The effect of chlorhexidine and some other detergents on the activity of dextransucrase from Streptococcus mutans

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The inhibitory effect of chlorhexidine and other bis-biguanides on the formation of dental plaque is not fully understood. The present paper describes the effect of chlorhexidine and some selected detergents on the activity of dextransucrase (EC 2.4.1.5.), an enzyme involved in the formation of important components of dental plaque. All detergents examined exerted an inhibitory effec' on dextransucrase activity, to some degree dependent on the presence of charged groups and their characters. The high concentrations of chlorhexidine necessary to inhibit dextransucrase activity seem to exclude the possibility that chlorhexidine exerts its plaque inhibiting effect by means of an effect on dextransucrase.

Key-words: Dental plaque; enzymes; microbiology

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INTRODUCTION

Chlorhexidine and a variety of other bis-biguanides possess the potential of inhibiting the formation of dental plaque, calculus, gingivitis and caries in human beings (*Schroeder*, 1969; *Løe & Schiøtt*, 1970 a, b; *Løe, Fehr & Schiøtt*, 1972; *Gjermo, Rølla & Årskaug*, 1973) and experimental animals (*Lindhe et al.*, 1970; *Davies & Hull*, 1973). Being a potent antibacterial, chlorhexidine depresses the oral microflora (*Schiøtt et al;* 1970) but this effect seems to play a minor role in its plaque-inhibiting potential (*Gjermo, Baastad & Rølla*, 1970). Additional explanations have therefore been sought.

The aim of the present study was to examine the effect of chlorhexidine and some detergents on the activity of extracellular soluble dextransucrase (α-1,6-glucan:D-fructose 2-glucosyltransferase, EC 2.4.1.5). Dextransucrase is involved in the production of extracellular glucans by certain plaque bacteria (*Carlsson, Newbrun & Krasse*, 1969). As such polysaccharides are important components of dental plaque (*Gibbons & Banghart*, 1967; *Gibbons & Nygaard*, 1968; *Guggenheim & Schroeder*, 1967), and probably play a significant role in the primary colonization of the tooth surface, a specific inhibition of dextransucrase activity would be a possible mecha-

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nism for the plaque-inhibiting effect of chlorhexidine.

MATERIALS AND METHODS

Bacteria and growth conditions

Streptococcus mutans strain Ingbritt (*Krasse*, 1966) was grown for 48 hr in 1 litre volumes of Tryptic Soy Broth (Difco) inoculated with a 100 ml preculture in the same medium. The broth was flushed with nitrogen prior to incubation. After incubation at 37°C, the bacteria were removed by centrifugation at 4°C and 10.000 × g for 15 min in a Serval centrifuge and the supernatant was used as the source of dextransucrase activity.

Determination of dextransucrase activity

The incubation consitions were a modification of those described by *Chludzinski, Germaine & Schachtele* (1974). A total volume of 5 ml consisting of 50 mM sodium acetate buffer pH 5.5, sucrose 100 mM, dextran T10 20μM, the respective detergents to be examined in appropriate concentrations (0–1 % w/v) and crude enzyme 0 –1 ml, was incubated at 37 °C in 25 ml conical flasks and shaken in a Dubnoff type metabolic shaking apparatus («Heto»). The reactions were initiated by the addition of enzyme. At intervals of from 2 to 10 min aliquots were withdrawn for the determination of reducing sugar by the Somogyi method (*Somogyi*, 1945), the proteins being precipitated and the reaction stopped as described by *Nelson*, 1944). The amount of reducing sugar released equivalent to fructose was calculated from fructose standards carried through the whole procedure. Zero-time controls with added enzyme were treated as the samples and used to correct the amount of fructose liberated. The release of reducing sugar was proportional with time for at least 30 min except for a lag period of from 1 to 5 min duration (Fig. 1). The velocity of the reaction was determined from the slope of the rectilinear portion of the curve using the methods of least squares to determine its slope. The velocity of the reaction was proportional with the amount of enzyme added and the K_m-value for sucrose was determined to 7.4 mM. The concentration of sucrose used was thus saturating. Addition of dextran T10 at a concentration of 20 μM increased the reaction rate about 20%.

In several experiments testing for formation of free glucose during the incubation was performed by a specific enzymatic method (Glucose oxidase method, GOD-Perid, Boehringer, kit. no. 15756). In no instance free glucose could be detected.

Chemicals used were as follows: Dextran T10 (Pharmacia Fine Chemicals, Uppsala, Sweden), Sodium desoxycholate (Difco Laboratories, Detroit, Michigan, USA), Chlorhexidine digluconate (ICI, Macclesfield, England), Sodium lauryl sulphate (Du Pont, Wilmington, Delaware, USA), Triton X-100 (*Rohm & Haas*, Philadelphia, USA).

Other chemicals used were analytical grade.

RESULTS

Fig. 1 shows the progress of the dextransucrase reaction with time for two different preparations of the enzyme. The lag period of from 2 to 5 min can be seen on the graphs. The rectilinear portions of the curves following the lag period extend to at least 30 min.

From Fig. 2 it can be seen that chlorhexidine and cetyltrimethylammonium bromide in concentrations higher than 0.04 mg/ml (0.004 %) cause a gradual decrease in the activity of *S.mutans* dextransucrase activity. The concentrations of the two compounds at which the enzyme activity is 50 % depressed (I_{50}) can easily be evaluated from the graphs as shown in the figure. At 10 mg/ml the enzyme activity in the presence of either of the compounds was depressed more than 90 %. It should be noted that the abscissa of the figure is logarithmic.

Table I shows the I_{50}-values of the various detergents as determined from plots of the same type as shown in Fig. 2. It is evident that cetyltrimethylammonium bromide and dodecyl sulphate on a weigt basis are about equally effective inhibitors of dextransucrase.

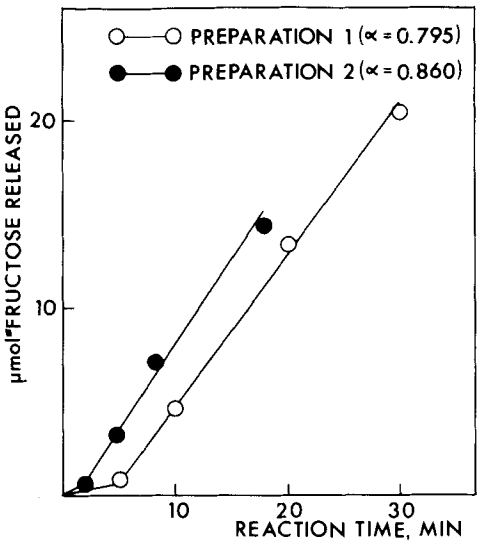

Fig. 1. Release of reducing sugar equivalent to fructose with time in the assay of dextransucrase from two different preparations of *S.mutans*. The velocity of the reaction was taken as the slope (α) of the rectilinear part of the progress curve. The conditions were as described in Materials and Methods using 1 ml enzyme preparation.

Table I. *Approximate concentrations (I_{50}) of various detergents at which activity of dextransucrase from S.mutans strain Ingbritt is 50 % inhibited.*

Detergent	I_{50} g/100 ml	mM
Chlorhexidine digluconate	0.065	0.72
CTAB*)	0.031	0.85
Sodium dodecyl sulphate	0.036	1.25
Sodium deoxycholate	> 1.0	> 24.1
Triton X-100	0.28	–

*) CTAB = cetyltrimethylammonium bromide

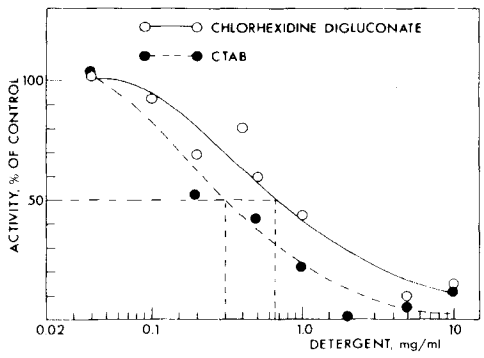

Fig. 2. Inhibitory effect of chlorhexidine digluconate and cetyltrimethylammonium bromide (CTAB) on the activity of dextransucrase from *S.mutans* strain Ingbritt. Each point on the curve represents the average of 2 to 3 separate determinations. The concentrations (I_{50}) at which the activity is depressed to half of the control value are shown on the figure.

The I_{50} of chlorhexidine is about twice as high as the other two compounds but on a molar basis all three compounds exhibit nearly identical I_{50}-values and chlorhexidine is the most active. Deoxycholate is decidedly less active as an inhibitor of dextransucrase and even at a concentration of 10 mg/ml the inhibition was only 20 %. The non-ionic detergent, Triton X-100, inhibited the dextransucrase reaction at a concentration about 10 fold higher than cetyltrimethylammonium bromide. At low concentrations (0.1 mg/ml) it actually stimulated the reaction about 20 %.

DISCUSSION

In the present study a crude, cell-free preparation of extracellular dextransucrase from *S.mutans* strain Ingbritt was used to test the influence of various detergents on the enzyme activity. The abscence of free glucose from the medium following incubation with sucrose rules out the presence of any significant amounts of invertase or levansucrase activities which have been shown to be produced by certain strains of *S.mutans (Fukui, Fukui & Moriyama*, 1974; *Carlsson*, 1970). Dextranase activity, which is known to be produced by many strains of *S.mutans*, (*Staat & Schachte-*

le, 1974), should also be considered as it might have released reducing sugars from dextran during the reaction either from the dextran added or from that formed during the reaction. However, no reducing sugars were produced when dextran T10 was incubated with the enzyme. We therefore concluded, that the activity measured was in fact dextransucrase and this conclusion is further supported by the Km-value of our enzyme for sucrose (7.4 mM) which is of the same order as the value (4.8 mM) reported by *Carlsson et al.* (1969) for dextransucrase.

In our test-system chlorhexidine exerted a concentration dependent inhibitory effect on the activity of dextransucrase but a similar and even slightly more pronounced effect was obtained with the cationic detergent cetyltrimethylammonium bromide. The anionic detergent, dodecyl sulphate was as effective as cetyltrimethylammonium bromide. This is in accordance with the recent observation by *Chludzinski et al.* (1974) who reported 95% inhibition of dextransucrase by 3.5 mM sodium dodecyl sulphate. The non-ionic detergent, Triton X-100, exhibited also an inhibitory effect on dextransucrase activity but at much higher concentrations. Finally, a weak inhibition of the enzyme was obtained with deoxycholate. The results may thus be summarized that all the detergents examined exert an inhibitory action on dextransucrase but the effect seems to some degree to be influenced by the presence of charged groups and their character.

The more pronounced effect of cetyltrimethylammonium bromide on the activity of dextransucrase than that of chlorhexidine is noteworthy because it has been established that cationic detergents do not inhibit plaque production in human beings, or do so to a very moderate degree. This has been observed with cetylpyridinium chloride by *Gjermo et al.* (1970) and with cetyltrimethylammonium bromide by *Rölla* (personal communication). These facts seem to exclude the possibility that chlorhexidine exerts its plaque-inhibiting effect by means of its inhibiting effect on dextransucrase. This is also supported by the much higher concentrations of chlorhexidine which are necessary for the inhibition of dextransucrase as compared with those found in saliva following mouth-rinses with chlorhexidine in plaque-inhibiting doses (*Jensen & Christensen*, 1971.)

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