Immunological Properties of the Primer-independent Glucosyltransferase of Streptococcus mutans Serotypes d and g

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Streptococcus mutans serotype g secretes at least three kinds of glucosyltransferase with different enzymological and immunological properties. One of them is a primer-independent enzyme and seems to be the source of primer for the others, both of which are primer-dependent enzymes. Recently, we purified the primer-independent enzyme, the third glucosyltransferase in this group from S. mutans strain AHT-k serotype g. In the present study, we examined the specificity of the antiserum against the primer-independent glucosyltransferase using extracellular culture-conditioned fluids of many strains of the various serotypes of S. mutans. The antiserum cross-reacted with the extracellular culture fluids from strains of serotypes d and a, in addition to serotype g, but not with those of other serotypes, indicating that the primer-independent glucosyltransferase is secreted by the S. sobrinus and S. cricetus, but not by S. mutans and S. rattus. The antiserum did not completely inhibit the activity of the enzyme, even at more than twofold antibody excess, determined by indirect precipitation with immobilized staphylococcal protein A.

INTRODUCTION

Streptococcus mutans secretes several kinds of glucosyltransferase which are important in cariogenicity (Gibbons & Banghart, 1967; Gibbons, 1968; Gibbons & Nygaard, 1968; Critchley et al., 1976). The various strains of S. mutans can be separated into seven or eight serotypes according to their cell surface antigenicities (Brathall, 1970; Perch et al., 1974; Beighton et al., 1981), the classification series ranging from serotypes a to g and/or h. These serotypes can in turn be classified into four groups according both to the specificities of their enzymes in extracellular polysaccharide synthesis (Mukasa, 1986; Kametaka et al., 1987) and to other biochemical properties (Coykendall, 1977; Shklair & Keene, 1974). Thus, serotypes d, g and/or h (S. sobrinus) form one group, serotype a (S. cricetus) another, serotypes c, e and f (S. mutans) a third, and serotype b (S. rattus) the fourth group. The mechanism of synthesis of extracellular polysaccharides in each group seems to be different.

Three kinds of glucosyltransferase have been purified from S. mutans serotype g; one is primer-independent water-soluble glucan synthase (McCabe, 1985), and the others are primer-dependent glucosyltransferase (Fukui et al., 1981; Shimamura et al., 1982). The former is thought to be the supplier of primer for the other two, and to have a significant role in the formation of S. mutans serotype g extracellular polysaccharide. Recently, we purified this enzyme, the third glucosyltransferase to be isolated from S. mutans strain AHT-k serotype g (Hanada et al., 1987).

In the present study, using antiserum against this enzyme, we examined the distribution of the enzyme in eight strains of S. mutans of various serotypes, and studied the effects of the antiserum on its activity.
**RESULTS**

Fig. 1 shows the results of double immunodiffusion analyses of crude enzyme preparations from various strains of *S. mutans*, using the antiserum against the primer-independent glucosyltransferase of strain AHT-k. With crude enzyme preparations of strains AHT-k, 6715 and OMZ176, the antiserum produced a single connecting line of precipitin, but formed a precipitin line spur with strain HS-1. In contrast, the antiserum did not cross-react with crude enzyme preparations from strains BHT, Ingbritt, B-14 (data not shown) or OMZ175 (data not shown).

Fig. 2 shows the inhibitory effects of the antiserum on enzyme activity. The antiserum only partially inhibited enzyme activity even when added in more than twofold excess. Thus, although addition of 2 μl antiserum caused complete immunoreaction between the enzyme and antiserum, about half of the original enzyme activity remained, and addition of 5 μl antiserum had little further effect.

Fig. 3 shows the results obtained by periodic acid Schiff staining of the immunoprecipitin line, with or without preincubation in enzyme assay buffer. When preincubation was done, the precipitin line was clearly stained with periodic acid Schiff (Fig. 3a), but when it was not done the single connecting precipitin line of purified enzyme and crude enzyme solution could not be stained (Fig. 3b).
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Fig. 1. Double immunodiffusion analysis of crude enzyme preparations from various strains of S. mutans. The centre well contained antiserum (20 µl) against the primer-independent glucosyltransferase from S. mutans strain AHT-k. Each outer well contained 400 µg crude enzyme protein solution derived from one of the strains of S. mutans: 1, 6715; 2, AHT-k; 3, OMZ176; 4, HS-1; 5, Ingbritt; 6, BHT.

Fig. 2. Inhibitory effect of antiserum against S. mutans strain AHT-k primer-independent glucosyltransferase on purified enzyme activity. Relative enzyme activities are given as percentages, compared with the activity in the absence of antiserum or control serum. Details of the enzyme assay are described in Methods. ○, Preincubated only with the antiserum; □, incubated with protein A cellulofine after preincubation with antiserum; △, preincubated with control serum.

Fig. 3. Detection of enzyme activity in immunoprecipitation of primer-independent glucosyltransferase. Precipitin lines were allowed to form in double diffusion gels between antiserum (20 µl) in the centre well, purified primer-independent glucosyltransferase (2 µg, wells numbered 1) and crude protein from S. mutans AHT-k (200 µg, wells numbered 2). Gel a was subsequently incubated in the assay mixture for glucosyltransferase activity and gel b was not, after which both gels were stained for carbohydrate with periodic acid Schiff reagent. Further details are given in Methods.

DISCUSSION

As a result of genetic (Coykendall, 1977) and biochemical (Shklair & Keene, 1974) investigations the serotypes of S. mutans have been classified into separate species: S. sobrinus (serotypes d and g), S. cricetus (serotype a), S. mutans (serotypes c, e and f), and S. rattus (serotype b). Recently, following studies with the two primer-dependent glucosyltransferases, a primer-independent water-soluble glucan synthase was purified from strain AHT-k (Hanada et
al., 1987) and from the 6715-13 mutant 27 (McCabe, 1985) of *S. mutans* serotype g, but its distribution in other serotypes of *S. mutans* remained unclarified. In the double immunodiffusion analyses, the enzyme/antiserum cross-reactions showed that the primer-independent glucosyltransferase of strain AHT-k was the same as that secreted by other strains of serotypes d, g, and a, but was different from that of the other serotypes (Fig. 1). However, the enzyme produced by serotypes d and g was immunologically slightly different from that of serotype a. These findings are similar to those obtained previously with the two primer-dependent glucosyltransferases (Fukui et al., 1974, 1983). The mechanisms of glucan synthesis are thought to differ from subspecies to subspecies, although those in *S. sobrinus* and *S. cricetus* have been found to be similar. In addition, immunological similarity between the glucosyltransferases of *S. rattus* and *S. mutans* was recently reported (Kumada et al., 1987). Thus, the mechanism of glucan synthesis in *S. mutans* must be resolved from at least two viewpoints.

There have been some reports on the effect of antiserum against the primer-dependent glucosyltransferases of *S. mutans* serotype a, d and g (Fukui et al., 1974, 1983; Linzer & Slade, 1976), while until now there have been no studies using antiserum against the primer-independent enzyme. In the present study, such an antiserum was found to be unable to completely inhibit the activity of the enzyme, even though complete immunoreaction between the enzyme and the antiserum occurred (Fig. 2). In order to confirm this finding, the precipitin line formed in double immunodiffusion gels was treated with periodic acid Schiff reagent. Precipitin lines preincubated with the enzyme assay buffer could be stained (Fig. 3a), while those not preincubated could not (Fig. 3b). These findings indicate that, even if the primer-independent glucosyltransferase is a glycoprotein, the line stained by periodic acid Schiff was due to glucan synthesized by the enzyme, and not to glycosyl residues on the enzyme itself. It is probable that the antigenic sites on the primer-independent glucosyltransferase that were bound by the antiserum used in this study did not correspond to the active site of the enzyme, in which case it is unclear why the enzyme activity was partially inhibited.

REFERENCES


McCabe, M. M. (1985). Purification and characteriza-
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