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Influence of Secretory Immunoglobulin A and Purified Secretory Component on Dextran-sucrase Activity of *Streptococcus mutans*[1]

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The net stimulation of dextransucrase EC 2.4.1.5) activity from *Streptococcus mutans* HS6 by dextran, secretory immunoglobulin A, or secretory component was investigated. Approximately equal stimulation resulted from treatment with these three components.

The production of extracellular glucans from sucrose (6) is an important attribute of oral bacteria, since it partially determines the adhesion of dental plaque to oral surfaces. Any substance that stimulates glucan production is therefore a candidate for intensifying some types of oral disease.

In a recent report, Fukui et al. (3) described the enhancement of dextransucrase activity by secretory IgA (SIgA), the major immunoglobulin component of saliva. Since this finding represents a previously undescribed biological activity of IgA with direct relevance to oral pathology, we initiated a study of the interaction of dextransucrase and SIgA. We also assayed purified secretory component (SC) for its activity, since SC is a polypeptide component of SIgA not found to any significant extent in serum IgA.

Dextransucrase was purified from cell-free spent medium from growth of *Streptococcus mutans* HS6, by 60% ammonium sulfate precipitation, and by column chromatography on hydroxylapatite and diethylaminoethyl-Bio-Gel A in a procedure similar to that used by Fukui et al. (2). Acrylamide disc gel electrophoresis of 10 μg of protein revealed an apparently pure preparation. SIgA was purified from human colostrum by the method of Pincus et al. (9). Free SC was isolated chromatographically from human colostrum and tested for purity by immunoelectrophoresis.

Dextransucrase was measured by the rapid filter-paper assay of Germaine et al. (5) for 20 min at 37°C. This method was used since it is specific for dextransucrase, whereas the assay used by Fukui et al. (2) cannot discriminate between invertase and dextransucrase activity. All assays were done in duplicate. All components were added in amounts that could be expected to produce maximum stimulation based on published values (3).

When zero-time values are compared to those found after 8 h of incubation, only a slight stimulation of dextransucrase activity resulted from treatment with either SIgA or purified SC (Table 1). SIgA resulted in a 36% stimulation of activity, whereas SC stimulated 37%. However, dextran at a 100-μg concentration stimulated dextran formation 27%, and a 19% increase in sucrose-derived glucose polymerization was observed with no additions to the reaction mixture. Thus, considering the controls (no addition) to be a zero stimulation, a net maximum stimulation of 21% was observed when both dextran and SC were added to the reaction mixture. This should be compared to the 735% increase reported by Fukui et al. (3). When data on additions are compared with controls at either zero time or after 8 h of incubation, the similarity of stimulation between dextran, SIgA, and SC suggests that all three may be acting as structural templates for synthesis of dextrans.

The results of this investigation do not support the initial report of Fukui et al. (3). Several factors should be considered in evaluating the differences. (i) Fukui et al. (3) obtained their immunoglobulins from other investigators, whereas we prepared all the components in the enzyme assay, with the exception of the [^{14}C]sucrose, in our own laboratories. (ii) If one considers only the values for 8-h preincubation with the various additions, greater than two-fold stimulation, as compared to no addition, is evident in some instances. However, much of the apparent stimulation could be due to different initial values that are not evident when

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TABLE 1. *Effect of dextran, SIgA, and SC of SIgA on dextransucrase activity from S. mutans strain HS6*[a]

Addition[b]	Dextransucrase activity (milliunits)[c]	
	0 h[d]	8 h
None	1.21	1.44
100 μg of dextran (10,000 mol wt)	2.22	2.68
50 μg of SC	2.50	3.43
100 μg of dextran and 50 μg of SC	2.25	3.18
50 μg of SIgA	2.12	2.88
100 μg of dextran and 50 μg of SIgA	2.73	2.80

[a] Values are means of duplicate experiments.

[b] Additions to standard reaction solution, which contained 25 μmol of sodium acetate buffer, pH 5.5, 3.5 μmol of NaF, 9.4 μmol of sucrose, and 0.6 μmol of [U-^{14}C]sucrose (4.3 mCi/mmol) in a total volume of 0.5 ml.

[c] One unit of dextransucrase activity is that amount of enzyme causing the polymerization of 1 μmol of sucrose-derived glucose per min at 37°C.

[d] Incubation time at 4°C of buffer and additions before adding sucrose (substrate) and start of assay time.

zero-time readings for the individual reaction mixtures are omitted. (iii) Since SIgA heated at 100°C/10 min had undiminished stimulatory activity in the original report, we considered that the stimulation might be due to the presence of a structural matrix for polymerization rather than to any activity of a native biological material. This hypothesis was borne out by the stimulation by dextran alone of dextransucrase activity (Table 1). (iv) The assay system utilized in these experiments, which directly measures isotope incorporation into insoluble dextran, may also account in part for the differing results. However, the basic method has been used with success by others (1, 4, 7, 8, 10). Since SIgA was without significant activity, we did not study the dextransucrase-enhancing effect of IgA monomers versus polymers, J chain, or the different IgA subclasses, all factors that differentiate SIgA from serum IgA.

In conclusion, we have been unable to repeat initial results of a sevenfold stimulation of dextransucrase by either SIgA or SC. We have been informed (personal communication from T. Moriyama) that recent data from the Fukui group also conflict with their earlier observations and are in agreement with our findings.

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