

SHORT COMMUNICATION

Streptococcal glucan-binding lectins do not recognize methylated α -1,6 glucans

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The glucan-binding lectin (GBL) of *Streptococcus sobrinus* is cell associated, enabling the bacteria to be aggregated by α -1,6 glucans. Glucans, such as amylose, pullulan, laminarin and nigeran, have no affinity for the lectin. High molecular weight α -1,6 glucans (dextrans) readily aggregate the bacteria, whereas low molecular weight glucans inhibit the aggregation brought about by the high molecular weight species. Methylated glucan T-2000 (an α -1,6 glucan with an average molecular weight of 2×10^6 Da) aggregated the bacteria very poorly when the extent of methylation (DS, or degree of substitution) was high, and less poorly when the DS was low. Similarly, methylated low molecular weight α -1,6 glucan was a poor inhibitor of aggregation induced by the high molecular weight glucan T-2000. Because the methylation occurred primarily on the hydroxyl of C-2, it is suggested that the hydroxyl is needed for formation of the lectin-glucan complex. It appears that the GBL is not only stereospecific in interaction with glucans, but also regio-specific, interacting only with the underivatized α -1,6-glucan.

Keywords: Glucan, lectin, methylation

Introduction

Several oral streptococci, such as *Streptococcus sobrinus*, and many other bacteria, express surface-associated lectins that enable the organisms to adhere to glycoconjugates. These streptococci are known to express a glucan-binding lectin (GBL) on their cell surfaces. The GBL of oral streptococci is primarily a cell wall protein capable of complexing with 6–10 internally-linked α -1,6 glucose residues, but not with other polysaccharides [1, 2]. It has been reported that the surface glucan-binding lectin is a virulence factor in promoting the colonization of the streptococci, because dextrans (glucans rich in α -1,6 linkages) are synthesized from sucrose by several oral streptococci [3–7]. A long-term goal of research performed in this laboratory is to characterize GBL combining sites. If the GBL combining site can be well-characterized, it may then be possible selectively to inactivate the site(s) and thereby provide a means of reducing sucrose-dependent adhesion. Methylated dextrans possessing various degrees of substitution (DS) have now been employed as probes for determining the fine specificity of the GBLs. The DS refers to numbers of methyl groups per hexose in the glucan. An inhibitor of the GBL, glucan T-10, molecular weight (MW) approximately 10 000 Da, was employed, along with glucan T-

2000, a dextran of about 2×10^6 average MW capable of aggregating the GBL⁺ bacteria. Derivatives of these polysaccharides were prepared by mild methylation procedures. The dextran derivatives were then used either to inhibit or promote the glucan T-2000 induced aggregation of *S. sobrinus* or *S. cricetus*.

Materials and methods

Bacteria and growth conditions In most of the experiments *Streptococcus sobrinus* 6715 (serotype g) was used. Some experiments were performed with *S. cricetus*, strain AHT. For daily use, bacteria were stored as turbid suspensions in Trypticase soy broth (TSB; BBL Microbiology Systems, Cockeysville, MD) at -20°C . Trypticase soy broth was treated as described by Drake *et al.* [1]. To eliminate possible sucrose and α -1,6 glucan contamination, TSB was incubated with fungal dextranase (Sigma Chemical Co., St Louis, MO), at a concentration of 0.65 U enzyme per g dry medium for 2 h at 37°C , then incubated with crude yeast invertase (Sigma) at 0.2 mg per g dry medium for 2 h at 55°C .

Bacteria were incubated statically in growth medium in a 5% CO_2 incubator at 37°C overnight. The bacteria were harvested by centrifugation ($7000 \times g$) for 5 min, washed with phosphate-buffered saline (PBS; 20 mM potassium phosphate,

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0.15 M NaCl, pH 7.2) and suspended in PBS. The washing process was repeated twice to ensure that the bacteria were free of metabolites. Bacteria prepared in the enzyme treated growth medium did not autoagglutinate.

Dextran aggregation assay The aggregation of the oral streptococci was determined by addition of high molecular weight dextran T-2000 (number average MW 2×10^6 Da) (Sigma Chemical Co., St Louis, MO, catalog D-5376) to washed cell suspensions. The kinetic assay of Drake *et al.* [1] was used. Suspensions of *S. sobrinus* 6715 (3 ml) were dispensed into a series of test tubes, and the initial absorbance (540 nm) was adjusted to approximately 0.8 (Spectronic 21D spectrophotometer, Milton Roy Company). Glucan T-2000 was then added to a final concentration of $5 \mu\text{g ml}^{-1}$. Each suspension was immediately vortexed for 5 s and the turbidity continually monitored for 10 min. The rate constants for the decrease in turbidity were obtained from the slopes of first-order plots of $\ln A_t/A_0$ (A_t = observed optical density; A_0 = optical density at time zero) versus time in minutes. Factors such as glucan concentration and inhibition by low MW glucans (number average MW 9700 Da, Sigma, catalogue D-9260) were similarly studied, using the rate assay of Drake *et al.* [1].

Methylation of dextran T-2000 Methylation has been an important method in structural analyses of polysaccharides for many years. Recently, with introduction of microtechniques for methylation and subsequent analysis by carbon ^{13}C nuclear magnetic resonance, the utility of the method has been greatly enhanced. In the present methylation studies, the method of Kobayashi and Sumimoto [8], which employs the methylsulphonyl methyl sodium and methyl iodide of Hakomori [9], was adapted to yield a low degree of substitution [10].

Results and discussion

Many bacteria possess lectins on their cell surfaces. Presumably, the evolutionary significance for microbial lectins is that they enable the microbes to adhere to surfaces. When microorganisms are adherent to surfaces, they seem to have more resistance to antibodies and antibiotics and are able to grow more rapidly [11]. The oral streptococci have evolved glucan-binding lectins, or GBLs. These lectins are specific for α -1,6 glucans, products of sucrose metabolism by the bacteria. Humans consume large quantities of starches (α -1,4 rich glucans), but starches are not recognized by the GBLs. The GBLs may be considered as virulence factors for the streptococci, as adhesion is a prerequisite for plaque formation and subsequent caries. If GBL could be inhibited by non-toxic analogues, there could be a means of preventing sucrose-dependent streptococcal adhesion. The experiments discussed in this paper were designed to examine the specificities of the GBLs of *S. sobrinus* and *S. cricetus*. Methylation was chosen because the extent of substitution can be readily controlled by modifying reagent concentrations. In addition, the extent of methylation can be monitored by NMR spectroscopy.

In Fig. 1, the aggregation of a suspension of *S. sobrinus* 6715 by a methylated glucan T-2000 is shown. The results show that the methylated derivative was not as effective as the unmodified glucan T-2000 in promoting cellular aggregation. When the results are plotted as first order reactions, the $K \cdot \text{min}^{-1}$ for the control dextran was 0.12, whereas the $K \cdot \text{min}^{-1}$ for the methylated derivative was 0.08. K values were plotted against DS (Fig. 2). The results reveal that methylation sharply reduces the ability of the high MW α -1,6 glucan to aggregate the bacteria. NMR results showed that monomethylation occurred primarily on the hydroxyl at the C-2 position [10]. At these low DS values the ratio of methylation at C-02:C-04:C-03 equalled 4.3:1.7:1.0. Similar to the foregoing results in which it was shown that methylated dextrans are poor cellular aggregating agents, methylated low molecular weight α -1,6 glucans are poor inhibiting agents, compared with the unmodified controls (Fig. 3). When the concentration of glucan T-10 or methylated glucan T-10 required for 50% inhibition of aggregation is plotted against the extent of methylation it is clear that methylation reduces the effectiveness of the glucan T-10 inhibitory power (Fig. 4).

The extent of interaction between the streptococci and the methylated glucans seemed to decrease as methylation increased. There are no rules for predicting the side chains of lectin combining sites, but carboxylate and phenolate (from tyrosine) are common. For the GBL of *S. sobrinus* 6715, carboxylates, phenolate and ammonium groups have been implicated as contact side chains [12]. Hydrophobic groups adjacent

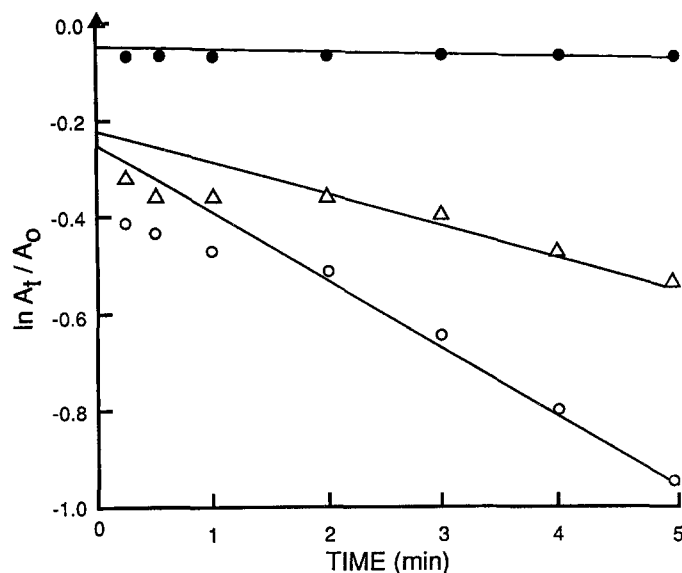


Figure 1. First order rate plots for the aggregation of *Streptococcus sobrinus* 6715 by glucan T-2000 preparations. A_0 : The initial absorbance of the cell suspension. A_t : the absorbance at time t after glucan T-2000 was added the cell suspension. Solid circle: control, PBS, but no glucan; open circle: $5 \mu\text{g ml}^{-1}$ unmodified T-2000; triangle: $5 \mu\text{g ml}^{-1}$ methylated T-2000. The glucan had a degree of substitution of 0.8.

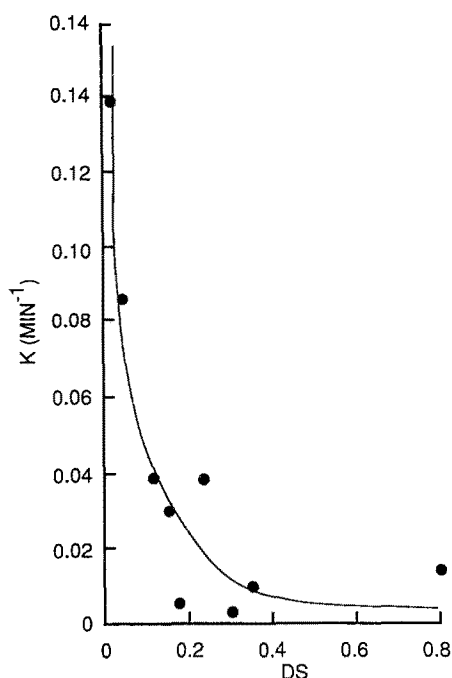


Figure 2. The kinetics of aggregation of *Streptococcus sobrinus* 6715 by glucan T-2000 containing different degrees of methyl substitution.

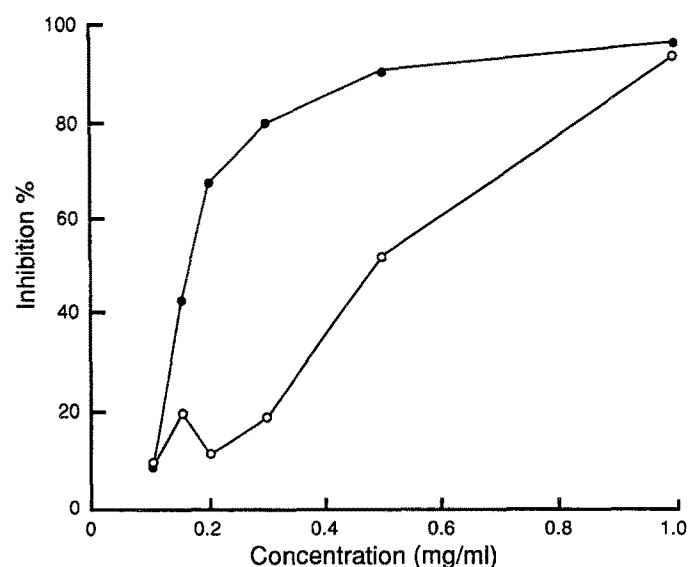


Figure 3. The per cent inhibition of *Streptococcus sobrinus* 6715 by unmodified glucan T-10 and by modified T-10 at different concentrations. Solid circle, unmodified glucan T-10; open circle, methylated glucan T-10 (DS = 0.18). Glucan T-10 (or methylated glucan T-10) and cells were mixed and then glucan T-2000 ($5 \mu\text{g ml}^{-1}$ final concentration) added. Turbidity changes were recorded as a function of time.

to phenolate groups might be expected to stabilize hydrogen bonds between hydroxyl donors and carboxylate acceptors. This was not the case, however, as shown by the reduced affinity of the streptococcal GBL for methylated glucans.

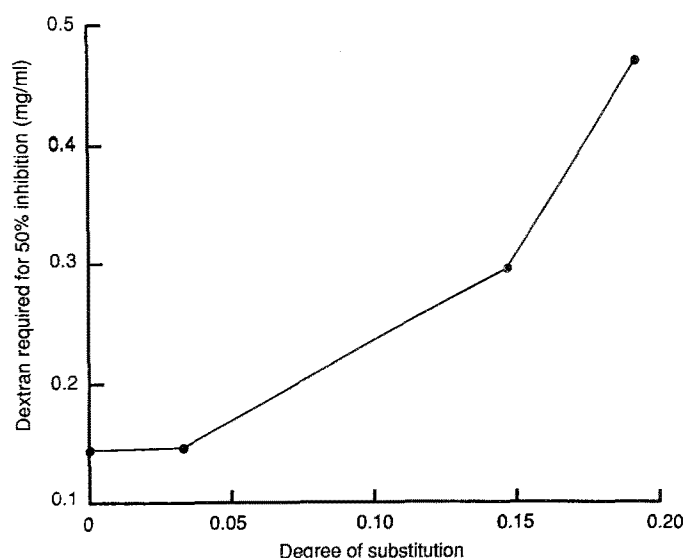


Figure 4. Inhibition of aggregation of *Streptococcus sobrinus* 6715 by methylated glucan T-10 preparations.

The results suggest that the hydroxyl of the C-2 of the polysaccharide is critical in saccharide recognition by the GBLs. To date, no papers have appeared showing the role of individual hydroxyls in the interaction of α -1,6 glucans with streptococcal GBLs. Drake *et al.* [1], employing various polysaccharides and oligosaccharide inhibitors, found that the GBLs of *S. sobrinus* and *S. cricetus* were specific for α -1,6 glucans. Mannans, starches, glycogens, pullulan, nigeran and other polysaccharides were refractory to the GBLs. They further showed that the combining site sizes were large enough to accommodate six to 10 hexose residues. Their data have now been extended to suggest that the hydroxyl of the C-2 is important in recognition by the lectins.

Finally, it is clear that methylated dextrans are poor candidates for anti-caries agents. It would be interesting however, to test the methylated dextrans for their effects on sucrose-specific glucosyltransferases. These enzymes convert sucrose into α -1,6 glucans (and to a lesser extent α -1,3 glucans) and their activities are enhanced by exogenously-added α -1,6 glucans.

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