

## Note

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### Detailed structure of a dextran from a cariogenic bacterium

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The broth dextran from the cariogenic bacterium *Streptococcus mutans* (E 49) is a highly branched polymer<sup>1</sup> About one fifth of its D-glucose residues are involved as branch points, as 18% of the D-glucose residues are resistant to periodate oxidation, and the hydrolyzates of the methylated dextran contain 2,4-di-O-methyl-D-glucopyranose in an amount representing about 20% of the D-glucose These results suggest two types of structures, *viz*, a "tree-like" (glycogen) structure with (1→3)-branches or a "comb-like" structure having a (1→3) backbone and branches from O-6 of every D-glucose residue The known stability of these dextrans to dextranases<sup>2</sup> suggests a "tree-like" structure These two postulated structures would be expected to yield different products after sequential periodate oxidation, borohydride reduction, and mild hydrolysis with acid Mild hydrolysis with acid would cause the hydrolysis of acyclic acetals, whereas cyclic acetals would remain intact<sup>3</sup> The "tree-like" dextran would yield a glycerol  $\alpha$ -D-glucopyranoside, whereas the "comb-like" dextran would give a (1→3)-D-glucan

#### EXPERIMENTAL

The E-49 broth dextran (2 mg/ml) was oxidized with sodium periodate (10 mg/ml) at room temperature in the dark<sup>4</sup> Periodate consumption was stable after 36–48 h, and the excess of periodate was decomposed by ethylene glycol (3 mg/ml) After 1 h, the solution was made slightly alkaline with 0.1M sodium hydroxide, and sodium borohydride (2 mg/ml) was added, a second addition of sodium borohydride (2 mg/ml) was made after<sup>5</sup> 2 h After reaction for 2 h, the excess of borohydride was decomposed by 0.05M sulfuric acid, and the solution was exhaustively dialyzed against distilled water The solution was then treated with 0.25M hydrochloric acid for 30 h at 25°, and then made neutral either with Dowex-400 (hydrogen carbonate) ion-exchange resin or by evaporation under diminished pressure The extent of hydrolysis was monitored by the amount of D-glucose formed (as measured by D-glucose oxidase), this indicated the hydrolysis of the cyclic acetal groups

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The components in the neutralized hydrolyzate were separated by paper chromatography with water-saturated butanol. Glycerol and a component having the  $R_G$  of a glycerol D-glucopyranoside<sup>6</sup> were detected by 0.25% ammoniacal silver nitrate spray. The D-glucoside spot was eluted, hydrolyzed with M hydrochloric acid for 1 h at 100°, and rechromatographed with the same solvent. Glycerol and D-glucose were the only compounds detected, indicating that the compound of  $R_G$  0.6 was a glycerol D-glucoside. This could result were a "tree-like" structure present, and the D-glucose were involved in a (1→3)-branch point. There was no material at the origin of the chromatogram of the partially hydrolyzed material, indicating the absence of a (1→3)-D-glucan.

A sample of the neutralized, partially hydrolyzed material (8 mg/ml) was separated by gel filtration on a column (2.0 × 36.0 cm) of Sephadex G-15. Fractions (3 ml) were collected, and assayed with D-glucose oxidase before and after complete hydrolysis with acid. D-Glucose was found only after hydrolysis, and was then present in the inclusion volume, where glycerol D-glucopyranoside would be expected. No material that was reactive with D-glucose oxidase (after hydrolysis) was present in the void volume, where a (1→3)-D-glucan might be expected. As Sephadex G-15 has a low exclusion range (10,000 MW), it is unlikely that a (1→3)-glucan was present.

The data strongly suggest a dextran having linear (1→6)-linkages, with random (1→3)-branches. The general enzymic resistance to dextranases also favors the concept of a "tree-like" structure.<sup>2</sup> Periodate oxidation of broth dextrans of *Streptococcus mutans* FA1, GF71, and HS6 show 61–67% of (1→6)-linkages and 21–32% of (1→3)-linkages. These organisms are very similar to E 49, and therefore, their dextrans probably have similar "tree-like" structures.

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#### REFERENCES

- 1 W. J. LEWICKI, L. W. LONG, AND J. R. EDWARDS, *Carbohydr. Res.*, **17** (1971) 175.
- 2 W. H. BOWEN, *Brit. Dent. J.*, **124** (1968) 347.
- 3 H. O. BOUVENG AND B. LINDBERG, *Advan. Carbohydr. Chem.*, **15** (1960) 87.
- 4 J. C. RANKIN AND A. R. JEANES, *J. Amer. Chem. Soc.*, **76** (1954) 4435.
- 5 I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohydr. Chem.*, **5** (1965) 361.
- 6 M. H. SAIER, JR., AND C. E. BALLOU, *J. Biol. Chem.*, **243** (1968) 4332.