REGULATION OF DEXTRANASE SYNTHESIS BY *STREPTOCOCCUS MUTANS*

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1. Introduction

The release of endo-dextranase in batch-grown cultures of *Streptococcus mutans* can influence the activity and products of D-glucosyltransferases that convert sucrose into α-D-glucans [1,2]. Although dextranase can be separated from the D-glucosyltransferases [3,4], the mechanism of glucan synthesis is often studied with preparations of D-glucosyltransferases that contain dextranase activity [2]. This paper reports on conditions of growth of *S. mutans* under which the production of D-glucosyltransferases was normal, whereas the release of dextranase could not be demonstrated.

2. Experimental

2.1. *Bacteria*

Strains of *S. mutans* AHT, B13, K1-R and OMZ176 were obtained from Dr D. D. Zinner, Dr S. Edwardsson, Dr R. J. Fitzgerald and Professor B. Guggenheim, respectively. The organisms were grown in continuous culture at 37°C in the medium of [5] with the limiting sugar, usually D-glucose, added at 0.5%. Anaerobic conditions were maintained with a gas mixture of N_2/CO_2 (95:5, v/v), and the pH was kept constant at 6.0. After the organisms had utilized all the glucose (batch-growth, stationary phase) a sample was withdrawn, and pumping of fresh medium through the vessel was commenced. When at least 5 culture vol. (equiv. 7 mean generation times) had passed through, the culture was considered to be in a steady state. Samples were then collected in an ice-cold bath, and the broth was centrifuged for 10 min at 12 000 × *g*. The cell-free filtrate was dialysed against 50 mM sodium citrate buffer (pH 6.0).

2.2. *Assay of enzymic activities*

Dextranase activity of the dialysed cell-free filtrate was determined from the release of reducing sugars [6] in a digest (1 ml) that contained Sigma 2000 dextran (15 mg) and enzyme (0.5 ml) incubated at 35°C. Samples were withdrawn at intervals up to 24 h. One unit of activity was defined as the amount of enzyme that released 1 μmol reducing sugar in 1 min.

The triplicate reaction mixtures (0.5 ml) for D-glucosyltransferase activity contained sucrose–[U-^{14}C]-glucose (5 mg, 0.006 μCi/μmol), Sigma 2000 dextran (50 μg) and dialysed cell-free filtrate (0.2 ml) in centrifuge tubes. After incubation at 35°C for 20, 40 and 60 min, respectively, ethanol (3 vol.) was added to precipitate polysaccharide, and the mixtures were stored at 4°C for at least 24 h. The precipitates were then collected by centrifuging, washed twice with methanol, and resuspended in methanol. The samples were filtered through 25 mm paper circles (Whatman 3MM) held in a Millipore assembly and further washed with methanol (20 ml). The dried papers were suspended in a toluene cocktail (8 ml), and the radioactivity of the glucans was counted in a Beckman Model LS 9000 liquid scintillation counter.

D-Glucosyltransferase activity was also assayed in similar digests containing unlabelled sucrose. The washed pellets of polysaccharide were dissolved in 7 M H_2SO_4 (1.0 ml) and their weight was determined with anthrone reagent [7,8]. The yield of glucan was obtained by subtracting the weight of fructan (cold anthrone) from that of total polysaccharide (hot anthrone). One unit of activity was defined as the amount of enzyme that transferred 1 μmol D-glucose from sucrose into glucan in 1 min under the assay conditions.

3. Results and discussion

3.1. *Effect of growth conditions on extracellular dextranase activity*

Fig.1 shows the decrease in dextranase activity of culture filtrate that occurred when a batch growth of *S. mutans* K1-R was converted into a continuously growing culture with glucose being the limiting nutrient. The volume of vessel contents was constant at 325 ml, and the speed of the nutrient pump was set to provide a medium flow rate of 16.25 ml/h. The dilution rate D, which is defined as the number of culture volumes of medium passing through the growth vessel in unit time, was therefore 0.05 h^{-1}. Since $D = (\log_e 2)/t_d$, where t_d is the mean generation time, the growth rate was controlled by the dilution rate so that t_d was 14 h. Cells which had released dextranase into the medium during batch growth no longer did so when their growth rate was controlled at a low value, giving a mean generation time close to that in [9] for organisms growing in dental plaque.

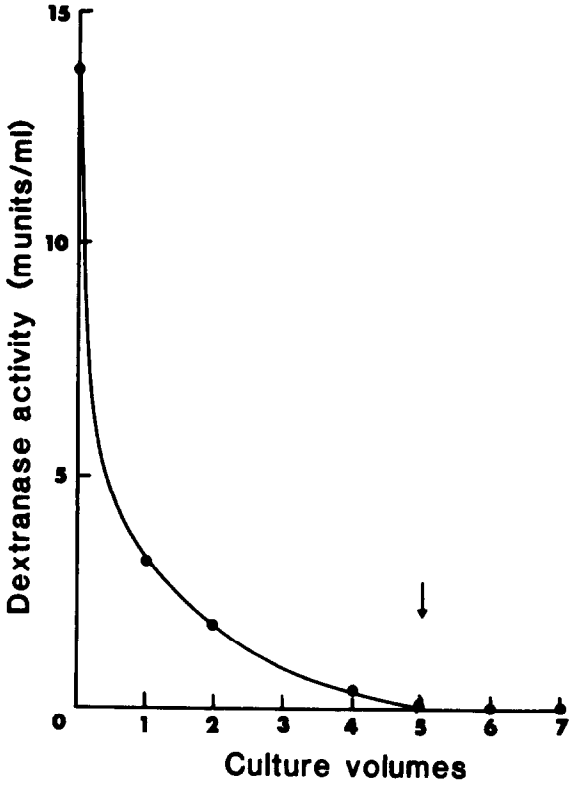

Fig.1. Extracellular endo-dextranase activity of *S. mutans* strain K1-R grown in a chemostat under conditions of glucose limitation. The initial value was obtained at the end of batch growth, then the nutrient pump was set to give D 0.05 h^{-1}. Three subsequent measurements of dextranase activity, taken during the transient stage, show the gradual loss of dextranase-producing cells. At the steady state (↓) and thereafter, the culture no longer produced dextranase.

S. mutans strains B13 and OMZ176, which are good producers of dextranase during batch growth, followed the same pattern as strain K1-R in continuous culture. No dextranase activity could be detected at D 0.05 h^{-1}. After *S. mutans* OMZ176 had become steady at D 0.05 h^{-1} in the glucose-limited medium, the reservoir was replaced by one that contained the same medium, supplemented with 4% glucose. Thus glucose became sufficient, and another of the substances essential for growth of the organism became limiting. Growth then continued at D 0.05 h^{-1}, but dextranase did not reappear. Thus, it may be a general rule that *S. mutans* strains do not release dextranase when the growth rate is kept low by a fixed rate of supply of a growth-limiting nutrient. If this condition were suitable for D-glucosyltransferase production, the culture of *S. mutans* at low growth rate would eliminate the need for the extensive chromatographic procedures that are necessary to remove dextranase from D-glucosyltransferase.

3.2. *Effect of growth rate on extracellular D-glucosyltransferase activity*

A preliminary study with strains OMZ176 and B13, which showed a 3.5-fold and 2.0-fold higher D-glucosyltransferase activity, respectively, at low growth rate (D 0.05 h^{-1}) than at high growth rate (D 0.5 h^{-1}), indicated that a low growth rate might be the best condition for D-glucosyltransferase production. However, the higher activity at low growth rate could have been due to the absence of dextranase, whereas the apparent lower activity at the higher growth rate might have been influenced by the presence of dextranase. The only way to assess the true effect of growth rate on D-glucosyltransferase activity was to use a strain of *S. mutans* that did not release dextranase under any condition.

A dextranase-deficient strain of *S. mutans* AHT was grown in medium supplemented either with glucose (0.5%) or with fructose (0.5%), and D was varied between 0.05–0.50 h^{-1}. D-Glucosyltransferase activity was maximal at the lowest growth rate in fructose medium, but did not vary greatly with growth rate in glucose medium (fig.2). A low growth rate was therefore a suitable condition for the production of D-glu-

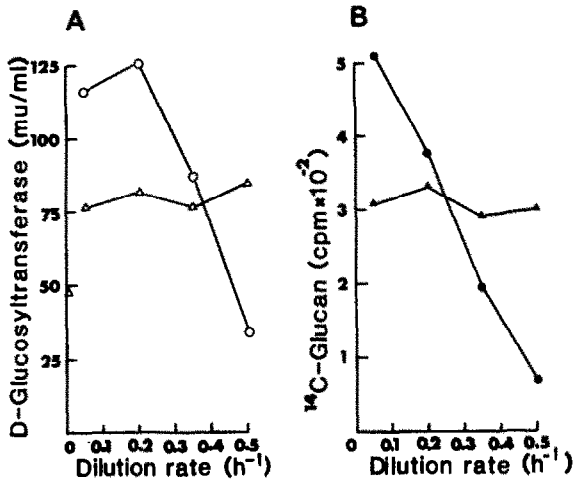

Fig.2. Effect of dilution rate on the extracellular D-glucosyltransferase activity of *S. mutans* strain AHT grown in a chemostat under conditions of glucose limitation (△, ▲) and fructose limitation (○, ●). The open symbols (A) represent activity units based on the weight of α-D-glucan synthesized, the value shown at *D* 0 being that obtained in batch culture. The closed symbols (B) represent incorporation of [^{14}C]glucosyl residues into glucan in 1 min.

cosyltransferase. Portions (300 ml) of cell-free filtrate produced at *D* 0.05 h^{-1} and 0.40 h^{-1} under glucose limitation were concentrated to 25 ml with hollow fibres, and the enzymes that precipitated with 50% saturated ammonium sulphate were separated on hydroxyapatite [4]. In both cases, a linear gradient of sodium phosphate permitted the isolation of an enzyme (GTF-S) that synthesized water-soluble glucan and another enzyme (GTF-I) that synthesized water-insoluble glucan, from sucrose. Peak activities of the two enzymes were eluted with 0.07 M and 0.26 M sodium phosphate (pH 6.5), respectively. This established that the two D-glucosyltransferases, previously isolated only from batch cultures, were also preduced in continuous culture.

Cell-free filtrate from *S. mutans* OMZ176, grown at *D* 0.05 h^{-1} in glucose-limited medium, was then treated as described above for AHT. Fractions eluted from hydroxyapatite that contained peak D-glucosyltransferase activity, were pooled and incubated with sucrose as in [4]. Methylation analysis of the glucan products proved that GTF-S had synthesized a highly branched 1,6-glucan, and that GTF-I had synthesized an essentially linear 1,3-glucan. The D-glucosyltransferases thus had the same specificity as the enzymes released in batch culture [10].

It is concluded that continuous culture of *S. mutans* at low growth rate is an appropriate technique for the production of cell-free filtrate that is completely devoid of dextranase activity. Since D-glucosyltransferases are apparently produced in optimal amounts, this condition gives suitable starting material for the study of these enzymes and their glucan products. Some serotypes of *S. mutans* give greatly increased yields of D-glucosyltransferases when cultured in excess glucose [11], whereas other strains give better yields in glucose-deficient medium [9]. We have confirmed that with both types of media, culture filtrates can be obtained free from dextranase.

Acknowledgements

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References

[1] Walker, G. J. (1972) J. Dent. Res. 51, 409–414.
[2] Germaine, G. R., Harlander, S. K., Leung, W. S. and Schachtele, C. F. (1977) Infect. Immunol. 16, 637–648.
[3] Pulkownik, A. and Walker, G. J. (1977) Carbohydr. Res. 54, 237–251.
[4] Walker, G. J. and Hare, M. D. (1977) Carbohydr. Res. 58, 415–432.
[5] Van Houte, J. and Saxton, C. A. (1971) Caries Res. 5, 30–43.
[6] Dygert, S., Li, L. H., Florida, D. and Thoma, J. A. (1965) Anal. Biochem. 13, 367–374.
[7] Van Handel, E. (1965) Anal. Biochem. 11, 266–271.
[8] Van Handel, E. (1967) Anal. Biochem. 19, 193–194.
[9] Hamilton, I. R., Phipps, P. J. and Ellwood, D. C. (1979) Infect. Immunol. 26, 861–869.
[10] Hare, M. D., Svensson, S. and Walker, G. J. (1978) Carbohydr. Res. 66, 245–264.
[11] Wenham, D. G., Hennessey, T. D. and Cole, J. A. (1979) J. Gen. Microbiol. 114, 117–124.