Identification of a Galactose-Binding Lectin on *Fusobacterium nucleatum* FN-2

PATRICIA A. MURRAY,* DAVID G. KERN, AND JAMES R. WINKLER

Department of Stomatology, Division of Periodontology, HSW 661, University of California San Francisco, San Francisco, California 94143-0515

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A previous study has suggested that *Fusobacterium nucleatum* FN-2 contains a galactose-binding protein (lectin) on the cell surface (P. A. Murray, V. Matarase, C. I. Hoover, and J. R. Winkler, FEMS Microbiol. Lett. 40:123–127, 1987). In the present study, the molecular specificity and size of this lectin were investigated by several techniques. Whole-cell affinity chromatography with asialofetuin covalently coupled to Sepharose 6MB demonstrated that 81% of 

$^{3}H$-labeled *F. nucleatum* were specifically eluted by 0.5 M galactose. Specific binding was calcium dependent and did not occur in the presence of calcium chelators. Binding was inhibited by preincubation with galactose. Agglutination of human parotid saliva by *F. nucleatum* was also inhibited by galactose and its structural analogs. Inhibition by lactose was 2 times that of galactose, inhibition by p-aminophenyl galactosides was 4 times that of galactose, and inhibition by asialoglycoproteins was 100 times that of galactose. Similar inhibition results were obtained for hemagglutination of neuraminidase-treated erythrocytes. These findings suggest that the binding specificity of *F. nucleatum* FN-2 is more complex than simply the recognition of the monosaccharide galactose. This is consistent with the concept that lectins considered identical in terms of monosaccharide specificity can recognize fine differences in more complex structures. To identify the specific bacterial component(s) involved in galactose recognition, proteins of *F. nucleatum* FN-2 were separated on a 4 to 11% gradient sodium dodecyl sulfate slab gel, transferred to nitrocellulose paper to renature bacterial binding sites, and then incubated with 

$^{125}$I-labeled asialofetuin. Autoradiographs of the nitrocellulose revealed a band at a range of $M_{r} 300,000$ to 330,000 which was not present when the blots were preincubated with galactose. These data support the concept that *F. nucleatum* FN-2 possesses a lectin that recognizes galactose and galactose-containing substrates.

The adherence of bacteria to host tissues is a specific interaction mediated by bacterial surface molecules (adhesins) which combine with complementary structures on the host cell surface (receptors) (2, 25). Several oral bacteria have been shown to contain surface adhesins, or binding proteins, and a lectin-like mechanism of adherence has been proposed on the basis of inhibition of attachment by specific sugars (8–10, 21, 22, 28). In gram-negative bacteria, these adhesins (lectins) include fimbriae and certain outer membrane proteins (13). Although it is recognized that surface components of oral bacteria participate in adherence and clearance phenomena, their properties remain largely obscure. The further identification and characterization of these binding proteins, as well as their receptor sites, will provide a more complete understanding of their role in host-parasite interactions.

Considerable data indicate that the monosaccharide moieties of salivary glycoproteins interact specifically with bacterial ligands and subsequently play a major role in adherence and clearance mechanisms in the oral cavity (3, 17, 18, 20–22, 29). Galactose (Gal)-binding lectins have been identified on several species of oral bacteria (4, 6, 10, 14, 22, 23). Yet, although these bacteria possess ligands that interact with β-galactosides, they differ in their properties of adherence and colonization of oral tissues (11). It appears that lectins considered identical in terms of monosaccharide specificity are able to recognize subtle structural differences (5). This is exemplified in the studies of Murray et al. (20, 21), who identified a sialic acid-binding lectin on *Streptococcus sanguis* and *Streptococcus mitis*, with the greatest specificity towards a NeuAc(a2,3)Gal(β1,4)GalNAc sequence (NeuAc, N-acetylmuraminic acid; GalNAc, N-acetylgalactosamine). Furthermore, Firon et al. (8, 9) elegantly demonstrated that bacterial lectins in the form of type I fimbriae on the various species of enterobacteria, typically classified as mannose specific, exhibited fine differences in actual sugar specificity.

In earlier studies, we described one such Gal-binding lectin on *Fusobacterium nucleatum* FN-2 (22). Very little is known about the detailed carbohydrate specificity of the receptor to which this adhesin binds (i.e., the recognition site). The purpose of this investigation was to analyze the sugar specificity patterns of *F. nucleatum* by using a whole-cell affinity chromatography technique, hemagglutination assays, and salivary agglutination assays. In addition, we have identified the bacterial lectin on the basis of size by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting.

**MATERIALS AND METHODS**

**Materials.** Sialyllactose was purchased from Boehringer Mannheim Biochemicals. Fetaun, enzymes, sugars, Nonidet P-40 (NP-40), and most reagents were obtained from Sigma Chemical Co. Electrophoresis reagents were from Bio-Rad Laboratories. Trypsin soy broth and brucella agar were from Difco Laboratories. CNBr-Sepharose 6MB was purchased from Pharmacia Fine Chemicals. Perlmest plastic impression material was from Kerr. [2,8-3H]Adenine and [125]Iodine were obtained from New England Nuclear Corp. Microtiter plates were purchased from Linbro, X-ray film was from Eastman Kodak Co., and Quanta III intensifying screens were from Du Pont Co. The nitrocellulose mem-

* Corresponding author.
brane (0.2 μm pore size) was purchased from Schleicher & Schuell, Inc.

**Bacteria and culture conditions.** *F. nucleatum* FN-2 was the kind gift of S. Syed, University of Michigan, Ann Arbor. This strain was stored frozen in tryptic soy broth containing 20% glycerol, 0.25% yeast extract, 2.5 μg of hemin per ml, 2.5 μg of menadione per ml, and 0.01% dithiothreitol. A stock culture was maintained anaerobically (85% N₂–10% H₂–5% CO₂) on laked rabbit blood brucella agar with weekly transfers to new plates. Cells were grown anaerobically at 37°C to late log phase of growth in tryptic soy broth containing 0.25% yeast extract, 2.5 μg of hemin per ml, 2.5 μg of menadione per ml, and 0.01% dithiothreitol. Radiolabeled bacteria were prepared by growing cells in the presence of [³²P]adenine (10 μCi/ml). The specific activity achieved was 3,000 to 5,000 cpm/10⁶ cells. Cells were harvested by centrifugation (8,000 × g for 15 min at 4°C) and washed either in PBS (10 mM sodium phosphate, 154 mM NaCl [pH 7.2], and 0.02% sodium azide) or in buffered KCl (5 mM KCl containing 2 mM sodium phosphate and 1 mM CaCl₂ [pH 6.0]).

**Whole-cell affinity chromatography.** Asialofetuin was covalently coupled to CNBr-activated Sepharose 6MB using 6 mg of ligand per ml of gel as previously described (22). The derivatized macrobeads (1.5 to 2 ml) were equilibrated in PBS in columns (0.5 by 5 cm) containing an 80-μm-pore-size nylon mesh filter, which allows bacterial cells to pass through but retains matrix beads. Radiolabeled bacteria (10⁹ cells) were suspended in 0.2% bovine serum albumin (BSA) in PBS (pH 7.2), carefully layered onto the affinity matrix, and incubated for 1 h at 4°C. In some experiments, to test the effect of calcium on bacterial binding, calcium or calcium chelators were added to the incubation buffer. After incubation, the columns were adjusted to a flow rate of 1 ml/min and the unbound fraction was recovered by washing with 10 volumes (2 ml each) of PBS containing 0.2% BSA. Bacteria specifically bound were eluted with 8 volumes of 0.5 M Gal in PBS containing 0.2% BSA (pH 7.2). The nonspecifically bound fraction was eluted with 10 volumes of 0.1% NP-40 in PBS (pH 7.2). The counts per minute recovered in the various fractions was determined by scintillation spectrometry. The percentage recovery was determined by dividing the counts per minute bound by the counts per minute added; the percentage specifically bound was calculated by dividing the counts per minute specifically eluted by the total counts per minute recovered.

**Saliva collection.** Whole paraffin-stimulated saliva from four donors was collected on ice into a protease inhibitor cocktail (5 mM benzamidine, 10 mM EDTA, 10 μM peptatin, 2 mM phenylmethylsulfonyl fluoride [pH 7.0]). The saliva was then pooled and centrifuged at 11,000 × g for 30 min at 4°C.

Human parotid saliva was obtained by means of a modified Carlson-Crittenden apparatus. Human submandibular-sublingual saliva was collected by means of a mouthpiece custom-made from Perlmaflex impression material. Salivary flow was stimulated by 2% citric acid applied to the lateral borders of the tongue at 30-s intervals. Samples of both types of saliva were collected on ice into the protease inhibitor cocktail and were centrifuged at 11,000 × g for 30 min at 4°C.

**Salivary agglutination and agglutination inhibition.** Visual assays for salivary agglutination were performed in 96-well round-bottom microtiter plates at room temperature. Freshly collected saliva samples (whole, parotid, or submandibular-sublingual) were serially diluted in PBS. Next, 25 μl of one type of saliva was added to an equivalent volume of bacterial suspension (10⁸ to 10⁹ cells per ml) in a checkerboard fashion in microtiter plates. Plates were allowed to sit for 1 h at room temperature, after which salivary agglutination was scored from 0 to 4+, with 4+ being 100% agglutination of bacteria and saliva. Agglutination scores of 4+ were assigned when there was an opaque, scalloped blanket of cells on the bottom of the well; whereas a score of 0 was assigned when there was a round, tight pellet of cells. Scores between these two numbers were based on the appearance of the cells relative to the two extremes. Inhibition studies were conducted by incubating 25 μl of serially diluted inhibitors (2 to 100 mM in PBS with pH adjusted to 7.2) with 25 μl of *F. nucleatum* FN-2 (final concentration, 5 × 10⁸ cells per ml) for 1 h at room temperature with gentle agitation. Saliva (50 μl) was added to each well, and the plates were again gently agitated for 1 h. Plates were scored as above. The MIC was determined to be the concentration of inhibitor required for 50% inhibition of agglutination.

**Hemagglutination and hemagglutination inhibition.** Rabbit erythrocytes (RBC) were treated with neuraminidase (type VI, *Clostridium perfringens*) in sodium acetate buffer (2 mM sodium acetate, 154 mM NaCl [pH 5.8]) for 1 h at 37°C to remove the terminal sialic acid residues and expose the penultimate Gal. Cells were then washed three times in PBS and either used immediately or stored in an equal volume of Alsever solution (University of California San Francisco Cell Culture Facility) at 4°C for no longer than 1 week. Removal of sialic acid was tested by hemagglutination of treated and untreated cells with Limulin lectin at a concentration of 2 μg/ml (1).

Washed asialo-RBC were diluted with PBS at a 4% (vol/vol) suspension. Visual assays for bacteria-mediated hemagglutination were performed in 96-well round-bottom microtiter plates at room temperature. Twofold serial dilutions of asialo-RBC (25 μl) were added to 25 μl of serially diluted bacterial suspensions in a checkerboard fashion. After the plates were allowed to sit for 1 h, hemagglutination was scored from 0 to 4+, with 4+ being 100% hemagglutination of asialo-RBC and bacteria (6, 7, 21). A score of 4+ was given to those wells in which the cells formed a scalloped blanket of cells at the bottom of the well. Those scored as 0 exhibited a tight, round pellet of cells at the bottom of the well. For hemagglutination inhibition studies, 25 μl of serially diluted inhibitors (2 to 100 mM in PBS with pH adjusted to 7.2) were added to 25 μl of bacteria (final concentration, 5 × 10⁸ cells per ml) and were incubated for 1 h with gentle agitation. Next, 50 μl of asialo-RBC were added, and the plates were again gently agitated for 1 h. Plates were scored, and the MIC was defined as the concentration needed to inhibit hemagglutination by 50%.

**Binding of ¹²⁵I-labeled asialofetuin to SDS-PAGE replicas of *F. nucleatum* on nitrocellulose.** *F. nucleatum* FN-2 (2 × 10⁶ cells) were solubilized in 20 μl of 0.125 M Tris hydrochloride containing 6 M urea, 4% SDS, 20% glycerol, 10% β-mercaptoethanol, and 0.01% bromophenol blue (pH 6.8) (loading buffer). The mixture was boiled for 10 min, and then proteins were separated by 4 to 11% gradient SDS-PAGE (16, 24). The molecular weight standards used were as follows: *M.*, 29,000, carbonic anhydrase; *M.*, 45,000, ovalbumin; *M.*, 66,000, bovine plasma albumin; *M.*, 97,400, phosphorylase b; *M. *, 116,000, β-galactosidase; *M.*, 205,000, myosin.

The resulting gels were cut into two pieces. One half was stained with 0.5% Coomassie blue in 45% methanol–10% acetic acid. The other half was transferred to nitrocellulose
by means of a semidry electroblotter (Satorius) for 1 h at 0.8 mA/cm² of gel (15, 30). The nitrocellulose blots were rinsed once with TBS (0.154 M NaCl-0.02 M Tris hydrochloride (pH 8.0)) and then were soaked with TBS containing 3% BSA-0.1% Tween 20 for 1 h at 37°C to block nonspecific attachment of ligand to the nitrocellulose blots. After blocking, the nitrocellulose blots were rinsed a total of five times for 6 min, each time with TBS containing 0.05% Tween 20. Asialofetuin was radiolabeled with [125I]iodide (specific activity, 17.4 Ci/mg) by the chloramine-T method (12). 125I-asialofetuin was dissolved in TBS containing 10 mM CaCl₂ (1.3 × 10⁶ cpm); blots were incubated in this mixture with gentle agitation for 1 h at 37°C and then were incubated overnight at 4°C. In some cases, to evaluate the specificity of binding, the incubations were done in the presence of 100-fold excess asialofetuin or 0.5 M Gal. The blots were then washed five times for 6 min each time with 0.05% Tween 20 in TBS containing 10 mM CaCl₂, dried in air, and exposed to X-ray film with an intensifying screen at −70°C for 24 h.

RESULTS

Affinity chromatography of F. nucleatum on asialofetuin-Sepharose 6MB. F. nucleatum FN-2 labeled with [³H]adenine was applied to a Sepharose 6MB column covalently bound to asialofetuin (Fig. 1a) and, as a control, to a Sepharose 6MB column alone (Fig. 1b). Of the cells added to the test column, 81% were specifically eluted from asialofetuin with 0.5 M Gal (Fig. 1a). The unbound fraction (eluted with PBS) represented 17%, whereas the nonspecifically bound fraction (eluted with NP-40) represented the remaining 2%, recovered. In the control columns containing no ligand (Fig. 1b), 95% of the added cells remained unbound, and no bacteria were eluted with 0.5 M Gal.

To investigate the potential role of calcium in mediating the attachment of F. nucleatum FN-2 to asialofetuin, we examined the effects of adding divalent cation chelators to our affinity chromatography system. When either 1 mM EDTA or 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) was added to the 0.2% BSA-PBS buffer before the addition of cells to the affinity column, the percentages of cells requiring elution by Gal (specific binding) were significantly reduced, i.e., by 91 and 95%, respectively (P < 0.001, Student’s t test). On the other hand, when 10 mM Ca²⁺ was also added to the buffer containing 1 mM EDTA or 1 mM EGTA, the percentages of cells requiring Gal for elution returned to near control levels (93%). This reversal of inhibition could not be duplicated by the addition of 1 mM Mg²⁺ or 1 mM Mn²⁺.

Effects of Gal concentration on binding of F. nucleatum to asialofetuin-Sepharose 6MB. F. nucleatum FN-2 was preincubated and eluted from affinity columns of asialofetuin-Sepharose 6MB with several different concentrations of Gal (Fig. 2). As the concentration of Gal used to preincubate and elute the bacteria was increased, the numbers of bacteria that bound to the column decreased. A concentration of approximately 20 mM Gal was needed for 50% inhibition of binding of the bacteria to the affinity column (Fig. 2).

Saliva-mediated agglutination of F. nucleatum. Serial dilutions of saliva were added to microtiter plates containing F. nucleatum FN-2 in a checkerboard fashion to compare the agglutination titers for whole, ductal parotid, and ductal submandibular-sublingual saliva (Table 1). For all cell concentrations of F. nucleatum FN-2 tested, parotid saliva proved to be the best agglutinator. At the optimal bacterial cell concentrations (5 × 10⁶ cells per ml), parotid saliva agglutinated F. nucleatum FN-2 at titers 8-fold higher than submandibular-sublingual saliva and 16-fold higher than whole saliva. These findings confirm that there are compo-

![FIG. 1. Elution profile of binding of F. nucleatum FN-2 to Sepharose 6MB covalently coupled to asialofetuin (a) or to Sepharose 6MB alone (b). F. nucleatum (10⁷ cells) were added to the column at room temperature and incubated for 1 h. (a) The nonadherent fraction (17%) was eluted off the column with PBS and is represented by the first peak on the graph. The major peak represents bacteria specifically bound and eluted by 0.5 M Gal (81%). The remaining two peaks are nonspecifically bound fractions of cells removed by 0.1% NP-40 (NP40) (2%). (b) The nonadherent fraction running through the column with PBS represented 95% of cells added (control). In this case, no bacteria were eluted with 0.5 M Gal. The arrows indicate when the eluent was added.](http://iai.asm.org/)
TABLE 1. Agglutinating activity of human salivas with *F. nucleatum* FN-2

<table>
<thead>
<tr>
<th><em>F. nucleatum</em> (no. of cells/ml)</th>
<th>Whole saliva</th>
<th>Parotid saliva</th>
<th>Submandibular-sublingual saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 10⁸</td>
<td>64</td>
<td>1.024</td>
<td>128</td>
</tr>
<tr>
<td>5 x 10⁸</td>
<td>128</td>
<td>2.048</td>
<td>256</td>
</tr>
<tr>
<td>1 x 10⁹</td>
<td>128</td>
<td>1.024</td>
<td>128</td>
</tr>
</tbody>
</table>

* Titers represent the reciprocal dilution giving 50% agglutination. The protein concentrations were 0.70, 0.67, and 0.60 mg/ml for whole, parotid, and submandibular-sublingual saliva, respectively.

potency of inhibition (4 times that of Gal), with both the α- and β-derivatives of p-aminophenyl galactopyranosides demonstrating equal inhibition. When the substitutions on Gal were examined and compared, the p-aminophenyl galac-
topyranosides were 10-fold better inhibitors than the p-
nitrophenyl-β-D-galactopyranosides. In addition, inhibition
by N-acetyl-D-galactosamine was fourfold that of Gal, and
inhibition by lactose and phenyl-β-D-thiogalactoside was
twofold that of Gal.

F. nucleatum-mediated hemagglutination and its inhibition.

Previous studies have demonstrated that many *F. nucleatum*
strains have the ability to hemagglutinate rabbit asialo-RBC (7).
We conducted similar studies confirming this point (data
not shown). Therefore, we developed a hemagglutination
inhibition assay to investigate the specificity of the adhe-
sin(s) of *F. nucleatum* FN-2. It appears that asialo-RBC
are suitable receptor analogs for *F. nucleatum* FN-2 (7, 19)
and we wanted to compare the sensitivity and specificity of
hemagglutination versus salivary agglutination. Asialo-RBC
were added to microtiter plates containing *F. nucleatum*
FN-2 and the same serially diluted sugars as in the salivary
agglutination assay. The results of these experiments are
also shown in Table 2. Although the same hierarchy of
potency of inhibitors was noted, hemagglutination proved to
be less sensitive than salivary agglutination. The one excep-
tion was fucose, which was a good inhibitor of salivary
agglutination but had no effect on hemagglutination.

Binding of 125I-labeled asialofetuin to *F. nucleatum* electro-
botted onto nitrocellulose membrane. To identify the bacte-
rial component(s) involved in recognition of galactosyl resi-
dues, we separated proteins of *F. nucleatum* FN-2 by
SDS-PAGE, electroblotted them to nitrocellulose, and then
incubated the blots with 125I-asialofetuin in the presence and
absence of 0.5 M Gal. Figure 3 shows the stained gel (lane 1)
and developed autoradiographs (lanes 2 and 3). The dark
band in lane 2, which showed binding of 125I-asialofetuin to
strain FN-2, had an electrophoretic mobility similar to that
of the three high-molecular-weight bands at approximately
300,000, 310,000, and 330,000 seen on the stained gel of
strain FN-2 (lane 1). When the FN-2 nitrocellulose replica
was preincubated in 0.5 M Gal (lane 3), the band was not
seen. Similar findings were obtained when the blots were
preincubated with a 100-fold excess of asialofetuin (data not
shown). Results demonstrate that the binding of 125I-asialo-
fetuin to the 300,000 to 330,000 bands was inhibited by the
presence of excess Gal, suggesting that this high-molecular-
weight bacterial complex might represent the Gal-binding
adhesin(s) of *F. nucleatum* FN-2.

**DISCUSSION**

Dental diseases are initiated by the colonization of the
tooth surface by pathogenic bacteria (11). The study of
factors influencing the attachment and distribution of organ-
isms in the oral cavity is therefore of considerable impor-
tance. In vivo the adherence of bacteria to the tooth surface
invariably occurs via the salivary pellicle. Yet, to combat
adherence, saliva interacts directly with bacteria, facilitating
clearance by mastication, movement of the tongue and
cheeks, and swallowing (11). It is now recognized that the
oligosaccharide moieties of salivary glycoconjugates play a
pivotal role in oral clearance and adherence mechanisms
(18, 29). In many instances, interactions are mediated by sur-
face structures present on the bacteria, involving the formation
of specific protein-carbohydrate complexes (3–5, 17, 20–23,
28). The molecular mechanisms by which these interactions
take place have been the subject of our investigations.
We report here that *F. nucleatum* FN-2 possesses a surface-associated lectin with specificities for galactosyl residues. Galactosyl residues are commonly found as terminal sugars on salivary glycoconjugates and appear to be functional receptor sites for bacterial Gal-binding lectins. We used an RBC model system of adherence to characterize the lectin activity of *F. nucleatum* because RBC possess receptors that are similar, if not identical, to the receptors on the normal target cells (6-9, 14, 19). However, the apparent sensitivity of the salivary agglutination assay was 1.5 to 2 times greater than that of the hemagglutination assay (Table 2). When select sugars were used as inhibitors, a similar pattern was seen, except for fucose, which inhibited salivary agglutination but not hemagglutination. The reasons for this remain unclear, and the role of fucose as an inhibitor is currently being investigated in our laboratory. The results of inhibition studies suggest that the lectin responsible for binding *F. nucleatum* to human parotid saliva is complex in nature and is capable of discerning subtle differences in structure. Alternatively, it is possible that multiple lectins recognizing different sugar determinants are present on this organism.

It is of note that in addition to the Gal-containing derivatives, fucose, NeuAc, and sialyllactose [NeuAc(2-3) and (2,6)Gal(1,4)Glc (Glc, glucose)] also demonstrated inhibition (30, 15, and 5 mM, respectively). However, the sialylglycopeptides of fetuin and transferrin did not show inhibition at any concentration. Consequently, the conflicting data are difficult to interpret and the role of NeuAc as a receptor recognition site remains unclear. One speculation is that the internal Gal residues of more complex structures may sometimes be recognized. Another possibility is that *F. nucleatum* has two separate adhesins, one for NeuAc and one for Gal. Further studies are required to address these possibilities. The fact that galacturonic acid and glucuronic acid did not inhibit agglutination of parotid saliva demonstrates that the NeuAc effect is not due to charge. It is tempting to speculate that NeuAc, fucose, and Gal, in combinations of two or three, may have a synergistic effect in inhibiting FN-2-mediated agglutination of parotid saliva.

It must be considered that the presence of specific antibodies to *F. nucleatum* may contribute to the saliva-induced agglutination. However, previous studies suggested that a high-molecular-weight mucinous glycoprotein was responsible for the aggregating activity for oral *F. nucleatum* strains, and the contribution from specific antibodies was minimal and not inhibitable by Gal (7). Studies by Smoot and Falkler (27) demonstrated that absorption of immunoglobulin M and immunoglobulin A from saliva did not remove salivary agglutinating activity. Furthermore, we have shown that binding of 125I-asialofetuin to nitrocellulose replicas of electrophoretically separated salivary proteins is only to the proline-rich glycoprotein of human parotid saliva (26).

To identify the bacterial surface component involved in the interaction with galactosyl residues, we separated proteins of *F. nucleatum* FN-2 by SDS-PAGE, transblotted the gels, and incubated the resultant blots with 125I-asialofetuin. This procedure apparently allowed the renaturation of the lectinlike components that presumably mediate adherence to Gal residues. Results suggest that this high-molecular-weight bacterial complex (300,000 to 330,000) represents the Gal-binding protein(s) of *F. nucleatum* FN-2 (Fig. 3).

The biological role of this lectin and its interaction in vivo with salivary glycoconjugates is a matter of speculation. Galactosyl residues are common constituents of salivary glycoconjugates and may serve as possible receptor sites for *F. nucleatum*. Falkler et al. (6) indicated that mucinous glycoproteins may play a role in aggregating *F. nucleatum* in the oral cavity. Other investigators have suggested that the serum glycoproteins containing Gal may bind to the surface of *F. nucleatum* and that this binding may influence colonization in the gingival sulcus (27). Studies in our laboratory have demonstrated that *F. nucleatum* adheres to saliva-coated cementum in high numbers, and this attachment is not inhibitable by Gal or Gal-containing glycopeptides (D. G. Kern, J. R. Winkler, and P. A. Murray, J. Dent. Res. 66 (Special Issue):1155A, 1987 [manuscript in preparation]). On the other hand, *F. nucleatum* FN-2 specifically binds to a proline-rich glycoprotein in human parotid saliva via galactosyl residues (26). Collectively, these data implicate a role for this Gal-binding lectin of *F. nucleatum* in bacterial mediated clearance and suggest that adherence and clearance of *F. nucleatum* in the oral cavity occur by different mechanisms.

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