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The inhibitory effect of magnolol from *Magnolia officinalis* on glucosyltransferase

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Summary Dental caries has been an intractable disease in spite of intense dental research. Glucosyltransferase (GTF) enzyme plays the most important role in the development of dental caries. In our previous studies, magnolol, a compound from *Magnolia officinalis* Rehder et Wilson (Magnoliaceae), was shown to possess a strong anti-GTF activity. The purpose of the present study was to examine the effect of magnolol on the functional domains of GTF for the purpose of defining its anti-GTF activity mechanism. GTF-I which was prepared from *Streptococcus milleri* transformant KSB8 cells expressing the *gtfB* gene was used. The results demonstrated magnolol reduced total glucan synthesis, depending on the magnolol concentration. There were no significant differences in Michaelis constant (K_m) values between the presence and absence of magnolol as determined by Lineweaver–Burk plot, and maximum velocity (V_m) in the presence of magnolol was lower than that in its absence. Magnolol significantly inhibited both sucrose hydrolysis and glucosyl transfer to glucan by GTF-I. Free glucose in the presence of magnolol was reduced by 33–48% as compared to in its absence, while the quantity of glucan was reduced by 75–82%. These findings suggested that magnolol inhibited both two sequential reaction phases of GTF non-competitively by operating on the glucan-binding domain, but not on the catalytic domain. Magnolol could be a valuable resource for the exploration of novel bioactive compounds in natural products.

Introduction

Dental caries, a bacterial infection has been an intractable disease. Glucosyltransferase (GTF; sucrose 6-glucosyltransferase, EC 2.4.1.5) produced

Abbreviations: GTF, glucosyltransferase; GTF-I, insoluble-glucan synthesis glucosyltransferase; K_m, Michaelis constant; V, initial velocity; V_m, maximum velocity

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by *Streptococcus mutans* has been recognized as a critical virulence factor in the pathogenesis of dental caries.[1] GTF is the key enzyme catalyzes sucrose to adhesive glucans and contributes significantly to the formation of dental plaque in which the accumulation of metabolic acids produced by bacterial colonies leads to local demineralization of the enamel surface.[2] Therefore, GTF activity is a potential up-stream target in the pathological cascade.[3] There are at least three kinds GTFs which are produced by cariogenic streptococci: GTF-I, synthesizing α-1,3-linked water-insoluble glucans[4]; GTF-SI, producing significant amounts of both water-soluble and insoluble glucans[5]; GTF-S, incorporating glucose in α-1,6-linkages into soluble glucan products.[6] Disruption analysis of genes encoding these GTFs revealed that the synthesis of water-insoluble glucan is essential to the cariogenesis of *S. mutans*.[1] Therefore, since GTF-I is more responsible for insoluble glucan synthesis than the other GTFs,[4] we used GTF-I in the present study. GTF is composed of two relatively independent functional domains, an amino-terminal catalytic domain, which binds and hydrolyzes the substrate of sucrose, and a carboxyl-terminal domain, which functions as an acceptor of glucan binding and also plays an important role in determining the nature of the glucan synthesized by a GTF.[7–9] GTF catalyze two sequential reaction phases: the cleavage of sucrose into fructose and an enzyme-bound glucosyl moiety (sucrase activity), and the subsequent transfer of the latter to the C-3/C-6 position of the glucose residue of glucan (transferase activity).[10]

Recently, natural products have attracted increased interest as an alterative choice in health care. A few recent studies have demonstrated inhibitory activity on GTF from natural sources such as proplis,[11] oolong tea polyphenols,[12] Chinese black tea and *Harrisonia perforata*.[13] Magnolol, a hydroxylated biphenyl compound isolated from the Chinese herb Hou'pu, cortex of *Magnolia officinalis* Rehder et Wilson (Magnoliaceae) is known to have many pharmacological activities. It can suppress the proliferation of tumor cell,[14] inhibit platelet aggregation,[15] and is an anti-microbial[16] and anti-asthmatic agent.[17] In our preliminary study, magnolol was found to inhibit growth of cariogenic bacteria in vitro and possess a strong anti-GTF activity.[18] Jun et al. reported that magnolol inhibited activities of streptococcal glucosyltransferases both in solution and adsorbed on an experimental pellicle.[19] However, until now, the inhibitory mechanism of magnolol on GTF has never been reported. The current study attempted to examine the effect of magnolol on the functional domains of GTF for the purpose of defining its anti-GTF activity mechanism so that inhibitor efficiency and prevention of caries can be predicted.

Materials and methods

Plant materials

The cortex of the root and stem of *Magnolia officinalis* Rehder et Wilson (Magnoliaceae), collected from Enshi (China) in June 2002, was purchased from Hubei Medicinal Material Company. Magnolol was isolated from *M. officinalis* as previously described.[20] Briefly, the ethanol extract from *M. officinalis* (5 kg) was partitioned between water and ether, and applied to a silica gel column eluted with petroleum ether/acetate ethyl ester 95:5. The fractions which comprised magnolol were chromatographed to a SiO_2 column eluted with acetone/*n*-hexane 5:95, 10:90, 15:85, and 20:80 (600 ml each). The eluted fraction was re-crystallized with cyclohexane to afford magnolol (yield, 386 mg). Magnolol was identified by TLC and mixed melting point determination by comparison with authentic magnolol. The purity of magnolol was identified by HPLC with 3D photodiode array (>99% purity) and by NMR (without impurity signals). All chemicals were purchased from Chinese Pharmaceutical and Biological Institute, China. Dimethylsulfoxide (DMSO) was the solvent for magnolol and the final volume of DMSO in the reaction mixture was less than 0.5% (v/v).

Enzyme preparation

GTF-I was prepared from *S. milleri* KSB8 cells, which expressed *gtfB* gene coding for GTF-I synthesis.[3] The microorganisms were grown in brain—heart infusion cultures, supplemented with 0.2 mmol/l $MnSO_4$, 1.0% Tween-20, 1 mg/ml glucose, 20 mg/ml sorbitol, 10 μmol/l phemylmethylsulfonylfluoride (PMSF) and 10 μg/ml erythromycin. After cultured anaerobically overnight at 37 °C, the cells were collected by centrifugation at $6000 \times g$ for 15 min, washed, and sonicated in 20 mM phosphate buffer (pH 6.0) for isolation of the cell-associated GTF-I. After centrifugation, the supernatant fluid was applied to a QAE column (Shimadzu, Japan) connected to a high performance liquid chromatography system. After the sample was loaded, the column was washed extensively with the equilibration buffer, and the adsorbed proteins were eluted with a gradient of 0—0.7 mol/l NaCl in 25 mmol/l Tris—HCl (pH 7.5). The fraction eluted at the concentration of 0.65 mol/l yielded a single band on SDS-PAGE. The purity of the enzyme preparation was analysed by SDS-PAGE and silver staining as