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Structural Preferences of β-Galactoside-Reactive Lectins on *Actinomyces viscosus* T14V and *Actinomyces naeslundii* WVU45

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Specificities of lectins on *Actinomyces viscosus* T14V and *Actinomyces naeslundii* WVU45 were compared by measuring the abilities of D-galactose, *N*-acetyl-D-galactosamine, 14 β-D-galacto-oligosaccharides, and 2 β-D-fuco-oligosaccharides to inhibit coaggregation between *Streptococcus sanguis* 34 and each actinomycete. Inhibition profiles were similar, but WVU45 was significantly more sensitive to several inhibitors. D-Galactose-β(1 → 3)-*N*-acetyl-D-galactosamine glycosides were most potent.

Reactions between lectins and specific carbohydrates appear to be very important in the adherence of certain species of bacteria to each other in dental plaque (1). When pure cultures of oral bacteria are mixed in pairs, some pairs aggregate together (coaggregate), whereas others do not (6); the coaggregations are regarded as examples of specific bacterial adherence. Many of the coaggregations are thought to depend upon lectin-carbohydrate interactions. They are inhibited by specific sugars, and they depend on a reaction between a protein on one coaggregation partner and a putative carbohydrate on the other partner (3, 10). The most thoroughly investigated are the coaggregations of various strains of *Streptococcus sanguis* with various strains of *Actinomyces viscosus* and *Actinomyces naeslundii*. From human plaque, a high percentage of all isolates of *A. viscosus* and *A. naeslundii* coaggregated with many human oral *S. sanguis* strains, and most of these coaggregations could be inhibited by lactose (3, 7).

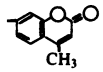
In nearly all lactose-inhibited coaggregations, the lectin was located on the actinomycete. With so many strains of *A. viscosus* and *A. naeslundii* carrying lactose-inhibitable lectins, one is compelled to wonder how similar those lectins are in their structural preferences. For a very limited exploration of this question we chose *A. viscosus* T14V and *A. naeslundii* WVU45, strains which coaggregate well with *S. sanguis* 34 (Ss34), but which are distributed somewhat differently in vivo, belong to different "clusters" by classification criteria (4, 5, 8), and are only distantly related with regard to the immunology of their lactose-sensitive fimbriae (2; J. O. Cisar, personal communication).

We compared the ability of D-galactose, *N*-acetyl-D-galactosamine (GalNac), β-D-galactosides, and β-D-fucosides to inhibit the coaggregation of T14V and WVU45 with Ss34. Because α-galactosides inhibit the coaggregations only very weakly if at all (10; unpublished data), the present study did not involve any such compounds.

The culturing of bacteria and the procedure for measuring inhibition of coaggregation have been published (9, 10). T14V and WVU45 were cultured with 0.05% glucose added to the medium. For the coaggregation inhibition studies, 5 mM sodium deoxycholate was added to the buffer to minimize loss of bacteria on glassware and to enhance the effectiveness of the inhibitors (9). The galactose and lactose used were Baker Analyzed; GalNac was from Sigma; compound N was kindly provided by V. Ginsburg, the National Institutes of Health; all other saccharides were synthesized in the laboratory of K. L. Matta (D. E. Sykes, S. A. Abbas, J. J. Barlow, and K. L. Matta, Carbohydrate Res., in press). An additional sample of compound F was kindly supplied by H. Flowers.

The data are summarized in Table 1. The ratio of means column gives a comparison of the two actinomycetes in terms of the millimolar concentration of each compound required for 50% inhibition of coaggregation with Ss34 (footnote *a* of Table 1). Of the ratios which were >1.2 or <0.8, all except one were highly significant statistically, with *P* values of 0.01 and 0.001; the

TABLE 1. Inhibition of coaggregation of Ss34 with T14V and with WVU45 by β-D-galactosides and β-D-fucosides

Saccharide[b]	Millimolar concn for 50% inhibition[a]				Ratio of means
	T14V		WVU45		T14V/WVU45[c]
	Mean (±SD)[d]	n[e]	Mean (±SD)[d]	n[e]	
Gal	14.6 (2.83)	6	12.0 (1.64)	10	1.22*
GalNAc	21.8 (3.57)	16	6.9 (0.25)	8	3.16***
A. Gal-β(1→4)-Glc (lactose)	2.68 (0.12)	26	3.13 (0.22)	24	0.86***
B. Gal-β(1→4)-GlcNAc	>10	2	>10	2	
C. Gal-β(1→4)-GalNAc	>10	2	>10	2	
D. Gal-β(1→3)-GalNAc	0.23 (0.013)	8	0.2 (0.02)	10	1.15**
E. Gal-β(1→3)-GalNAcαOC_6H_5	0.158 (0.014)	6	0.109 (0.019)	8	1.45***
F. Gal-β(1→3)-GalNAcα$OCH_2C_6H_5$	0.132 (0.012)	18	0.124 (0.08)	18	1.06
G. Gal-β(1→3)-GalNAcα$OC_6H_4NO_2$(*o*)	0.16 (0.016)	6	0.11 (0.038)	4	1.45**
H. Gal-β(1→3)-GalNAcα$OC_6H_4NO_2$(*p*)	0.16 (0.025)	6	0.07 (0.016)	4	2.29***
I. Gal-β(1→3)-GalNAcβ$OC_6H_4NO_2$(*p*)	0.15 (0.027)	6	0.08 (0.027)	4	1.88***
J. Gal-β(1→3)-GalNAcβO 	0.075 (0.001)	12	0.073 (0.004)	10	1.03
K. Gal-β(1→3)-GalNAcα$OC_6H_4NO_2$(*o*) 6 ↑ GlcNAcβ-1	0.148 (0.024)	4	0.163 (0.005)	6	0.91
L. Fuc-β(1→3)-GalNAcα$OCH_2C_6H_5$	0.35 (0.038)	12	0.158 (0.017)	8	2.21***
M. Fuc-β(1→3)-GlcNAcα$OCH_2C_6H_5$	1.32 (0.07)	12	0.30 (0.03)	8	4.4***
N. Gal-β(1→3)-GlcNAc-β-(1→3)-Gal-β(1→4)-Glc (LNT)	3.2 (0.15)	8	1.65 (0.38)	4	1.94***
O. Gal-β(1→3)-Gal-β$OC_6H_4NO_2$(*p*)	1.35 (0.29)	8	0.70 (0.04)	8	1.93***
P. Gal-β(1→6)-Galβ$OC_6H_4NO_2$(*p*)	1.6 (0.13)	4	2.08 (0.28)	6	0.77**

[a] Coaggregation in the absence of inhibitors was usually 80 to 90%. Thus, 80 to 90% of the cells originally mixed in suspension were removed by sedimentation of the coaggregates; only 10 to 20% remained in suspension as measured by absorbance at 650 nm. In the case of 50% inhibition of 90% coaggregation, only 45% of the cells would be removed by coaggregation, and 55% would remain in suspension (9).

[b] Abbreviations: Gal, D-galactose; Glc, D-glucose, Fuc, D-fucose, GalNAc, *N*-acetyl-D-galactosamine, GlcNAc, *N*-acetyl-D-glucosamine.

[c] The statistical significance with which each ratio differs from 1.0 is indicated as follows: *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$.

[d] Range with 95% confidence.

[e] Number of measurements.

one exception had a *P* value of 0.05. None of these differences between T14V and WVU45 was of great magnitude; one was 4.4-fold, one was 3-fold, and the rest were 2-fold or less.

In nearly all of the differences noted, WVU45 was more sensitive to inhibition by the given compound. Most notably, T14V required the higher concentration of inhibitor when: (i) GalNAc was the inhibitor; (ii) D-fucose instead of D-galactose (Gal) was in the nonreducing terminal position (cf. compounds F and L); (iii) *N*-acetyl-D-glucosamine instead of GalNAc was in the penultimate position (cf. compounds F, L, M, and N); (iv) Gal was in the penultimate position and the linkage was 1 → 3 (cf. compounds O and P); and (v) the aglycone carried a nitro group (cf. compounds F, G, H, and I). The attachment of the aglycones increased the inhibitory potency significantly but not greatly, and there were no large differences between α and β linkage of the aglycones. Apparently the attachment of GlcNAc at the 6 position of penultimate GalNAc (cf. compounds K and G) had no effect on the inhibition of T14V coaggregation but did affect the inhibition of WVU45 coaggregation with Ss34.

Although there were only modest differences between T14V and WVU45, there were large differences among the various saccharides in the concentration required for 50% inhibition of coaggregation. Most striking was the 50-fold (or greater) difference in potency between Gal-β(1 → 4)-GalNAc and Gal-β-(1 → 3)-GalNAc. In having a strong preference for the latter structure, the T14V and WVU45 lectins resemble the peanut agglutinin (11). However, both of the actinomycete lectins were inhibited much more by methyl-β- than by methyl-α-galactoside and in this regard, they differ from the peanut agglutinin.

These data demonstrate some similarities and differences between the lectins on the two different actinomycetes, but many questions remain unanswered because the appropriate saccharides were not available for testing. For a more nearly complete definition of the specificity of these lectins, it would be desirable to test at least: (i) disaccharides representing all possible points of linkage in Gal-β-GalNAc, Gal-β-*N*-acetyl-D-glucosamine, Gal-β-Gal, and Gal-β-D-glucose; (ii) selected disaccharides of Gal in beta linkage with other sugars; and (iii) a few disaccharides of Gal in alpha linkage to other sugars. Because WVU45 is twofold more sensitive to GalNAc than to Gal, especially desirable would be a set of disaccharides with GalNAc instead of Gal at the nonreducing terminal. Our study illustrates the need for a great variety of oligosaccharides of known structure for the purpose of defining the structural specificities of lectins.

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