

The Effects of anti-Glucosyltransferase (anti-GTF-I_b) antibody on Growth of Mutans Streptococci *Streptococcus sobrinus* (serotype G) N₁₀ Strain and Purified GTF-I_b enzyme activity

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Abstract: In this study the effect of different concentrations of anti-GTFI_b antibody was tested on the growth of *Streptococcus sobrinus* serotype G N10 strain and the enzymatic activity of purified GTF-I_b enzyme which was isolated from the *Streptococcus sobrinus* serotype G N10 strain. It was found that different concentrations of these compounds and the anti- GTF-I_b antibody were able to inhibit the enzymatic activity of the purified GTF-I_b with an exception of EDTA. The result was proved that anti- GTF- I_b antibody at concentration of (1.5x10³ ml/ mol) has a great influence to inhibit (87.33%) of the enzymatic activity of the purified GTF I_b enzyme followed by sodium fluoride (18ml M), chlorohexidine (20ml M) and ZAK mouthrins which were able to inhibit (81.64%), (75.55%) and (60.40%) respectively from the enzymatic activity of purified GTF- I_b enzyme.

Key words: *Streptococcus sobrinus*, Glucosyltransferase (GTF), Anti-GTFI_b.

I. Introduction

Mutans Streptococci is implicated as the primary etiological agents of human caries within this group , *Streptococcus sobrinus* and *Streptococcus mutans* were the most commonly isolated species from human.[1]

Most oral streptococci possess Glucosyltransferase (GTF) enzyme that use sucrose as substrate to synthesize extracellular polysaccharide [2,3] which facilitate the accumulation and adhesion of oral bacterial cells on the tooth.[4]

Several molecular components has been used as an antigen to stimulate the immune system against cariogenic bacteria, GTF preparations were attractive possible vaccine that may constitute important target of the antibacterial mechanism [5]. Also a variety of compounds capable to controlling dental caries have been extensively used on the basis of the following criteria:

So the aim of this study to determine the antienzymatic activities of different concentrations of inhibitors and anti- GTF antibody on the growth of mutans streptococci *Streptococcus sobrinus* local Iraqi isolated bacterial strain from teeth and against the activity of purified GTF enzyme which that isolated and purified from the same strain.

II. Materials and Methods

Bacterial isolate : *Streptococcus sobrinus* (serotype G) N₁₀ was isolated from dental plaque and identified by growing on the surface of MS-agar[6], testing their tolerance to high concentration of sodium chloride, utilization of different carbohydrate sources , antibiotic sensitivity test and Latex test (PASTOREX STREP) for serotype identification.

Anti-GTF-I_b antibody: It was prepared by Al-Mudallal *et al.*, [6,7]. The antigen was a concentrated purified GTF-I_b enzyme which was isolated and purified also by (Al-Mudallal *et al.*, [8] from the previous bacterial isolate. The antigen preparation and the immunization schedule were done following the method describes by Wunder and Bowen, [9].

The effects of Inhibitors and Anti-GTF-I_b antibody on Purified GTF-I_b enzyme

The effect of inhibitors and anti-GTF antibody on the GTF activity was estimated following the method described by Evans and Genco, [10], as follow:

A mixture of (200 µl) of purified (GTF) from *S.sobrinus* (Serotype G) N₁₀ strain with (200 µl) of each concentration of previous inhibitors and anti-GTF-I_b antibody was incubated at 37°C for an hour. Then a (20 µl) of (50%) solution sucrose was added for each concentration of inhibitors and dilutions of anti-GTF-I_b with (0.02%) sodium azide and incubated at 37°C for 18 hrs. Centrifugation was done at (5000 xg) for 30 minutes

and precipitation of glucan was done with an absolute ethanol then GTF activity was measured by phenol-sulfuric acid method [11]. The blank was prepared using the same procedure which contained the same amount of each concentration of the previous inhibitors and anti-GTF-I_b antibody with (200 µl) of phosphate buffer saline (0.3M) (pH 7.5) instead of enzyme with (20 µl) of (50%) sucrose.

III. Results and Discussion

The Effect of Inhibitors and Anti-GTF-I_b antibody on The Activity of Purified GTF-I_b

The effect of different concentrations of EDTA, sodium fluoride, chlorohexidine dichloride, ZAK (mouthrinse) and anti-GTF-I_b antibody was done as described previously. Results shown in figures (1), (2), (3) and (4), indicated that different concentrations of the inhibitors and anti-GTF-I_b antibody were capable to inhibit the GTF-I_b antibody activity with the exception of EDTA. Sodium fluoride at concentration (18mM) was capable to inhibit (81.64%) of the GTF-I_b enzyme activity, chlorohexidine at concentration of (20mM) was capable to inhibit (75.55%) of the GTF-I_b activity, ZAK (mouthrinse) at concentration of (12mM) was capable to inhibit (60.4%) of the GTF-I_b enzyme activity and anti-GTF-I_b antibody at concentration of (1.5×10^{-3} mM) was capable to inhibit (87.33%) of the GTF-I_b enzyme activity. No complete inhibition was recognized with any concentrations of these inhibitors and anti-GTF-I_b antibody.

Accordingly, anti-GTF-I_b antibody was considered as the best inhibitor on the activity of purified GTF-I_b enzyme followed by sodium fluoride, chlorohyexidine and ZAK (mouthrinse) respectively.

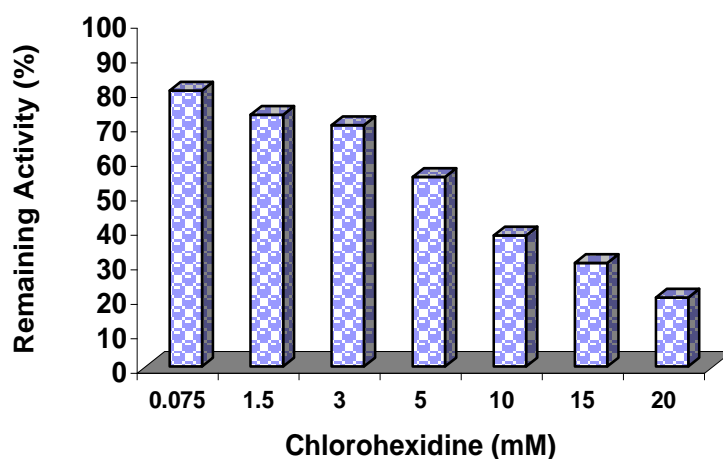


Figure (1): The effect of Chlorohexidine (CHX) mouthrinse on GTF-I_b activity.

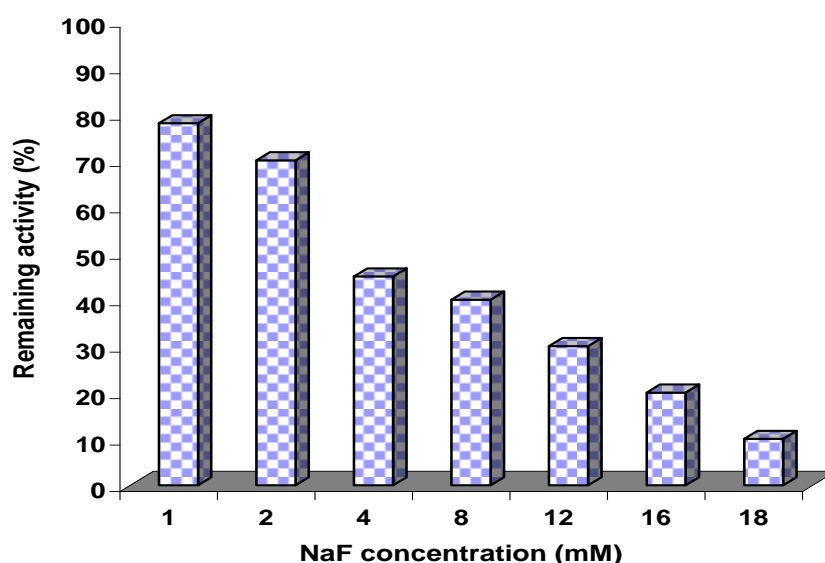


Figure (2): The effect of Sodium Fluoride (NaF) mouthrinse on GTF-I_b activity.

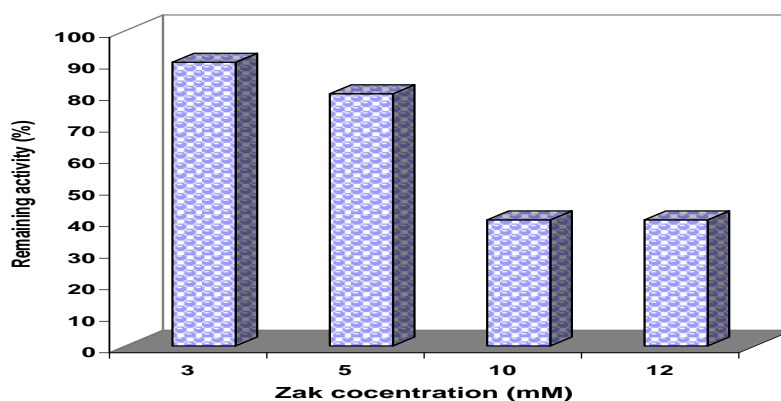


Figure (3): The effect of ZAK mouthrinse on GTF-I_b activity.

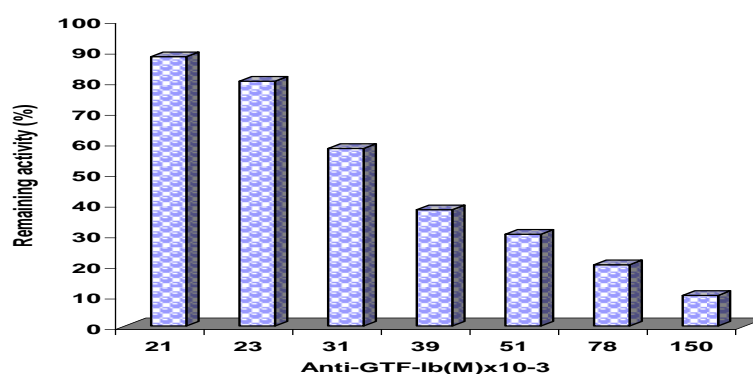


Figure (4): The effect of Anti-GTF-I_b mouthrinse on GTF-I_b activity.

Wunder and Bowen [9] demonstrated the inhibitory effect of antibodies specific for GTF-C, GTF-B and GTF-D enzymes of *S.mutans* on the activity of these enzymes in solution and on the surface of hydroxyapatite. Antibodies to GTF-B, GTF-C and GTF-D were found to inhibit (75%), (90%) and (90%) of the activity of these enzymes respectively. No complete inhibition was found with any one of these antibodies against the activity of GTF. These antibodies were capable to block the synthesis of insoluble and soluble glucan polymers. So by inhibiting the activity of GTF the adsorption of bacteria on the tooth surface will be prevented.

Fluoride was widely used as a highly effective anticaries agent [12]. It was found to inhibit the activity as well as the production of GTF enzymes from the mutans streptococci through a set of action with fundamentally different mechanisms. It inhibits the glycolytic enzyme enolase which had a vital role in the breaking of the glucose moieties during glycolysis process, so it is capable to inhibit the formation of glucan. Another mode of action was involved in the formation of metal-fluoride complexes, most commonly AlF₄⁻. These complexes were responsible for the fluoride inhibition of proton-translocation F-ATPases and were thought to act by mimicking phosphate to form complexes with ADP at reaction center of the enzyme. The linkage between the ATPs molecules and the F group was able to prevent the formation of glucan from the glucose-6-phosphate, so inhibition of GTF was recognized. [13]

Chlorohexidine was defined as an effective antiplaque agent [12]. Chlorohexidine in solution at concentration (1.25mM) was found to inhibit (100%) of GTF-C, (36%) of GTF-B and (15%) of GTF-D enzyme activities of *S. mutans* bacteria [14]. The clinical effectiveness of chlorohexidine could be due to interaction of antiplaque agent with the GTF-C enzyme prior to the adsorption of the enzyme onto tooth and apatitic surfaces, thus inhibiting the enzyme before adsorption onto the tooth surface.[15]. This suggestion could be consistent with the staining frequency absorbed on the tooth surfaces of persons who used chlorohexidine [16] (i.e chlorohexidin interacte with GTF-C in solution and bound to tooth surface with GTF-C).

Many potential anti-plaque agents might be ineffective when they became in the form of mouthrinse, because they were incompatible with other ingredients or materials that had found in the constituents of the mouthrinse [17]. ZAK (mouthrinse) although it contains sodium fluoride and chlorohexidine, it did not reflect the same effect as chlorohexidine or sodium fluoride alone.

In spite of the wide distribution of the antiplaque and anticaries agents, dental caries remained the most prevalent oral disease in different parts of the world especially in developing countries [18]. Although the

concept of using the GTF of mutans streptococci as a possible immunogen against dental caries was attractive, several major problems continue to plague the field including:

1. Vaccination against caries was based on the idea that the same principles that applied to mucosal immunity were applicable for protection against caries.
2. Although the occurrence of dental plaque disease was not on a mucosal surface but on hard, non-shedding, largely non-reactive surfaces, protective antibody was required to react on a solid surface in a largely hostile environment with large variation in pH values, active proteases and limited diffusion into and out plaque.
3. Furthermore, Antibodies which were reacted with epitopes on putative protective bacterial proteins in solution might not identify the same epitopes when the proteins adsorbed to a surface undergo conformational changes. Such changes were known to occur for example with GTF adsorbed on saliva-coated hydroxyapatite [19]. It was assumed that even partial inhibition of GTF by antibodies might be beneficial. However, it was cleared that the presence of antibody which partially inhibits or simply react with the enzyme, a glucan of novel structure might be formed there by providing a distinctive structure to which microorganism might bind [20]. There had been an increasing interest in the possible use of topically applied antibodies as a mean of controlling dental caries [21]. This approach certainly had attractions in that immunogens did not have to be administered systematically. Nevertheless, although the approach was technically feasible, it shared many of the same problems mentioned above. In addition, depending on the method of administration, it might suffer from the same problem as many mouthrinse or topically application, in that it did not remain in the mouth for a sufficient time to exert its therapeutic effect. Perhaps antibodies could be used as a "homing agent" to deliver therapeutic substance to specific area of the mouth [20].

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