ml.), dialysed against tap water and concentrated under vacuum to a small volume (5 ml.). The concentrate was hydrolysed with 3*N*-H₂SO₄ (5 ml.) at 100° for 9 hr. and the hydrolysate neutralized and concentrated to a syrup for paper-chromatographic analysis.

Optical rotations. These were measured with a Hilger polarimeter in a 1 dm. tube containing dextran (20–40 mg.) dissolved in *N*-NaOH (10 ml.).

Infrared spectra. Spectrograms were obtained over the range 700–900 cm.⁻¹ on a Perkin-Elmer, model 21, double-beam infrared spectrophotometer with Nujol (liquid paraffin) films of the dextrans.

Authentic Leuconostoc dextran. A specimen of *Leuconostoc* dextran, prepared by the author in Professor M. Stacey's Laboratory, Birmingham University, was used for comparative purposes and as a source of authentic isomaltose and isomaltotriose. This dextran, produced from sucrose at 25° by *Leuconostoc arabinosaceus* (*Betacoccus arabinosaceus*, Birmingham strain), contained about 15% of α -1:3 linkages but no α -1:4 linkages (Barker *et al.* 1954*a*).

RESULTS

Yields and analyses of the dextrans produced by strains of Streptococcus bovis. The yield of purified dextran produced by each strain of *S. bovis* (as reported by Bailey & Oxford, 1958) together with the optical rotation and glucose content of the dextran is listed in Table 1. Results obtained from the periodate oxidation of the dextrans, after oxidation for 96 hr., are also listed in Table 1. Periodate consumption and formic acid production showed no change after 96 hr. Acid hydrolysates of the final, concentrated, oxidized dextran solutions showed on paper chromatograms only a trace component corresponding to glucose. The amount of glucose present on the papers from all six oxidized dextrans was estimated, by visual comparison with standards, to correspond to less than 0.5% of the original dextran. In contrast with these results oxidized *Leuconostoc* dextran hydrolysate showed on the papers a strong glucose component corresponding to 10–15% of the original dextran.

Effect of cultural conditions on dextran structure. *S. bovis* (strain I) was grown in the standard medium at 28°. At this temperature growth was slower and the yield of dextran only 65% of that at 37°. Strain I was also grown at both 28° and 37° in a modified medium containing in addition magnesium acetate (0.008*M*-Mg²⁺ ions). Both the dextrans produced at 28° were noticeably less soluble in water than any of the dextrans produced at 37°. All three dextrans had the same optical rotation as the strain I dextran listed in Table 1 and consumed after oxidation for 96 hr. 1.99–2.00 moles of periodate and produced 0.98–0.99 mole of formic acid/mole of anhydroglucose. Only trace amounts (less than 0.5%) of glucose were detected in acid hydrolysates of the oxidized dextrans.

Infrared spectra of the dextrans. Spectrograms prepared from dextran from each strain of *S. bovis* showed two absorption peaks at 760–765 cm.⁻¹ and at 842–844 cm.⁻¹ Barker, Bourne, Stacey & Whiffin (1954*b*) record three absorption peaks for dextrans in this region at 841, 794 and 768 cm.⁻¹ and assign them to the α configuration, α -1:3-glucosidic linkage and α -1:6-glucosidic linkage respectively. The second of these three absorption peaks was absent from the spectrograms of all six *Streptococcus* dextrans but present on that of the *Leuconostoc* dextran.

Paper-chromatographic analyses of dextran hydrolysates. Total acid hydrolysates of all six *Streptococcus* dextrans showed on the papers a single reducing component chromatographically identical with glucose. Partial acid hydrolysates of each dextran showed components corresponding to glucose and an apparently homologous series of oligosaccharides (detected with silver nitrate or aniline hydrogen phthalate). The first two of these oligosaccharides were chromatographically identical with authentic isomaltose and isomaltotriose on papers developed with either solvent (*a*) or (*b*) or by the benzylamine–ninhydrin technique. No sign of the disaccharides derived from possible branch linkages (nigerose or maltose) was seen on any of the papers.

Table 1. *Yields and analyses of dextrans produced by rumen strains of Streptococcus bovis in liquid sucrose media in the presence of carbon dioxide at 37°*

<i>S. bovis</i> strain no.	Dextran yield (% conversion of available glucose)	Glucose content (%)	$[\alpha]_D^{25}$ (in <i>N</i> -NaOH)	Moles of periodate consumed/mole of dextran an- hydroglucose	Moles of formic acid produced/ mole of dextran anhydroglucose
1010Y	37.9	96.0	+187	1.94	0.97
18M2	83.5	99.1	+189	2.00	0.95
A1	17.9	96.05	+190	1.97	0.98
A2	67.3	96.50	+194	2.00	0.98
I	50.8	97.0	+190	2.00	0.96
293C	27.2	97.0	+192	1.99	1.005
<i>Leuconostoc</i> dextran	—	96.0	+202	1.67	0.74

Oligosaccharides isolated from a dextran hydrolysate. Dextran (strain I; 1.5 g.) dissolved in 1.5N-H₂SO₄ (100 ml.) was heated for 2 hr. at 100°, and the hydrolysate, after neutralizing (BaCO₃) and filtering, was adsorbed on a charcoal-Celite column (40 cm. × 3.5 cm.; Whistler & Durso, 1950). Sugars were eluted from the column with water and water-ethanol mixtures. After removal of glucose (water 3 l., followed by ethanol-water, 2%, v/v; 2 l.) chromatographically pure disaccharide was eluted with ethanol-water, 5%, v/v (1 l.). Stronger ethanol solution (7.5%, v/v; 1 l.) eluted a trisaccharide fraction. The two fractions were concentrated under vacuum and freeze-dried, then treated with anhydrous methanol to remove inorganic material, and finally freeze-dried again and vacuum-dried. The yield of disaccharide was 200 mg. of glassy solid and of trisaccharide 150 mg. of solid. The disaccharide showed on paper chromatograms a single component chromatographically identical with authentic isomaltose, the trisaccharide showed a single component identical with authentic isomaltotriose. No sign of any other disaccharide was detected in any of the washings from the column.

The disaccharide had $[\alpha]_D^{20} + 120^\circ$ (c, 0.20, in water); Montgomery, Weakly & Hilbert (1949) report $[\alpha]_D^{20} + 120^\circ$ for isomaltose. The disaccharide (70 mg.) was acetylated with acetic anhydride and sodium acetate (Barker *et al.* 1954a), and worked up in the usual manner and crystallized from ethanol to yield 60 mg. of crystalline solid. The crystalline acetate had m.p. 142–143° (uncorr.), unchanged by admixture with an authentic sample of β -isomaltose octa-acetate kindly supplied by Dr S. A. Barker, and had $[\alpha]_D^{26} + 100^\circ$ (c, 0.4, in chloroform). Barker *et al.* (1954a) report for β -isomaltose octa-acetate m.p. 144–145° and $[\alpha]_D^{20} + 99.6^\circ$ (in chloroform).

DISCUSSION

That the glucose polymer produced by *S. bovis* from sucrose is a dextran is clearly established by the identification of isomaltose as the sole disaccharide in partial acid hydrolysates of the polysaccharide. In the absence of methylation studies periodate oxidation is probably the most useful technique for studying dextran structures. An unbranched dextran, containing only α -1:6-glucosidic linkages consumes 2 moles of periodate and produces 1 mole of formic acid/mole of anhydroglucose. Branch linkages, either α -1:3 or α -1:4, cause a significant lowering of both periodate consumption and formic acid production. The presence of free glucose in the hydrolysate of the oxidized dextran is also a useful confirmatory indication of the presence of α -1:3-glucosidic linkages in the

original dextran. The values obtained for periodate consumption and formic acid production with all six *S. bovis* dextrans clearly indicate the absence of more than traces of α -1:3 or α -1:4 linkages. The absence of α -1:3 linkages is confirmed by the low level (less than 0.5%) of glucose in the oxidized dextrans, and by the infrared spectra.

Highly branched dextrans would show disaccharides other than isomaltose (i.e. nigerose or maltose) on paper chromatograms of their partial hydrolysates (see Barker *et al.* 1954a). These other disaccharides were never detected. Specific optical rotations (+187 to +194°) confirm that the glucosidic linkages have an α configuration and are in the same range as values reported for other unbranched dextrans; e.g. unbranched *Streptococcus* dextran +194° (Barker *et al.* 1955b), unbranched *Leuconostoc* dextran +194° (Barker, Bourne, Jeanes, Neely & Stacey, 1955a). The specimen of *Leuconostoc* dextran, containing about 15% of α -1:3-glucosidic linkages, had $[\alpha]_D^{20} + 202^\circ$ (in N-sodium hydroxide). Higher values, up to 220°, have been reported for branched dextrans of this type (Bailey, Barker, Bourne & Stacey, 1957).

All of the results obtained indicate that the dextrans produced by six strains of rumen *S. bovis* are unbranched polysaccharides containing only α -1:6-glucosidic linkages. The structures of the dextrans do not appear to reflect any differences between the strains.

It is possible that incubation temperature has an effect on the branching of dextrans. Both Barker *et al.* (1955b) and Jeanes *et al.* (1954) grew their streptococci at 25°. Working with a *Leuconostoc* dextranucrase, Bailey *et al.* (1957) reported that the enzyme producing the branch linkage (α -1:3) was much less stable, even at 0°, than the enzyme producing the main chain (α -1:6). Our streptococci were grown at 37°; the temperature of the rumen, the organism's natural habitat, is just below 40°. When *S. bovis* (strain I) was grown at a lower temperature (28°) the dextran produced was still unbranched. Barker *et al.* (1955a) also reported that magnesium was essential for the production of a highly branched dextran by one strain of *Leuconostoc*. The medium used in the present work would contain traces of magnesium. When the medium was enriched with magnesium *S. bovis* (strain I) still produced an unbranched dextran at both 28° and 37°.

Under the cultural conditions used in the present work it seems that rumen strains of *S. bovis* are useful organisms for the synthesis of unbranched dextran. They may also prove a useful source of a dextranucrase free from enzymes which synthesize α -1:3- and α -1:4-glucosidic linkages. The properties of the dextranucrase secreted by *S. bovis* are being investigated.

SUMMARY

1. The polysaccharide produced by rumen strains of *Streptococcus bovis* from sucrose at 37°, in the presence of carbon dioxide, yielded only glucose on hydrolysis.

2. Isomaltose was isolated and identified as the sole disaccharide produced by partial acid hydrolysis of the polysaccharide, which is therefore a dextran.

3. On the evidence of periodate oxidation, infra-red spectra and optical rotations it is concluded that the dextrans produced by six strains of *S. bovis* contain only α -1:6-glucosidic linkages and are therefore unbranched.

4. Growth of one strain of *S. bovis* at a lower temperature (28°) or in a medium containing added magnesium still produced an unbranched dextran.

My thanks are due to Dr A. E. Oxford, of this Laboratory, who grew the additional cultures used in this work; and to Mr R. I. M. Fraser, of the Dominion Laboratory, Lower Hutt, who provided the infrared spectrograms. Freeze-dried strains of the organism were kindly provided by Dr P. N. Hobson of the Rowett Research Institute, Aberdeen, and the specimen of β -isomaltose octa-acetate by Dr S. A. Barker, Chemistry Department, Birmingham University. The technical assistance of Miss J. Michael is gratefully acknowledged.

REFERENCES

- Bailey, R. W., Barker, S. A., Bourne, E. J. & Stacey, M. (1957). *J. chem. Soc.* p. 3530.
 Bailey, R. W. & Oxford, A. E. (1958). *J. gen. Microbiol.* **19**, 130.

- Barker, S. A., Bourne, E. J., Bruce, G. T., Neely, W. B. & Stacey, M. (1954a). *J. chem. Soc.* p. 2395.
 Barker, S. A., Bourne, E. J., Stacey, M. & Whiffen, D. H. (1954b). *J. chem. Soc.* p. 171.
 Barker, S. A., Bourne, E. J., James, A. E., Neely, W. B. & Stacey, M. (1955a). *J. chem. Soc.* p. 2096.
 Barker, S. A., Pautard, F., Siddiqui, I. R. & Stacey, M. (1955b). *Chem. & Ind.* p. 1450.
 Bayly, R. J. & Bourne, E. J. (1953). *Nature, Lond.*, **171**, 385.
 Dain, J. A., Neal, A. L. & Seely, H. W. (1956). *J. Bact.* **72**, 209.
 Hehre, E. J. & Neil, J. M. (1946). *J. exp. Med.* **83**, 145.
 Jeanes, A. R., Haynes, W. C., Wilham, C. A., Rankin, J. C., Melvin, E. A., Austin, M. J., Cluskey, J. E., Fisher, B. E., Tsuchiya, H. M. & Rist, C. E. (1954). *J. Amer. chem. Soc.* **76**, 5041.
 Jeanes, A. R. & Wilham, C. A. (1950). *J. Amer. chem. Soc.* **72**, 2655.
 Jermyn, M. A. & Isherwood, F. A. (1949). *Biochem. J.* **44**, 402.
 Montgomery, E. M., Weakly, F. B. & Hilbert, G. E. (1949). *J. Amer. chem. Soc.* **71**, 1682.
 Niven, C. F., Smiley, K. L. & Sherman, J. M. (1941). *J. biol. Chem.* **140**, 105.
 Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
 Pirt, S. J. & Whelan, W. J. (1951). *J. Sci. Fd Agric.* **2**, 224.
 Shaffer, P. A. & Hartmann, A. F. (1921). *J. biol. Chem.* **124**, 425.
 Topley, W. W. C. & Wilson, G. S. (1946). *Principles of Bacteriology and Immunity*, 3rd ed., p. 600. Ed. by Wilson, G. S. & Miles, A. A. London: Edward Arnold and Co.
 Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature, Lond.*, **166**, 444.
 Whistler, R. L. & Durso, D. F. (1950). *J. Amer. chem. Soc.* **72**, 677.

The Anaerobic Metabolism of Citrate in Rat Liver

By W. BARTLEY, M. SOBRINHO-SIMÕES,* BRENDA M. NOTTON AND GRAZIA MONTESI†
Cell Metabolism Research Unit, Department of Biochemistry, University of Oxford

(Received 10 June 1958)

In experiments with washed particles of rat liver, Vignais, Vignais & Bartley (1957) showed that under aerobic conditions the uptake of oxygen was insufficient to account for the removal of tricarboxylic acids. This paper is concerned with the examination of the products of anaerobic citrate metabolism in washed particulate preparations of rat liver.

* Present address: Faculdade de Medicina, Universidade de Porto, Porto, Portugal.

† Present address: Biochemistry Dept., Vanderbilt University Medical School, Nashville, Tenn.

EXPERIMENTAL

Washed particles. Rats were stunned and beheaded and the livers were rapidly removed and chilled in ice. After removal of surplus ice the livers were minced in the Fischer mincer (Jouan, Paris). The pulp from one liver (8–10 g.) was homogenized with 30 ml. of 0.9% KCl soln. in a stainless-steel homogenizer of the Potter–Elvehjem type. The homogenate was made to 60 ml. with 0.9% KCl soln., and the particulate material that sedimented after centrifuging at 0° for 15 min. at 2000 g was collected and washed twice on the centrifuge with 30 ml. of 0.9% KCl, the same centrifugal conditions being used for both washings.