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Specific and Nonspecific Inhibition of Adhesion of Oral Actinomyces and Streptococci to Erythrocytes and Polystyrene by Caseinoglycopeptide Derivatives

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Various caseinoglycopeptide derivatives prepared from mammalian milk were evaluated as inhibitors of hemagglutinations mediated by *Actinomyces viscosus* Ny1, *Streptococcus sanguis* OMZ9, and, for comparative purposes, plant lectins from *Arachis hypogaea* and *Bauhinia purpurea*. It was found that recognition of the β-D-galactose-(1→3)-2-acetamido-2-deoxy-D-galactose carbohydrate chain by *Actinomyces viscosus* Ny1 organisms and *Arachis hypogaea* and *B. purpurea* agglutinins had similar structural requirements; in all cases, the desialylated bovine caseinoglycomacropeptide, on which several units of the above mentioned disaccharide are clustered, behaved as the most potent hemagglutination inhibitor. By contrast, none of the preparations tested inhibited erythrocyte agglutination by *S. sanguis* OMZ9. Thus, the desialylated bovine caseinoglycomacropeptide acts as a potent and specific inhibitor of oral *Actinomyces* adhesion to cell membranes (a soft surface) and could be used as a probe for the study of recognition mechanisms mediated by *Actinomyces* galactose-binding lectins. During the present study, both native and desialylated variants of the same bovine glycomacropeptide also totally prevented the adhesion of *Actinomyces viscosus* Ny1, *S. sanguis* OMZ9, and *S. mutans* OMZ176 to polystyrene surfaces. Comparative evaluations of various structurally different compounds gave the following results. Neither mono- nor disaccharides related to caseinoglycopeptide carbohydrates prevented adhesion; highly positively or negatively charged polypeptides and polysaccharides were either not or only moderately active. Besides these glycomacropeptides, an inhibitory activity was also exhibited by other mucin-type glycoproteins carrying short *O*-linked carbohydrate chains (including bovine submaxillary mucin), polyethylene glycol, and bovine serum albumin. Consequently, caseinoglycopeptide prevention of oral bacterial adhesion to polystyrene tubes (a hard surface) takes place with no species specificity and can be compared to nonspecific inhibition exhibited by various polymers with very different structural characteristics.

During the past 10 years, it has become apparent that the in vivo expression of specific microbial lectins could be one of the main determinants of the composition and distribution of bacterial communities in the buccal cavity (6, 30, 46). A recent screening of multigeneric coaggregations among oral bacteria also emphasized that lectin-carbohydrate interactions form the molecular basis of most of these cell-cell recognitions (23). The attribution of precise roles for the various microbial lectins expressed in such complex systems requires the preparation of different molecular probes which are able to selectively neutralize each of these adhesins. Among such biological tools, antibodies to fimbriae have been successfully used (8, 42), as well as common and abundantly available saccharides, such as lactose (23). However, the fact that complex carbohydrate structures (preferentially recognized by the microbial lectins) are usually not routinely available and easily prepared limits the use of such specific and potent inhibitors in microbiological studies. In this context, the aim of the present work was to take advantage of the natural abundance of a glycopeptide family originating in mammalian milk caseins, the structural characteristics of which are already known.

Among the first of the oral bacterial lectins which have gained attention were the galactose-binding fimbriae expressed by *Actinomyces* species (6, 29). These adhesins mediate coaggregations between *Actinomyces* strains and oral streptococci (7, 29), hemagglutinations (9, 11), and bacterial adhesion to cultured epithelial cells (3, 4). Independent studies established this fimbrial specificity for the β-D-galactose-(1→3)-2-acetamido-2-deoxy-D-galactose [Galβ(1→3)GalNAc] disaccharide (the T hapten) (13–15, 26, 27). Also, the reported inhibition of the attachment of *Actinomyces naeslundii* WVU45 to cultured epithelial cells by *Arachis hypogaea* and *Bauhinia purpurea* lectins is relevant (3, 4), since both of these plant lectins are known to readily recognize the Galβ(1→3)GalNAc disaccharide and its glycosides (20, 37).

Investigations of the interactions between *Streptococcus sanguis* G9B and salivary glycoproteins suggested the recognition of glycoprotein carbohydrate chains by a streptococcal lectin (2), also enabling numerous *S. sanguis* strains to agglutinate guinea pig erythrocytes. A study of its carbohydrate specificity indicated a preference for the sequence *N*-acetylneuraminic acid [NeuNAcα](2→3)Galβ(1→3)GalNAc (31). This trisaccharide is a sialylated derivative of the above-mentioned T hapten, and it occurs as a carbohydrate chain on human salivary mucins (2, 41).

It has long been known that rennin digestion of casein leads to the release of a soluble glycomacropeptide (CGP) containing the casein carbohydrates, all originating from the

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κ-casein component (1, 17). Three oligosaccharides have been identified on κ-casein from bovine mature milk after reductive alkaline borohydride cleavage (12, 43, 47). One of these sugar sequences corresponds to the trisaccharide preferentially recognized by the *S. sanguis* lectin (see Fig. 1). In addition, since all of these structures are mono- and disialylated derivatives of the Galβ(1→3)GalNAc unit, desialylation of such carbohydrates would lead in all cases to the disaccharide specifically recognized by *Actinomyces viscosus* fimbrial lectins. Consequently, the interest of evaluating native CGP as a potential inhibitor of a widely distributed streptococcal lectin, the hope of specifically neutralizing *Actinomyces* galactose-binding fimbriae with desialylated variants of the same glycopeptides, and the ease of preparing these compounds on a large scale from raw dairy materials prompted us to undertake the present investigation.

In performing our experiments, we observed that some CGP derivatives totally prevented adhesion of the strains studied to polystyrene tubes without any species specificity. Such nonspecific interactions are also of major importance, especially for bacterial binding to hard surfaces during dental plaque formation. Thus, the effects of CGP derivatives on the adhesion of *Actinomyces viscosus* Ny1, *S. sanguis* OMZ9, and *S. mutans* OMZ176 to plastic tubes were investigated in more detail and compared with those of various mono-, di-, and polysaccharides, structurally defined glycopeptide mixtures, polypeptides, glycoproteins, and also polymers known to nonspecifically inhibit bacterial adhesion to solid surfaces, such as bovine serum albumin (BSA) (38) and polyethylene glycol (Carbowax 20M) (16).

MATERIALS AND METHODS

Materials and analytical methods. Methyl-α-D-mannoside, *N*-acetylneuraminic acid (type VI), GalNAc, polygalacturonic acid (from oranges), bovine submaxillary mucin (type I), glycophorin A (from type B negative human blood), asialoglycophorin A, and BSA (fraction V; 98 to 99%) were obtained from Sigma Chemical Co., St. Louis, Mo. D-Galactose and D-lactose monohydrate were purchased from Fluka, Buchs, Switzerland. Among the complex disaccharides, Galβ(1→4)-*N*-acetylglucosamine (GlcNAc), Galβ(1→4)GlcNAc-β-*O*-ethyl, and GalNAcβ(1→3)Gal-α-*O*-methyl were from Carbohydrates International, Arlöv, Sweden, whereas Galβ(1→3)GalNAc was from BioCarb, Lund, Sweden. Poly-L-glutamic acid (molecular weight, 15,000 to 50,000) and poly-L-lysine (molecular weight, 8,000 to 30,000) were obtained from Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.), chitosan (cosmetic grade) was from Atomergic Chemical Co., Farmingdale, N.Y., and Carbowax 20M (CW-20M) was from Supelco, Bellefonte, Pa. Antifreeze glycoprotein of Antarctic fish was donated by W. Hösel (Boehringer, Mannheim, Federal Republic of Germany).

B. purpurea and *Arachis hypogaea* agglutinins were affinity-purified lectins purchased from E.Y. Laboratories, San Mateo, Calif., and Boehringer, respectively. Both lectins were used at a concentration of 2 mg/ml, the former in phosphate-buffered saline (10 mM phosphate buffer, pH 7.2, containing 150 mM NaCl), and the latter in 150 mM NaCl.

Bovine CGP was isolated by a single protein precipitation with a trichloroacetic acid solution, starting from a concentrate of whey proteins produced by ultrafiltration of bovine sweet cheese whey (18, 36). With milk samples obtained from local farmers just after collection, ovine and caprine CGP were prepared by rennin digestion of sheep and goat whole caseins, respectively (17). Desialylation by mild acid hydrolysis (H_2SO_4, 25 mM, 2 h at 80°C) and pronase digestion of these CGP were performed as previously described (33). In addition, three typical mixtures of structurally defined *N*-linked glycopeptides were prepared as previously reported (33), starting from common bean phaseolin (34, 40), chicken egg ovalbumin (grade V; Sigma), and bovine fibrinogen (type I-S; Sigma).

Total neutral sugars (10) and total sialic acids (19) were quantitatively estimated by colorimetry. Gas-liquid chromatographic quantifications of neutral and amino sugars were performed (35) together with that of *N*-acetylneuraminic acid when necessary (32).

Bacterial strains and culture conditions. The three bacterial strains used in this study were *Actinomyces viscosus* Ny1, *S. sanguis* OMZ9, and *S. mutans* OMZ176, and all three were maintained as multiple frozen stocks. The *Actinomyces viscosus* strain was cultured in actinomyces broth (Difco Laboratories, Detroit, Mich.), whereas both *S. sanguis* and *S. mutans* strains were grown in Difco brain heart infusion supplemented with 0.5% glucose.

For hemagglutination and inhibition studies, *Actinomyces viscosus* Ny1 was precultured for 24 h at 37°C and then subcultured for 16 h before harvesting. Similarly, *S. sanguis* OMZ9 was precultured for 7 h at 37°C and then cultured for 14 h before harvesting. For assays of adhesion to a solid surface and inhibition studies, *Actinomyces viscosus* Ny1 cells were cultured for 36 h, whereas cells of both streptococcal strains were harvested after 24 h.

Hemagglutination and inhibition studies. Hemagglutinations by *Actinomyces viscosus* Ny1 were performed with neuraminidase-treated erythrocytes from a blood group A donor. The latter cells were desialylated by incubation of washed, packed erythrocytes (0.5 ml) suspended in 150 mM NaCl containing 10 mM $CaCl_2$ with 0.4 U of neuraminidase from *Vibrio cholerae* (Behring, Marburg, Federal Republic of Germany). After incubation for 60 min at 37°C with occasional shaking, the cells were washed three times in 150 mM NaCl and suspended at 1% (vol/vol) in the same solution. Hemagglutinations by *S. sanguis* OMZ9 were performed with intact (freshly drawn) guinea pig erythrocytes, washed in 150 mM NaCl, and suspended before the tests, as described above. Just after harvesting, the bacteria were also washed three times in this saline solution, and hemagglutination tests were performed by mixing serial twofold dilutions of the final bacterial suspension (50 μl) with 50 μl of the appropriate erythrocyte suspension. Inhibition studies were performed by mixing 25 μl of serial twofold dilutions of a saline solution containing the potential inhibitor to be tested (previously neutralized with an NaOH solution, when necessary), 25 μl of a bacterial suspension corresponding to four hemagglutinating doses, and 50 μl of the erythrocyte suspension. The results were recorded after 1 h at room temperature.

Hemagglutinations by *B. purpurea* and *Arachis hypogaea* agglutinins were performed with neuraminidase-treated NN erythrocytes from blood group O donors (all typed as SS and obtained from the Centre National de Transfusion Sanguine, Paris, France) (39). The cells were desialylated as described above and then washed three times in phosphate-buffered saline. Hemagglutination inhibition studies were performed by mixing serial dilutions of a neutralized solution of the potential inhibitor in 25 μl of phosphate-buffered saline, with 25 μl of the lectin-containing solution (four hemagglutinating doses). After agitation, the mixture was allowed to settle for

TABLE 1. Carbohydrate compositions of the various derivatives of bovine, ovine, and caprine CGP tested in this study[a]

Origin of derivative and derivative	Gal[b]	GlcNAc[b]	GalNAc[b]	Total Gal-GalNAc	Total sialic acids[c]	NeuNAc[b]	NeuNGl[d]	Total carbohydrates
Bovine whey								
CGP	5.8 (0.9)	1.1 (0.1)	7.6 (1)	13.4	13.2 (1.3)	13.2	—	27.7
As-CGP	7.5 (0.9)	0.9 (0.1)	10.0 (1)	17.5	—	—	—	18.4
CGP-Pr	11.5 (0.9)	2.9 (0.2)	15.0 (1)	26.5	26.5 (1.3)	26.5	—	55.9
As-CGP-Pr	16.0 (0.9)	3.0 (0.1)	22.5 (1)	38.5	—	—	—	41.5
Ovine casein								
CGP	1.3 (1.2)	0.1 (0.1)	1.3 (1)	2.6	2.4 (1.3)	<0.5	>1.9	5.1
As-CGP	3.2 (1.1)	0.5 (0.1)	3.4 (1)	6.6	—	—	—	7.1
Caprine casein								
CGP	2.7 (1.1)	0.2 (0.1)	3.1 (1)	5.8	5.1 (1.2)	2.5	2.6	11.1
As-CGP	3.8 (1.1)	0.4 (0.1)	4.3 (1)	8.1	—	—	—	8.5

[a] Expressed in percent (wt/wt). The numbers in parentheses represent molar ratios relative to GalNAc. —, Not detected.
[b] Determined by capillary gas-liquid chromatography.
[c] Determined by the periodate-resorcinol method.
[d] NeuNGl, *N*-glycolylneuraminic acid; calculated by subtraction.

1 h at room temperature. Then, 50 μl of a 1% suspension of the neuraminidase-treated NN erythrocytes in a 10 mM $NaHCO_3$ solution containing 150 mM NaCl was added and incubation was performed at room temperature. The results were recorded 30 min later.

Assay of adhesion to plastic tubes and inhibition studies. After harvesting, the bacteria were washed three times with buffer (150 mM NaCl, 1 mM Tris, 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 0.02% NaN_3) and their final suspension was adjusted to an optical density of 0.8 (λ = 650 nm). The 10-ml polystyrene tubes (Milian Instruments SA, Geneva, Switzerland) were first filled with 0.5-ml volumes of the above-described buffer with or without inhibitor, with the pH corrected to 7.3 unless otherwise specified; then, 0.5-ml volumes of the bacterial suspension were added, and the tubes were stoppered and vigorously mixed with a Vortex mixer (Scientific Industries Inc., Bohemia, N.Y.) to provoke a flowing contact for 10 s between the bacterial suspension and the internal tube surface. The mixture was then allowed to settle for 4 h at room temperature. Vigorous mixing was repeated once each hour. Finally, the residual bacterial suspension was measured and bacterial adhesion was calculated as follows: adhesion (percent) = [(0.4 − residual optical density) × 100]/0.4. Adhesion inhibition was determined by calculating the ratio between adhesions obtained with or without an inhibitor. Mean values of triplicate determinations are reported.

RESULTS

Analysis of sugar constituents of caseinoglycopeptide derivatives. Since it has been known for a long time that strong similarities exist between the monosaccharide compositions of all bovine, ovine, and caprine CGP (1), derivatives originating from the milk of these three species were prepared. To investigate the influence of sialic acids on the biological properties of these glycopeptides, desialylations were performed, leading to asialo derivatives (As-CGP). To study the effect of the sugar chain multivalency exhibited by bovine CGP, the glycopeptide mixtures yielded by extensive pronase digestions of the macrostructures (CGP-Pr and As-CGP-Pr) were also prepared. The carbohydrate compositions of the above-described glycopeptides are summarized in Table 1. In all cases, we found total carbohydrate contents, homogeneity or heterogeneity among sialic acid constituents, and ratios for the monosaccharide components to be in complete agreement with previous reports (1, 48) and also with the occurrence of the primary structures established for the cow κ-casein carbohydrate chains (47) (Fig. 1).

Hemagglutination inhibition studies. Inhibition of neuraminidase-treated human erythrocyte agglutinations by *Actinomyces viscosus* Ny1 led to the results in Table 2, which show that most of the caseinoglycopeptide preparations tested served as excellent hemagglutination inhibitors. These results also demonstrated the very high inhibitory

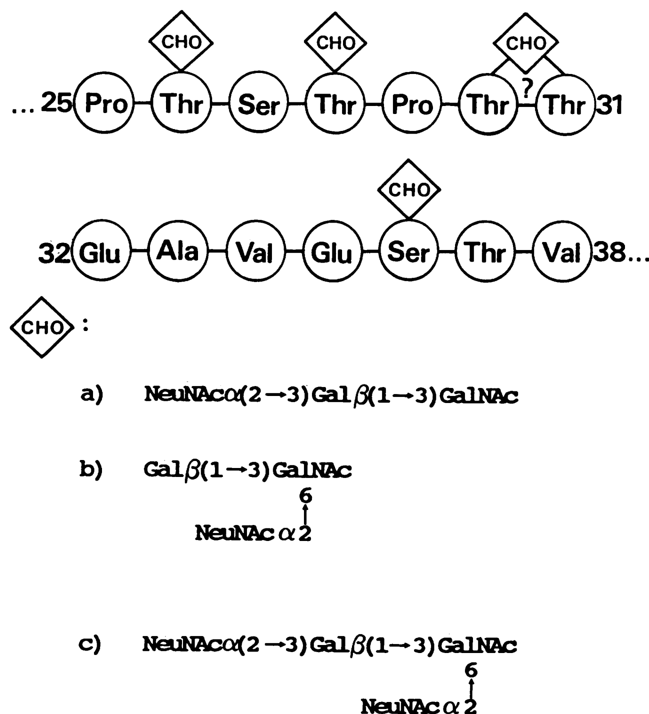

FIG. 1. The sugar-bearing region of the highly glycosylated bovine CGP subcomponents (adapted from references 21, 22, and 48). CHO, Carbohydrate.

TABLE 2. Inhibitions of hemagglutination of various erythrocytes by two oral bacterial strains and two plant lectins

Source of inhibitor and inhibitor	Concn (μg of Gal/ml) needed to inhibit agglutination of:[a]			
	Neuraminidase-treated human A erythrocytes by *Actinomyces viscosus* Ny1	Guinea pig erythrocytes by *S. sanguis* OMZ9	Neuraminidase-treated human NN erythrocytes by:	
			Arachis hypogaea agglutinin	*B. purpurea* agglutinin
Cow				
CGP	360 (2)	>730	5.4 (50)	2.7 (50)
As-CGP	8 (100)	>1,040	2.7 (100)	1.4 (100)
CGP-Pr	2,120 (0.4)	>2,840	180 (1.5)	720 (0.2)
As-CGP-Pr	970 (0.8)	>4,280	90 (3)	180 (0.8)
Sheep				
CGP	>340 (<2.5)	>340	45 (6)	45 (3)
As-CGP	230 (3)	>470	5.4 (50)	5.4 (25)
Goat				
CGP	>1,460 (<0.5)	>1,460	90 (3)	180 (0.8)
As-CGP	NT	NT	10.8 (25)	10.8 (12.5)
Lactose	>10,000	>10,000	NT	NT
Galactose	>20,000	>20,000	NT	NT

[a] Minimal concentration necessary for complete inhibition. Expressed in micrograms of Gal contained in the inhibitor tested per milliliter of the final suspension. The numbers in parentheses represent the activities relative to that of bovine As-CGP, which is defined as 100. NT, Not tested.

potency of bovine As-CGP, compared with those of the other compounds tested. For example, it appears to be 30 times more active than its ovine analog, 50 times more active than the native (sialylated) CGP as it naturally occurs in bovine whey, and over 100 times more active than smaller glycopeptides obtained by pronase digestion of the macrostructures. In these experimental conditions, lactose and galactose had no inhibitory activity.

The results of the hemagglutination inhibition tests with *S. sanguis* OMZ9 revealed the following. Among the seven different caseinoglycopeptide preparations tested against this streptococcal lectin, none was able to achieve complete agglutination inhibition. In this case, the values reported in Table 2 are therefore consistently related to the maximal concentration tested. These results unambiguously indicate that the oligosaccharide structures that characterize this glycopeptide family are not recognized by the *S. sanguis* OMZ9 agglutinin.

To document the similarities among the carbohydrate specificities of *Actinomyces* galactose-binding fimbriae and *Arachis hypogaea* and *B. purpurea* agglutinins (3, 4), the same caseinoglycopeptide preparations were independently evaluated against hemagglutinations mediated by both of these plant lectins. Such tests were performed with neuraminidase-treated human NN erythrocytes (39). All of the caseinoglycopeptide derivatives tested were able to inhibit these erythrocyte agglutinations (Table 2). Moreover, the relative potencies of the compounds against both of these β-D-galactose-specific lectins were analogous to those exhibited against hemagglutinations mediated by *Actinomyces viscosus* Ny1 (Table 2). Indeed, the three As-CGP were potent hemagglutination inhibitors, and that of bovine origin was the most effective. Desialylation always resulted in an increase of the glycopeptide inhibitory activity, whereas pronase digestion led to dramatic losses of activity. However, it is interesting that desialylation of bovine CGP increased its inhibitory potency against plant agglutinins only weakly, whereas against *Actinomyces*-mediated hemagglutinations an increase corresponding to a factor of 50 was observed.

Inhibition of adhesion to polystyrene surfaces. We observed that both bovine CGP and bovine As-CGP were able to totally prevent the adhesion of *S. sanguis* OMZ9 and *Actinomyces viscosus* Ny1 to polystyrene tubes, even at low concentrations. Under the present experimental conditions, adhesion of 90% of *S. sanguis* OMZ9 cells or 43% of *Actinomyces viscosus* Ny1 cells to polystyrene tubes was observed. With bovine CGP or bovine As-CGP (1 mg/ml), adhesion inhibition levels of more than 95% were calculated. Finally, similar experiments performed with *S. mutans* OMZ 176 showed an inhibition of bacterial adhesion of the same order of magnitude (71% adhesion without inhibitor; 96% inhibition in the presence of CGP or As-CGP).

Prompted by the desire to better understand which molecular characteristics of bovine CGP and As-CGP could be related to such an inhibitory property, we tested the effects of various (and structurally very different) compounds at a concentration of 1 mg/ml in the same assay. Among the numerous mono- and disaccharides tested, none exhibited inhibition at a significant level (Fig. 2). Among polymers, we found polyanionic and polycationic polypeptides (poly-L-glutamic acid and poly-L-lysine, respectively) to be almost totally devoid of activity; polyanionic and polycationic polysaccharides (polygalacturonic acid and chitosan, respectively) were moderately active, and only amphoteric BSA and neutral CW-20M were potent inhibitors. On the other hand, Fig. 3 shows the results of a comparative evaluation of bovine CGP and bovine As-CGP and glycopeptide mixtures of completely different structures, such as, for example, *N*-linked oligomannosides derived from kidney bean phaseolin and *N*-linked complex type biantennary chains prepared from bovine fibrinogen. Tested at a concentration corresponding to 10 μg of neutral sugars per ml, the potency of these latter glycopeptide preparations was very weak in comparison with those of bovine CGP and As-CGP. Thus, among various glycopeptides, the most potent inhibitors of adhesion of oral bacteria to polystyrene tubes appeared to be those bearing several short *O*-linked carbohydrate chains. Several glycoproteins known to carry clusters of short *O*-linked carbohydrate units were tested for comparative

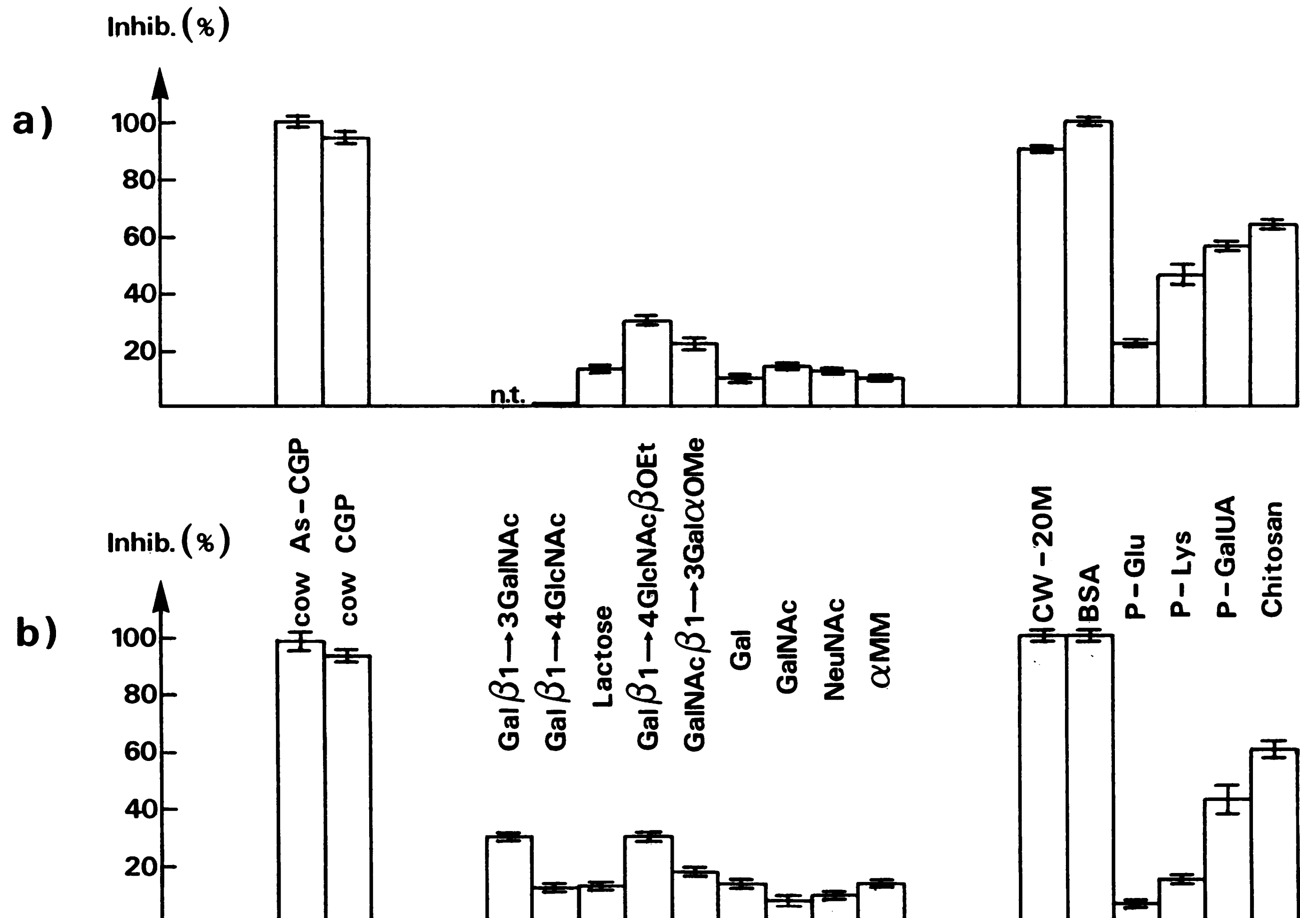

FIG. 2. Inhibition (Inhib.) of adhesion of *Actinomyces viscosus* Nyl (a) and *S. sanguis* OMZ9 (b) to polystyrene tubes. The relative inhibitory potencies of 1 mg of bovine CGP and As-CGP, mono- and disaccharides, and various polymers per ml are shown. For evaluation of chitosan, the pH was corrected to 6.0. NeuNAc, *N*-Acetylneuraminic acid; αMM, methyl-α-D-mannoside; P-GalUA, polygalacturonic acid.

reasons. The results (Table 3) show that bovine CGP and its desialylated analog share with other mucin type glycoproteins, as well as BSA and CW-20M, this ability to act as nonspecific inhibitors of adhesion of oral bacterial strains to hard surfaces.

DISCUSSION

In the first studies on the ability of most *Actinomyces* strains to agglutinate erythrocytes, these agglutinations were reported to be reversed by lactose (9) and other β-galactosides (11), and agglutination inhibition of neuraminidase-treated erythrocytes required higher inhibitor concentrations (11). The present assay conditions were ideal for selection and comparison of compounds exhibiting more potent and specific activities; looking for complete inhibition and using four hemagglutinating doses of bacterial suspension increased the difficulty of inhibiting these agglutinations. This point is demonstrated by the inability of lactose (60 mM) to act as an inhibitor under our conditions. In their comparative study on the inhibition of coaggregations between *Actinomyces viscosus* T14V (or *Actinomyces naeslundii* WVU45) and *S. sanguis* 34, McIntire et al. (26, 27) showed that all α-glycosides derived from the Galβ(1→3)GalNAc disaccharide were more than 15 times more active than lactose. In the present study, the As-CGP-Pr preparation can be regarded as a mixture of aminoacyl-α-glycosides of this T disaccharide, produced by pronase digestion of bovine As-CGP. Thus, our results not only are in complete agreement with the carbohydrate specificity previously established for *Actinomyces viscosus* type 2 fimbriae (15, 26, 27), but they also demonstrate that bovine As-CGP exhibits a potency 100 times higher than that of its constitutive α-glycoside units for neutralizing such galactose-binding lectins, certainly because of its multivalent structural pattern.

The fact that caseinoglycopeptides are not recognized by the *S. sanguis* OMZ9 agglutinin appears surprising. Indeed, the trisaccharide which corresponds to the reported specificity of this widely distributed streptococcal lectin (structure a, Fig. 1) (31) has been proposed as being the major sugar chain of bovine κ-casein (43). On the other hand, three oligosaccharides were isolated from the same glycoprotein and identified by nuclear magnetic resonance spectroscopy (structures a to c, Fig. 1) (47), but their relative ratios were not reported. Our results could be explained by assuming either that trisaccharide a (Fig. 1) contributes only as a minor constituent of the CGP sugar chains or that the *S. sanguis* OMZ9 agglutinin exhibits a different carbohydrate specificity than that of *S. sanguis* G9B (31).

Comparative evaluations of glycopeptides against plant and bacterial lectins demonstrate that recognition of the

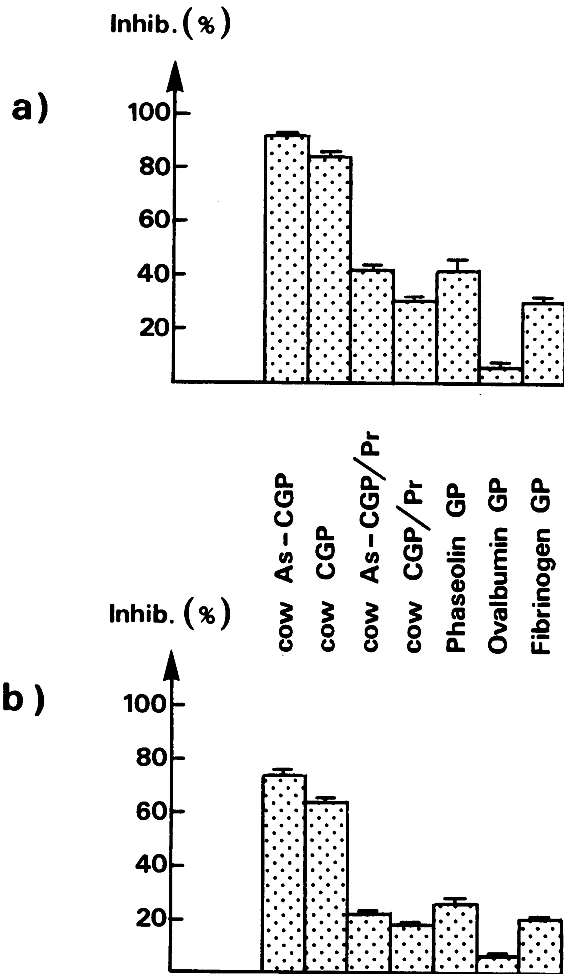

FIG. 3. Inhibition (Inhib.) of adhesion of *S. mutans* OMZ176 (a), and *S. sanguis* OMZ9 (b) to polystyrene tubes. The relative inhibitory potencies of 10 μg of total neural sugars of selected glycopeptide (GP) mixtures per ml (determined as described in reference 10) are shown.

Galβ(1→3)GalNAc sequence by *Actinomyces viscosus* Nyl, *Arachis hypogaea*, and *B. purpurea* agglutinins follows similar structural requirements, a result which corroborates those of previous studies (3, 4). However, it is noteworthy that desialylation of bovine CGP increases its potency against the *Actinomyces* agglutinin much more than its potency against plant lectins; in other words, native bovine CGP is much better recognized by *Arachis hypogaea* and *B. purpurea* lectins than by *Actinomyces viscosus* Nyl (Table 2). This last point indicates that bovine CGP certainly contains a significant number of chains terminating in nonsubstituted Gal residues (structure b, Fig. 1), which are known to be recognized by *Arachis hypogaea* agglutinin (37). Desialylation of such a sugar unit seems not to be required for its binding to both β-D-galactose-specific plant lectins, whereas it could be crucial for its binding to *Actinomyces* fimbriae.

TABLE 3. Inhibition of adhesion of *S. sanguis* OMZ9 to polystyrene tubes

Inhibitor	Mean ± SD (μg [dry wt]/ml)[a]
Bovine submaxillary mucin	3.4 ± 0.2
CW-20M	3.4 ± 0.1
BSA	3.8 ± 0.2
Asialoglycophorin A	20 ± 2
Glycophorin A	30 ± 3
Bovine As-CGP	23 ± 1
Bovine CGP	28 ± 1
Antifreeze glycoprotein	140 ± 3
Bovine As-CGP-Pr	560 ± 39
Bovine CGP-Pr	>1,000 ± 42

[a] Concentration necessary for 50% inhibition.

Functional differences between both fimbriae expressed by closely related oral *Actinomyces* species have been correlated with the known preference of *Actinomyces naeslundii* for soft (epithelial) surfaces and that of *Actinomyces viscosus* for hard (tooth) surfaces (6, 30). Recently, several studies were devoted to other cell-cell recognition mechanisms mediated by the galactose-binding fimbriae of *Actinomyces viscosus* T14V (28, 44, 45). Our present results revealed a compound (bovine As-CGP) which can be seen as a potent inhibitor of galactose-specific adhesion of oral *Actinomyces* species on cell membranes (a soft surface). The presence of large amounts of bovine CGP in whey-based raw materials and the unproblematic chemical desialylation make the preparation of As-CGP easy. Thus, our results should prompt further investigation of the relative roles of fimbriae on oral bacteria by taking advantage of bovine As-CGP, which enables the specific blockade of recognition mechanisms mediated by these *Actinomyces* fimbrial lectins.

Handling oral bacterial strain suspensions in polystyrene tubes immediately permitted us to observe the antiadhesive effects of bovine CGP and As-CGP. The polystyrene tubes used in the present study are certainly not an ideal model for imitating the enamel surfaces of teeth. However, among the multiple mechanisms involved in bacterial adhesion to dental enamel (5), some are certainly shared by all "microbial cell/hard surface" interactions (24). It is well known that bacterial adhesion at solid-water interfaces is sensitive to extracellular polymers, which can play either adhesive or antiadhesive roles (25). In the field of oral microbiology, polymers such as salivary agglutinins and glycoproteins modulate both bacterial clearance from the mouth and bacterial adhesion to teeth (5). Our comparative evaluations first show that bovine CGP and As-CGP exhibit the same inhibitory property as other polymers that are structurally very different, BSA (38) and CW-20M (16). However, it is noteworthy that both bovine caseinoglycomacropeptide derivatives behaved also as other mucin type glycoproteins, including a major salivary component, namely, bovine submaxillary mucin. This should incite further investigation of the effects of caseinoglycopeptides on the adhesion of oral bacterial strains on saliva-coated hydroxyapatite.

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