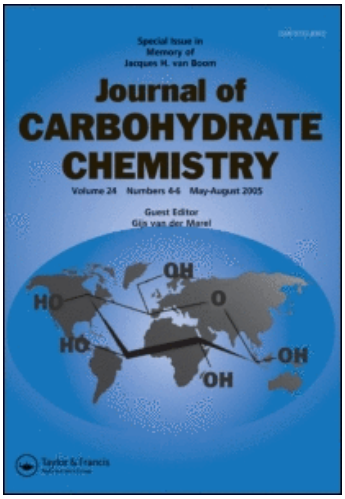

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ACARBOSE BINDING SPECIFICITY WITH ORAL BACTERIAL GLUCOSYLTRANSFERASE

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ABSTRACT

Mutans streptococcus glucosyltransferases are the significant virulent factors in causing dental caries. The binding specificity of acarbose was probed with glucosyl and fructosyl sub-site binding ligands using multiple inhibition kinetics. The results indicate that acarbose and a glucosyl subsite binding ligand (1-deoxynojirimycin) are mutually or partially exclusive. On the other hand, acarbose with a fructosyl subsite ligand (fructose) might induce a conformational change leading to enhanced binding at the adjacent subsite.

INTRODUCTION

Oral bacteria referred to as mutans streptococci are remarkable for their central role in the initiation of dental caries on smooth enamel surfaces. They derive this potential in large measure from the production of extracellular glucosyltransferases (GTF). The enzyme taps the high free energy of the sucrose glycosidic bond to synthesize long-chain glucans.

The glucans accumulate on tooth enamel surfaces and form a scaffolding for bacterial colonization. In the protected environment of the glucans, the mutans streptococci and other oral microorganisms form a stable community (dental plaque) and may release sufficient quantities of metabolic acids to demineralize tooth enamel and initiate dental caries.[1-3]

Acarbose is a strong inhibitor of glucoamylase and binds exclusively at the catalytic site.[4] It has been used as an inhibitor in various enzyme kinetic studies.[5-8] Some strains of Actenomycetales synthesize amino sugar derivatives that inhibit the activity of α-glucosidases.[9] One of the derivatives, acarbose, a pseudotetrasaccharide consisting of an unsaturated cyclitol unit, a 4-amino-4,6-dideoxyglucose unit and two glucose units, has pronounced inhibitory effects on intestinal α-glucosidases such as sucrase, maltase and glucoamylase.[10] Acarbose causes reduction of blood glucose and triglyceride levels in diabetics and thus is a useful adjunct to dietary control in non-diabetic patients affected by severe hypertriglyceridaemia.[11] The efficacy of acarbose for improving metabolic control was observed in type 2 diabetic patients.[12,13] Inhibition of GTF from *S. mutans* by acarbose, nojirimycin and 1-deoxynojirimycin (dNJ) has been reported.[14,15]

In the present communication, we report the binding specificities of acarbose at the subsites of the active site of glucosyltransferase from *Streptococcus mutans*.

RESULTS AND DISCUSSION

Dissociation constant of acarbose. The inhibition constant for acarbose with glucosyltransferase was measured and the ligand dissociation constant was calculated according to equation 1. The dissociation constant for acarbose with glucosyltransferase is 0.07±0.01 mM. Our previous studies indicated that *N*-methyl-dNJ, a potential glucose subsite ligand is the strongest glucosyltransferase inhibitor reported to date with a dissociation constant of 0.031±0.008 mM.[16] Our studies with potential GTF inhibitors revealed that *N*-methyl-dNJ has the lowest reported K_i followed by acarbose. Fig. 1 shows GTF inhibition by acarbose with varying sucrose concentrations.

Multiple inhibition kinetics. In multiple inhibition kinetic analysis, the concentration of two reversible inhibitors is varied in a single experiment to distinguish between exclusive and non-exclusive binding. Glucose transition-state analogue, 1-

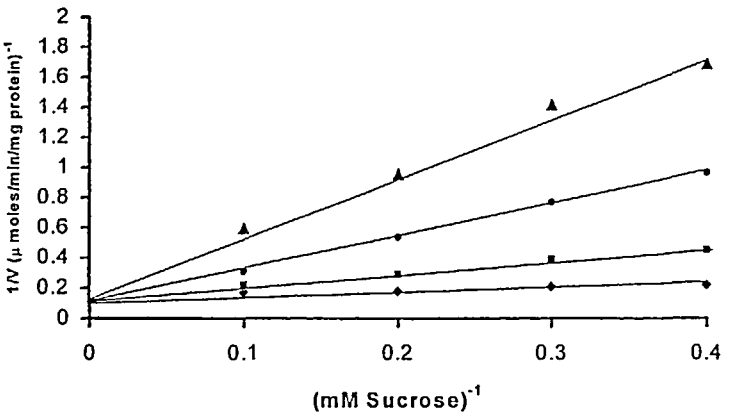

Fig. 1 GTF inhibition by acarbose with variable sucrose. Acarbose concentrations are 0 (◆), 0.1(■), 0.5 (●), and 1.0 mM (▲).

deoxynojirimycin (dNJ) assumes a planar configuration at C-1, approximating the glucosyl oxocarbonium ion transition state.[16] 1-Deoxynojirimycin is a potent inhibitor of glucosidases and greatly inhibits the formation of complex *N*-linked oligosaccharides in intact cells.[17, 18] GTF analysis with acarbose and fructose is shown in Fig. 2. The α value for acarbose paired with fructose is less than 1, indicating that the two inhibitors induce a conformational change that enhances binding at the adjacent subsite. In contrast, the α is greater than 1 when acarbose is paired with dNJ, which might indicate that the two inhibitors are mutually or partially exclusive.[19] The interaction factor for acarbose paired with dNJ and fructose is listed in the Table.

The interaction factor α with subsite ligands fructose and dNJ with acarbose provides potential details of the enzyme structure at the active site of glucosyltransferase. Among the ketohexoses, free D-fructose is a strong inhibitor of glucosyltransferase with a dissociation constant of 6.0 ± 0.9 mM.[16] dNJ is a relatively strong glucosyltransferase inhibitor with a dissociation constant of 0.56 ± 0.07 mM.[16] Acarbose has been used as an inhibitor in solving crystal structures of human pancreatic alpha-amylase (HPA) and cyclodextrin glucanotransferase (CGTase).[20, 21] Since the GTF crystal structure has not been solved, studies exploring the subsites of the active site of GTF would contribute to the understanding of the catalytic site. Based on our results acarbose is a strong binding

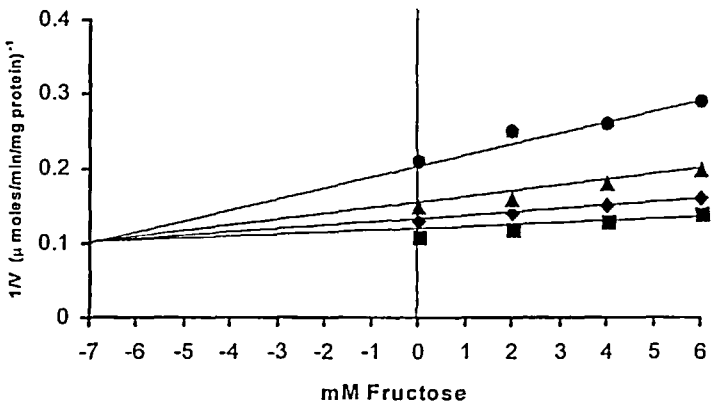

Fig. 2 Multiple inhibition kinetic data were plotted as the reciprocal initial velocity as a function of fructose concentration. GTF-I was assayed with 10 mM sucrose at pH 6.0 in the presence of fructose (0, 2, 4, 6 mM) and acarbose. Acarbose concentrations are 0 (■), 0.05 (◆), 0.1 (▲), and 0.2 mM (●).

Table. The interaction factor, α, between glucose and fructose subsite inhibitors

Acarbose[a] paired with	Interaction factor α
Fructose[b]	0.88 ± 0.01
dNJ[c]	5.46 ± 0.35

a. Acarbose concentrations: 0, 0.05, 0.1, 0.2 mM
b. Fructose concentrations: 0, 2, 4, 6 mM
c. dNJ concentrations: 0, 1, 2, 3 mM

inhibitor of glucosyltransferase; we propose that it could be used to prevent dental caries by inactivating the enzyme glucosyltransferase from *Streptococcus mutans.*

EXPERIMENTAL

Kinetic assays. Glucosyltransferase was purified as previously described.[16] Kinetic assays were based on ^{14}C-isotope transfer from uniformly labeled sucrose to

glucan, similar to a previously described procedure.[16] The reactions were carried out in 0.1 M MES (2-*N*-morpholinoethanesulfonic acid) buffer at pH 6.0 with 10 mM sucrose. Linear competitive inhibition data were resolved according to the following equation 1.

$$V = VA/(K(1 + I/K_i) + A) \qquad \text{(Eq. 1)}$$

Multiple inhibition kinetics. Multiple inhibition kinetics were performed at 10 mM sucrose and four or five concentrations (including zero) of each of the two inhibitors. In multiple inhibition kinetics I and J are competitive inhibitors of substrate, S. I and J can be exclusive, non-exclusive, or partially exclusive based on the interaction factor, α. α is 1 when I and J are nonexclusive, greater than 1 when I and J are mutually or partially exclusive, and less than 1 when I and J induce a conformational change that enhances binding at the adjacent subsite.[19] The interaction between two competitive inhibitors was determined by multiple inhibition kinetic analysis according to equation 2.

$$V = V_{app}/(1+I/K_{iapp}+J/K_{japp}+ IJ/(\alpha K_i K_j)) \qquad \text{(Eq. 2)}$$

where α is the interaction factor, and I and J are inhibitor concentrations. The specific program used for multiple inhibition kinetic data analysis was kindly supplied by Dr. W. W. Cleland[22] and was translated from FORTRAN into BASIC.

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