Dental caries suppression effect of highly branched and modified oligosaccharides

서은성, 박현정[1], 김지희[1], 이기영[1], 김도만[1,2,3]

전남대학교, 물질 · 생물화학공학과, 화학공학부[1], 공업기술연구소[2], 촉매연구소[3]
전화 (062)530-0874, Fax (062)530-1849

Abstract

We have synthesized branched oligosaccharides (BOS) by the mixed-culture fermentation (MBOS), fructosyltransferase (FBOS) or glucosyltransferase (GBOS) with high concentration of sucrose (3M). MBOS was further modified as iron- and sulfate-oligosaccharides. The modified MBOS were stable at high temperatures (up to 140℃) and low pHs (2 to 4). Most highly branched and modified oligo-saccharide (0.34%, w/v) effectively inhibited fructose release from sucrose by *Streptococcus mutans* 6715 mutansucrase. FBOS, GBOS, iron-MBOS inhibited the mutansucrase activities from *Streptococcus sobrinus* about 46.8%, 49.2% and 43.1%, respectively. Most highly branched and modified oligo- saccharides (0.5%, w/v) effectively inhibited the formation of insoluble glucan and adherence of *S. mutans* or *S. sobrinus* cell in the presence of sucrose. Modified oligosaccharides affected the growth and acid production of oral pathogens. Cytotoxicity test showed that highly branched and modified oligosaccharides was non-toxic.

Introduction

Dental plaque is a film of microorganisms on the tooth surface that plays an important part in the development of caries and periodental diseases[1)]. Mutans streptococci can colonize on the tooth surface and initiate plaque formation by their abilities to synthesize extracellular polysaccharides from sucrose, mainly water-insoluble glucan, using glucosyltransferase[2)]. This sucrose-dependent adherence and accumulation of cariogenic streptococci is critical to the development of pathogenic plaque. Therefore, antibacterial agents against these oral pathogens could play an important role in the prevention of dental caries and periodontal disease, particularly those that can affect plaque formation. Carbohydrates contribute about 20% of dry weight to the dental plaque[3)]. Structural studies of the extracellular glucans produced *in vitro* have shown that

they have mainly α-(1→3), α-(1→4), and α-(1→6)-D-glucosidic linkages[4]. Thus, mutanolytic, amylolytic, and dextranloytic activities are required for the efficient removal of dental plaque. However, enzyme activity easily lost while it stored for a long time and during food processes. So the material that can prevent the formation of dental plaque and maintain activity for a long time is required. In this work, we have investigated the inhibition effect of modified highly branched oligosaccharides for dextransucrase or mutansucrase activity and for the growth of oral pathogens.

Materials and Methods

Effect of highly branched and modified oligosaccharides on the inhibition of glucosyltransferase activity: Reactions were carried out in 500 μℓ digests containing 34 mM sucrose, 0.34 % (w/v) various oligosaccharides and 0.1 U of dextransucrase or mutansucrase per ml in 20 mM sodium acetate buffer (pH 5.2) or 20 mM imidazole-HCl buffer (pH 6.5) at 28℃ or 37℃. The reaction mixture of mutansucrase contained 0.1% dextran T10 as a primer. Samples were then analyzed by TLC as described previously[5].

The effects of the highly branched and modified oligosaccharides in cell growth and acid production: *Streptococcus mutans*, *Streptococcus sobrinus*, *Eikenella corrodens*, *Prevotella intermedia* and *Actinobacillus actinomycetemcmitans* were grown in their medium plus 0.5% glucose, various highly branched and modified carbohydrates with the pH and temperature at 7.0 and 37℃, respectively. At the same time, the acid formation was monitored by the pH changes in the culture of *S. mutans* and *S. sobrinus*[6].

Sucrose-dependent, glucan-mediated adhesion: For adhesion experiments for *S. sobrinus*, 2.5×10^6/ml of cell inoculated in BHI medium containing 10% (w/v) sucrose, 0.5% (w/v) three highly branched and modfied oligosaccharides were incubated 24 hr at 37℃ without shaking. The adherent biomass was measured by the absorbance at 550 nm.

The pH and heat stability of modified MBOS: In case of pH stability, the modified MBOS (highly branched oligosaccharide using mixed fermentation) were mixed with buffer (pH 2 to 4). The mixture was incubated at different temperatures (60℃ to 120℃) for 15 min. In case of heat stability, modified oligosaccharide incubated at different temperatures (60℃ to 140℃) for 30 min and 60 min[4]. The remaining quantity of modified oligosaccharides was analyzed

by TLC to determine the degree of hydrolysis after treatments[4].

Results and Discussions

The amount of fructose released from sucrose by mutansucrase of *S. mutans* 6715 was measured at various intervals. Some modified oligosaccharides significantly reduced the mutansucrase activity: for iron- and sulfate-MBOS, 40.5% and 17.3%, respectively [Fig. 1-(A)]. FBOS, GBOS and iron-oligo saccharides inhibited the mutansucrase activities from *S. sobrinus* about 46.8%, 49.2% and 43.1%, respectively [Fig. 1-(B)]. By the addition of iron- and sulfate-oligosaccharide the growths of *S. mutans* and *S. sobrinus* were inhibited and the pH in *Streptococcus sp.* culture dropped slowly comparing to that of pH in cultures without the addition of oligosaccharides. The formation of insoluble glucan by *S. mutans* 6715 were decreased about 70, 90, and 80% by the addition of MBOS, iron- and sulfate-oligosaccharides, repectively [Fig. 2-(A)]. All highly branched and modified oligosaccharides effectively inhibited the formation of insoluble glucan by *S. sobrinus* NRRL 14555 [Fig. 2-(B)]. Modified BOS were stable at high temperatures (up to 140℃) and low pHs (2 to 4). Each highly branched and modified oligosaccharides was introduced into the medium. MBOS was not cytotoxic upto the addition of 10㎕. Iron- and sulfate-oligosaccharides was also not cytotoxic.

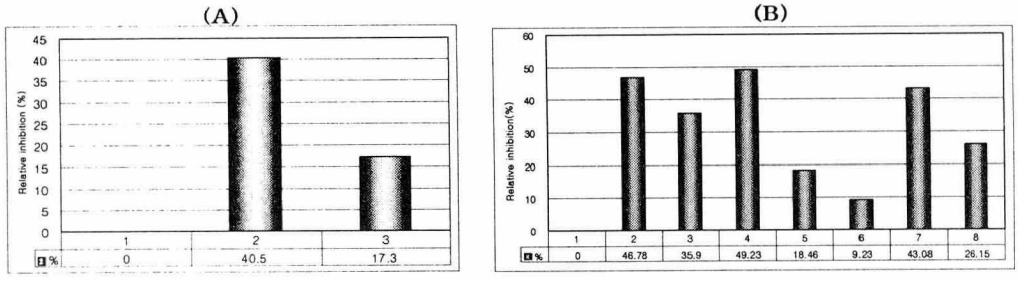

Fig. 1. The inhibition percentage of modified carbohydrates for mutansucrase activity (U/ml) from *S. mutans* 6715(A) and from *S. sobrinus* NRRL 14555(B)

(A) Bar 1: control (in reaction mixture without the addition of highly branched and modified carbohydrate), Bar 2: containing iron-MBOS, Bar 3: containing sulfate-MBOS.

(B) Bar 1: control in reaction mixture without highly branched and modified carbohydrate, Bar 2: oligosaccharide prepared using fructosyltransferase (FBOS), Bar 3: containing sulfate-FBOS, Bar 4: containing oligosaccharide prepared using dextransucrase (GBOS), Bar 5: containing sulfate-GBOS, Bar 6: containing oligosaccharide prepared using mixed-culture fermentation (MBOS), Bar 7: containing iron-MBOS, Bar 8: containing sulfate-MBOS.

(A) (B)

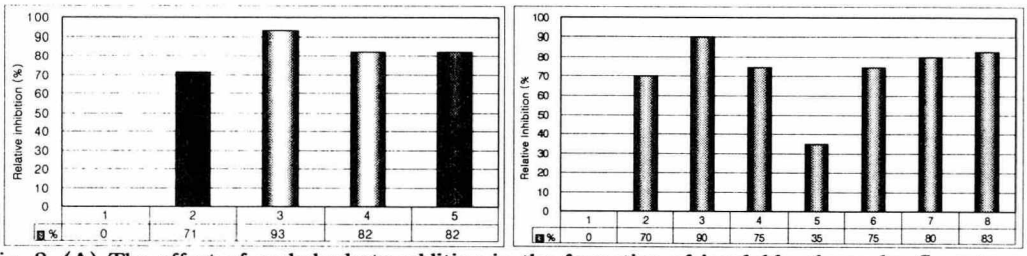

Fig. 2. (A) The effect of carbohydrate addition in the formation of insoluble glucan by *S. mutans*

Bar1: control(without the addition of highly branched and modified carbohydrates),

Bar2: containing oligosaccharide prepared using mixed-culture fermentation(MBOS),

Bar3: containing iron-MBOS, Bar4: containing sulfate-MBOS.

(B) The effect of carbohydrate addition in the formation of insoluble glucan by *S. sobrinus*

Bar 1: control (in reaction mixture without the addition of highly branched and modified carbohydrate),

Bar 2: containing oligosaccharide prepared using fructosyltransferase (FBOS), Bar 3: containing sulfate-FBOS,

Bar 4: containing oligosaccharide using glucosyltransferase (GBOS), Bar 5: containing sulfate-GBOS,

Bar 6: containing oligosaccharide prepared using mixed-culture fermentation (MBOS),

Bar 7: containing iron MBOS, Bar 8: containing sulfate-MBOS.

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