INFECTION AND IMMUNITY, Oct. 1980, p. 117-124
0019-9567/80/10-0117/08$02.00/0

Vol. 30, No. 1

# Further Characteristics of $\beta_2$-Microglobulin Binding to Oral Streptococci

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A total of 85 strains of oral bacteria representing *Streptococcus mutans*, *S. sanguis*, *S. mitior*, *S. salivarius*, *S. milleri*, *S. infrequens*, *S. durans*, *S. lactis*, *S. faecalis*, *S. faecium*, *S. equinus*, *Streptococcus* species group E, *Actinomyces*, and one group A *Streptococcus* were tested for binding of aggregated human $\beta_2$-microglobulin. Positive affinity between bacteria and aggregated human $\beta_2$-microglobulin was detected in 36% of the strains. No apparent correlation with bacterial species, serotype, or group was noted. No positive strains were detected among seven group I:A *S. sanguis* strains ($P < 0.01$). Binding constants for one *S. mutans* strain indicated heterogeneous binding structures on the bacterial surface. The number of binding sites for aggregates of human $\beta_2$-microglobulin involving multipoint attachment varied from 70 to 1,700 per bacterial cell. With whole saliva as buffer, a general increase in affinity was seen. Variations in salt concentrations of the buffers revealed different salt-dependent species-associated uptake patterns. Oral bacteria tended to have an uptake maximum at a salt concentration similar to that seen in saliva. Binding structures for aggregated $\beta_2$-microglobulin on oral streptococci were sensitive to pepsin, heat, and formaldehyde treatment. Bacterial binding structures for aggregated $\beta_2$-microglobulin might represent one of several factors of importance for bacterial attachment in the oral cavity. Experimental conditions reflecting the salivary milieu increased the degree of interaction, emphasizing the importance of physiological test systems for such studies.

Surfaces of the oral cavity are colonized by large numbers of bacteria of various species. A common and fundamental characteristic of these bacteria is their capacity to attach to host surface structures (10). Different mechanisms responsible for bacterial attachment in the mouth have been increasingly studied during the past few years. Considerable interest has been directed toward the effects of saliva on bacterial attachment. Secretory immunoglobulin A in saliva is, for example, capable of inhibiting the attachment of *S. sanguis* to oral epithelial cells (25). High-molecular-weight glycoproteins in saliva, termed agglutinins, are capable of aggregating bacteria (12) and could thereby possibly prevent bacterial attachment (26). The agglutinins may also be absorbed onto teeth in pellicle formation, providing receptors for oral bacteria and perhaps facilitating the formation of dental plaque (18, 19). Other substances may interact with bacteria or host surfaces and affect the ability of bacteria to colonize the surfaces of the oral cavity (11, 17).

Relatively little is known about host surface substances involved in bacterium-host interactions in the oral cavity. $\beta_2$-Microglobulin, the light chain of HL-A antigens, is present on oral epithelial cell surfaces and in saliva (for references, see reference 2) and might therefore be worth considering when approaching this problem. Interactions between several strains of oral streptococci and aggregated $\beta_2$-microglobulin have been described earlier (8). In the present investigation, we studied the occurrence of such receptors as well as the kinetics of the interactions and the physicochemical characteristics of the receptor structures on several species of oral bacteria with affinity for aggregated $\beta_2$-microglobulin.

## MATERIALS AND METHODS

**Bacterial strains.** A total of 85 strains of oral bacteria were selected for study: 22 strains of *Streptococcus mutans* (AHT and 3720 [serotype *a*], Fa-1 and BHT [serotype *b*], KPSK2, NCTC 10449, PK1, B21, P8, S8, At3, At6, and OMZ70 [serotype *c*], B13, OMZ176, OIHI, ME1, and MX [serotype *d*], LM7 and P4 [serotype *e*], OMZ175 [serotype *f*], and OMZ65 [serotype *g*]); 22 strains of *S. sanguis* (OPA1, HPA1, MPD1, MPG1, LPA1, KTD1, and MPB1 [group I:A] [5], KPB1, MPC1, ATCC 10556, 804, 903, MPH1, KPE2, PPE21, OPC1, OSH1, HPC1, PPF1, and GVE1 [group I:B] [5], G26-R, and G26-S [24]); 11 strains of *S. mitior* (ATCC 9811, Nt62, LV61, and KPD1 [group V:A] [5], Nt63, Nt61, Mt61, and Pt51 [group V:B]

[5], Nv71 and Hv81 [group IV] [5] and CHT [15]); 11 strains of *S. salivarius* (PVS2, GSS2, MVS1, KTS1, KPS1, NCTC 8618, ATCC 9759, HTS1, MPS1, MSS2, and HHT [group III] [5] [15]); and 5 strains of *S. milleri* (NCTC 10708, ATCC 9895, MCP1, MCP9, and 1624 [freshly isolated from a brain abscess]). Seven other *Streptococcus* species were also used: Lancefield's group E strains NCTC 5385, NCTC 5968, 012, 073, 074, 0232, and 0239. In addition, we studied *S. infrequens* strain NCTC 4678, *Streptococcus durans* strain NCDO 596, *S. lactis* strain NCTC 6681, *S. faecalis* strain NCTC 775, *S. faecium* strain NCTC 7171, *S. equinus* strain ATCC 9812, and *Actinomyces* strain T6. The strains were selected from the culture collection of the Department of Cariology, University of Lund, Malmö, and from a fresh isolate from the Department of Medical Microbiology, University of Lund, Lund, Sweden. The origins of the strains have been described earlier (4–6, 15, 24).

Bacterial strains were kept on blood agar plates and transferred to tubes containing 10 ml of Todd-Hewitt broth. After incubation in anaerobic jars overnight the bacteria were washed twice in phosphate-buffered saline (0.12 M NaCl–0.03 M phosphate, pH 7.2; PBS) containing 0.02% $NaN_3$ and 0.05% Tween 20 (PBSAT) and resuspended in the same buffer to a concentration of $10^{10}$ bacteria per ml as determined by measuring the optical density at 530 nm in a Beckman colorimeter model CP-1. A standard curve was obtained through direct counting of bacterial cells in a Petroff-Hausser chamber. In some experiments, alternative buffers were used as indicated.

**Protein preparations.** Human $\beta_2$-microglobulin and $\alpha_1$-microglobulin were purified as previously described (3, 7). Proteins were radiolabeled with $^{125}I$ by the chloramine-T method (13) and aggregated with glutaric dialdehyde according to Avrameas (1) as earlier described (8, 16). Aggregates with a molecular weight of approximately 280,000 were used as determined from gel filtration on a Sephadex G-200 column.

**Binding assays.** To 25 μl (0.3 μg) of radiolabeled aggregated protein solution, 200 μl of bacterial suspension was added. After incubation for 1 h at room temperature, the suspension was washed with 2 ml of PBSAT and centrifuged for 10 min at 2,000 × *g*, and the radioactivity in the pellet was measured in a LKB-Wallac 1270 Rackgamma gamma counter (AB Biotec, Stockholm, Sweden). The radioactivity in the pellet was expressed as percentage of the total radioactivity added.

**Calculation of binding constant and number of receptors.** Radiolabeled, aggregated $\beta_2$-microglobulin with an apparent molecular weight of 280,000 was diluted serially in PBSAT. To 100 μl of the different dilutions of aggregated $\beta_2$-microglobulin, 200-μl bacterial suspensions of *S. mutans* ME1 were added. The suspensions were incubated, washed, and spun down as described above. Binding constants and the approximate number of binding structures on each bacterium were calculated according to Scatchard (22).

**Influence of pH changes in the buffer environment.** Buffers with different pH values were prepared by adjusting 0.15 M acetate and 0.15 M primary phosphate containing 0.05% Tween 20 with 5 N NaOH. The uptake of aggregated $\beta_2$-microglobulin to two strains of oral streptococci was tested at different pH values from 3.0 to 8.0. Then 250-μl samples of the bacterial suspensions were washed, spun down, and resuspended in the respective buffers. For uptake assays as described above, 200-μl samples of the bacterial suspensions were assayed using the respective buffers throughout. *Staphylococcus albus* strain L603 (heat killed) and tubes containing no bacteria served as negative controls.

**Saliva as buffer.** To investigate the possible effects of saliva on the uptake of aggregated $\beta_2$-microglobulin to oral streptococci, 15 different strains were tested in binding assays as described above. Freshly collected paraffin-stimulated whole saliva from five persons was centrifuged to remove debris, pooled, and used as the buffer throughout the assay. From conductivity measurement of saliva samples, a buffer with the same conductivity was prepared. With this 0.066 M PBS buffer containing 0.05% Tween 20 used throughout the assay, 77 strains of bacteria were tested for binding of human aggregated $\beta_2$-microglobulin.

**Influence of changes in ionic strength.** Series of buffers with different ionic strengths were prepared in order to investigate the effect of different salt concentrations on the binding of aggregated $\beta_2$-microglobulin to oral streptococci as compared with one group A *Streptococcus* strain, AR-1, and *Staphylococcus aureus* Cowan I. To 0.01 M phosphate buffer containing 0.05% Tween 20, various amounts of NaCl were added, giving buffers with ionic strengths from 0.015 to 0.48 M. Twelve strains of bacteria were included in uptake assays as described above, using the respective buffers throughout the assay. Tubes containing no bacteria served as negative controls.

**Fibrinogen inhibition studies.** To 200-μl suspensions of four different oral streptococci, various amounts of fibrinogen (AB Kabi, Stockholm, Sweden) were added to study the possible inhibition of bacterial binding of aggregated $\beta_2$-microglobulin. The suspensions were incubated at room temperature for 30 min and washed in 2 ml of PBSAT. Bacteria were resuspended in 200 μl of PBSAT and used for uptake assays as described above.

**Pepsin digestion.** Pellets from 250-μl bacterial suspensions of three strains of oral streptococci were suspended in 0.1 M acetate buffer (pH 4.5) containing various amounts of pepsin (Sigma Chemical Co., St. Louis, Mo.; N:o P-7012, lot. 26C-8045) and incubated in a water bath for 1 h at 37°C. Pepsin digestion was stopped by the addition of 50 μl of 1 M tris-(hydroxymethyl)aminomethane buffer at room temperature. The bacterial suspensions were spun down, washed in 2 ml of PBSAT, and resuspended in 250 μl of PBSAT. For uptake assays as described above, 200-μl samples of the suspensions were used.

**Formaldehyde treatment.** Washed pellets of 250-μl bacterial suspensions of two strains of oral streptococci were resuspended in 2 ml of PBSAT containing formaldehyde concentrations of 0 to 0.5%. The suspensions were incubated at room temperature for 30 min, washed, and resuspended in PBSAT. For uptake assays as described above, 200-μl samples of the suspensions were used.

**Heat treatment.** The possible heat sensitivity of the bacterial binding structures for aggregated $\beta_2$-mi-

croglobulin was investigated. For this assay, 400-μl bacterial suspensions of *S. mutans* B13 and ME1 and *S. sanguis* MPC1 were incubated at different temperatures in a water bath for 10 min. For uptake assays as described above, 200-μl samples of the bacterial suspensions were used.

## RESULTS

**Binding of aggregated $\beta_2$-microglobulin to oral bacteria.** A total of 84 strains of oral streptococci were tested for binding of human aggregated $\beta_2$-microglobulin (Fig. 1). Protein uptake varied from 1.5 to 31% of the added protein, with 36% of the strains showing an uptake of aggregated $\beta_2$-microglobulin of more than 4%. In Fig. 1, a cluster of negative strains is seen around the 2.5% uptake level, clearly separated from the positive strains with an uptake of over 4%. When comparing levels of binding among different groups or serotypes of bacteria, consistent binding was seen within certain serotypes and groups in repeated assays. All strains of *S. mutans* serotypes *d*, *f*, and *g* showed positive binding, whereas all strains of serotypes *b* and *e* were negative. Both positive and negative strains were seen within serotypes *a* and *c*. *S. mitior* group V:A and IV strains were all negative; in group V:B, one of four strains was positive. Two of 11 *S. salivarius* strains tested were positive, as were 3 of 5 *S. milleri* strains. The one strain of *Actinomyces* showed a positive binding with a 14% uptake. Within *S. sanguis* group I:A, all seven strains were negative in contrast to group I:B, which showed both positive and negative strains. With so few strains of each serotype and group tested, a statistically significant correlation between group and $\beta_2$-microglobulin binding was only seen in a negative direction for *S. sanguis* group I:A ($P < 0.01$). Other species of streptococci tested were nonhomogeneous in binding of the protein.

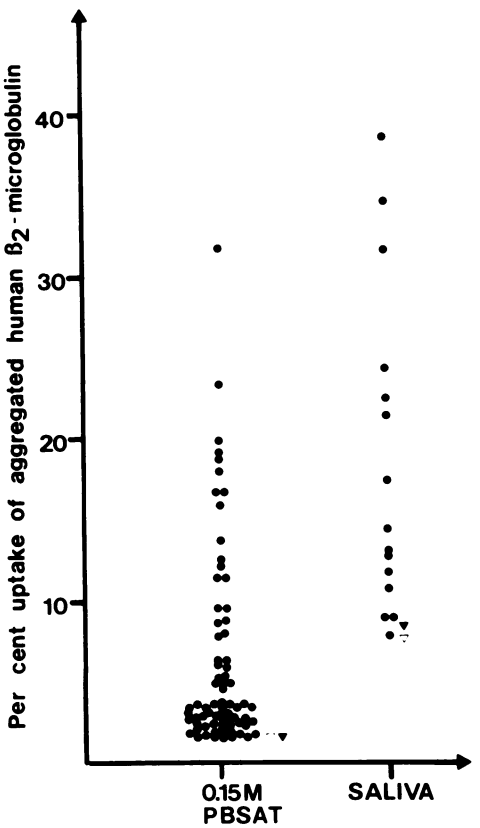


FIG. 1. *Uptake of labeled aggregated $\beta_2$-microglobulin to oral streptococci in PBS and in saliva. Symbols: ▽, empty tube; ▼, L603.*

The monomeric form of human $\beta_2$-microglobulin was studied in control experiments. Radiolabeled monomeric $\beta_2$-microglobulin was tested for affinity to seven strains of oral streptococci, using 0.066 M PBS containing 0.05% Tween 20 as the buffer throughout the assay. Four strains of oral streptococci were also tested for affinity for the protein in a pH 5.6 acetate buffer (0.15 M) containing 0.05% Tween 20. None of the oral streptococci tested showed affinity for the protein, not even at the low pH. Previous studies have shown that monomeric $\beta_2$-microglobulin does not interfere with binding of the aggregated form of the protein (8, 16). Eight strains of oral streptococci, both positive and negative in affinity for aggregated $\beta_2$-microglobulin, were tested for affinity for aggregated human $\alpha_1$-microglobulin to investigate the possible effects of aggregation of proteins. In this control experiment, none of the strains showed affinity for aggregated human $\alpha_1$-microglobulin.

**Determination of binding constant and binding capacity for aggregated human $\beta_2$-microglobulin.** *S. mutans* ME1 was tested for binding of varying amounts of aggregated $\beta_2$-microglobulin, using a preparation with an apparent molecular weight of 280,000 achieved by rechromatography on a Sephadex G-200 column. Bound (*b*) and free (*f*) fractions of radiolabeled protein were determined as described by Scatchard (22), using the relationship $b/f = nK - bK$ (Fig. 2). The regression curve in Fig. 2 is divided into two linear regression lines, a and b, suggesting a heterogeneous receptor on bacteria for the protein. The slope of the regression line a ($r^2 = 0.88$) indicates one type of receptor on bacteria with a high affinity but with a very low capacity. The slope of line b ($r^2 = 0.78$) indicates a receptor with lower affinity but higher capacity. The interceptions of these lines with the *x*-axis give the value *n*, the amount of aggregated $\beta_2$-microglobulin bound at saturation. Calcula-

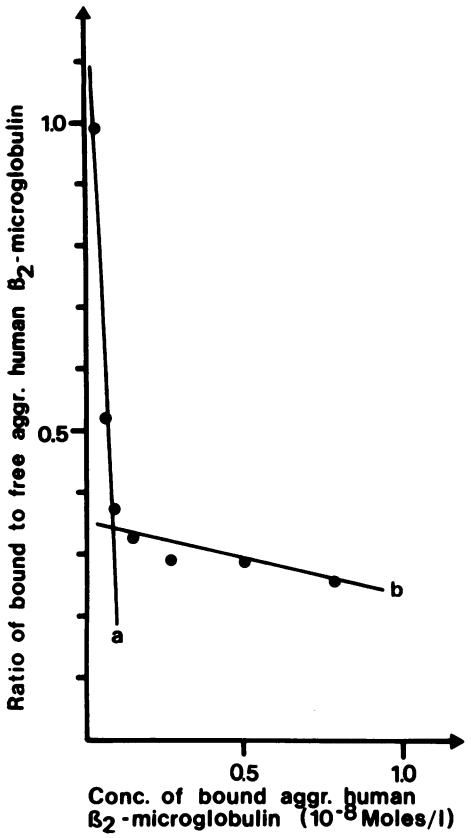


FIG. 2. *Scatchard plots of binding experiments performed with varying concentrations of labeled aggregated $\beta_2$-microglobulin. The S. mutans strain ME1 was studied.*

tion of binding constants for the two receptors according to the formula above, using a molecular weight of 280,000, gives apparent binding constants ($K$) of $11 \times 10^8$ liters/mol for the high-affinity receptor and $0.12 \times 10^8$ liters/mol for the low-affinity receptor. The binding capacities were calculated to $0.12 \times 10^{-8}$ mol/liter for the high-affinity receptor and to $2.9 \times 10^{-8}$ mol/liter for the low-affinity receptor. This gives a maximum binding capacity of 70 aggregates of $\beta_2$-microglobulin with a mean molecular weight of 280,000 on each bacterium to the high-affinity receptor and of 1,700 aggregates to the low-affinity receptor. Because of the multivalent nature of aggregated $\beta_2$-microglobulin, the calculated constants should be used only for comparative purposes and not taken as absolute figures.

**Effect of changes of hydrogen ion concentration on the binding of aggregated $\beta_2$-microglobulin to oral bacteria.** Two strains of oral streptococci were suspended in buffers with different pH values and tested for affinity to aggregated human $\beta_2$-microglobulin. In Fig. 3, the effect of pH changes on the affinity of aggregated $\beta_2$-microglobulin for oral streptococci is presented. Generally, a decrease of pH below pH 6 gave an increase of affinity for both positive and control strains. The $\beta_2$-microglobulin-positive strains of oral streptococci seemed to increase in affinity faster than did the control strain, *Staphylococcus albus* L603, when pH was decreased. With an increase of pH over 6, *S. sanguis* MPC1 showed a slow increase in affinity for the protein. The buffers used in the systems were changed between pH 5.75 and 6.0 from 0.15 M acetate buffer to 0.15 M phosphate buffer, both containing 0.05% Tween 20. At pH 5.5, which represents a hydrogen ion concentration not uncommon in the oral cavity, a clear discrepancy in affinity for aggregated $\beta_2$-microglobulin was seen between the control strain and the positive oral streptococci. At this pH there was also a high background affinity for the plastic tubes.

**Effect of saliva as buffer on the uptake of normal human aggregated $\beta_2$-microglobulin.** Fifteen strains of oral streptococci were tested for affinity to aggregated $\beta_2$-microglobulin, using saliva as the buffer throughout the

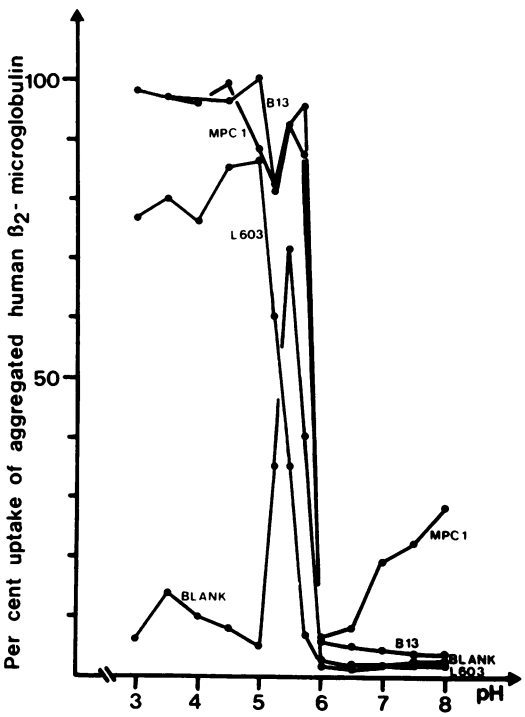


FIG. 3. *Effects of pH variation in buffers on the uptake of labeled aggregated $\beta_2$-microglobulin to Streptococcus mutans B13 and Streptococcus sanguis MPC1. Negative controls were Staphylococcus albus L603 and empty tubes.*

assay (Fig. 1). A general increase in affinity was seen in this system. As compared with a positive control strain, group A *Streptococcus* strain AR-1, the general increase of affinity between oral streptococci and the protein was 2.2 times the increase of AR-1 binding when compared with the uptake in 0.066 M PBS with 0.05% Tween 20 (see below).

**Effect of ionic strength on the uptake of aggregated $\beta_2$-microglobulin.** A total of 77 bacterial strains were tested for affinity to aggregated normal human $\beta_2$-microglobulin, using 0.066 M PBS containing 0.05% Tween 20 as the buffer throughout the assay. The salt concentration in this buffer is similar to that in saliva. Compared with the binding of the protein in a 0.15 M PBS buffer with Tween 20 to the same bacteria, a general increase of affinity was noted. A clustering of negative strains was seen around the 2.5% uptake level. Compared with the affinity seen in 0.15 M PBS, the correlations between species, serotypes, or groups were similar with two notable exceptions. All strains of *S. milleri* showed positive binding in 0.066 M PBS, but only three of five strains did so in 0.15 M PBS. Similar effects were seen for the *S. salivarius* strains: only 2 of 11 strains were positive in 0.15 M PBS, whereas 9 of 10 strains became positive in 0.066 M PBS. Twelve strains of bacteria were tested for affinity to aggregated $\beta_2$-microglobulin in buffers with ionic strength ranging from 0.015 to 0.48 M. The oral streptococci had, in general, a maximum uptake at a lower salt concentration than did the group A *Streptococcus* strain AR-1 (Fig. 4). At least four patterns of salt concentration dependence were seen in Fig. 4: (i) the positive strains *S. sanguis* MPC1 and PPE21 and *S. milleri* MCP9 and 9895 showed an uptake maximum at a salt concentration close to 0.03 M, which decreased when more salt was added in the buffers; (ii) the positive strains *S. mutans* B13 and ME1 showed an uptake maximum close to 0.08 M; (iii) the negative strains *S. mutans* LM7 and *S. sanguis* OPA1 showed an uptake of protein only at 0.015 M, and when salt was added no uptake was seen; and (iv) the $\beta_2$-microglobulin-positive group A *Streptococcus* strain AR-1 showed an uptake maximum at 0.15 M. *S. milleri* strain MCP1 showed an uptake maximum at 0.015 M but did not lose affinity as quickly as negative strains when more salt was added. The two negative strains *Staphylococcus aureus* Cowan I and *S. albus* L603 did not show any uptake of added protein except for a small increase at 0.48 M.

**Inhibition studies using fibrinogen pretreatment.** Four positive strains of oral streptococci were pretreated with fibrinogen before uptake assays with aggregated normal human $\beta_2$-microglobulin to determine whether the receptor on oral streptococci could be blocked with fibrinogen similar to the receptor on group A, C, and G streptococci (16). No effect of pretreatment with fibrinogen was seen on the binding of

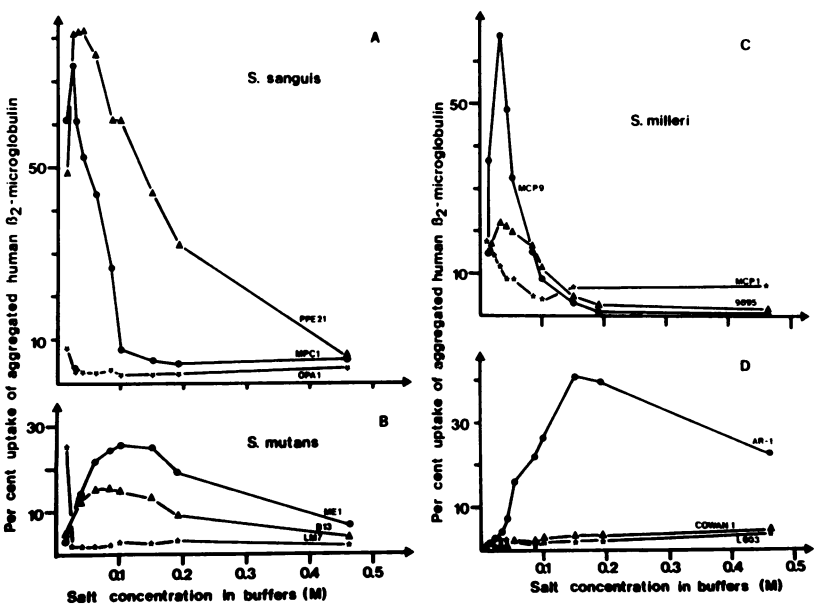


FIG. 4. *Effects of variation of salt concentration in buffers on the uptake of labeled aggregated $\beta_2$-microglobulin to oral streptococci (A–C) to a positive control strain AR-1 (a group A Streptococcus), and to negative controls Staphylococcus aureus Cowan I and Staphylococcus albus L603 (D).*

aggregated $\beta_2$-microglobulin to oral streptococci.

**Effects of pepsin digestion on $\beta_2$-microglobulin binding.** *S. mutans* B13 and *S. sanguis* MPC1 and PPE21 were digested with different amounts of pepsin and then tested for binding of aggregated $\beta_2$-microglobulin in 0.15 M PBSAT (Fig. 5A). The receptor for aggregated $\beta_2$-microglobulin on all strains was pepsin sensitive. Almost all reactivity was lost for *S. mutans* B13 and *S. sanguis* MCP1 at pepsin concentrations of 14 and 1.7 μg/ml, respectively, in the buffer.

**Effect of formaldehyde treatment on $\beta_2$-microglobulin binding.** *S. mutans* B13 and *S.*

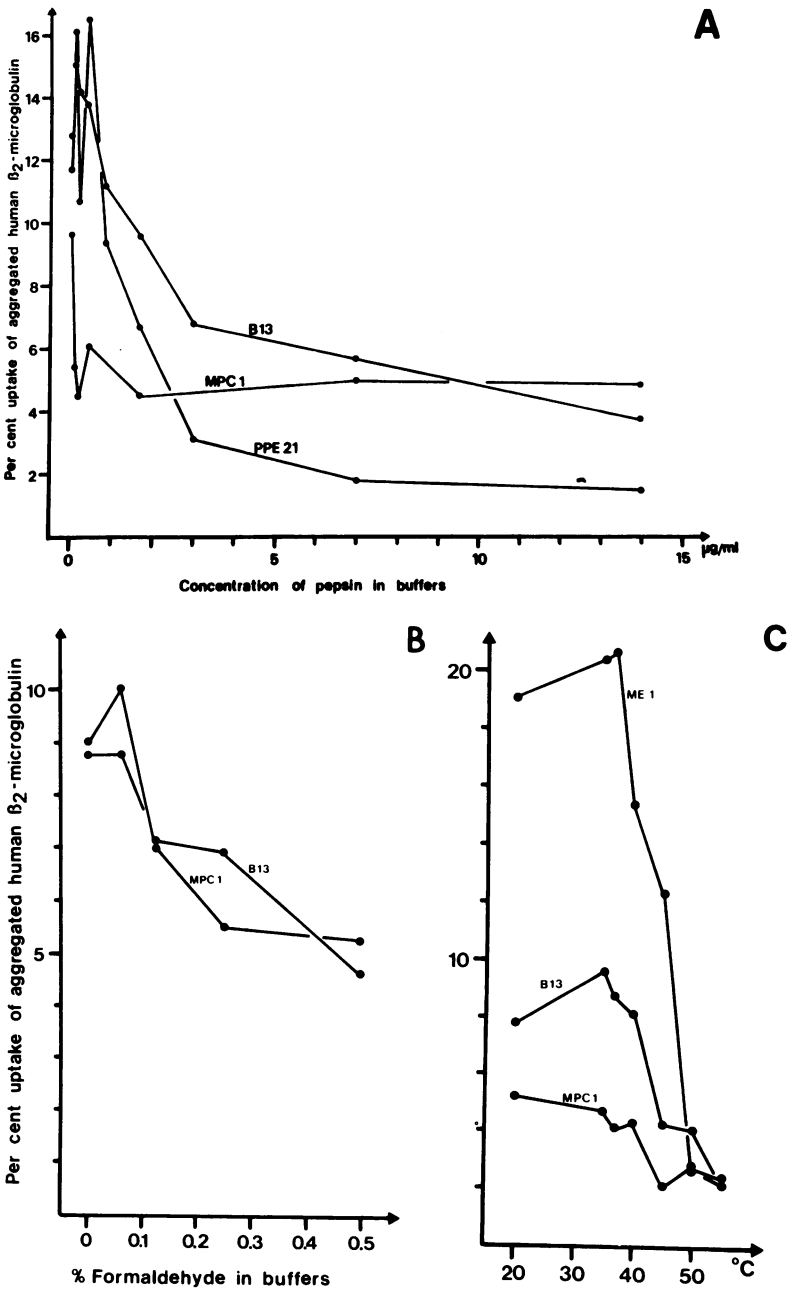


FIG. 5. *Effects of pepsin digestion (A), formaldehyde treatment (B), and heat treatment (C) on the uptake of labeled aggregated $\beta_2$-microglobulin to oral streptococci.*

*sanguis* MPC1 were suspended in PBSAT containing formaldehyde from 0 to 0.5%. Both strains showed a decreased affinity for normal aggregated human $\beta_2$-microglobulin after treatment with formaldehyde. At a concentration of 0.5% in the buffers, the levels of reactivity were close to negative control values (Fig. 5B).

**Effect of heat treatment on $\beta_2$-microglobulin binding.** *S. mutans* B13 and ME1 and *S. sanguis* MPC1 were heat treated before uptake assays for aggregated $\beta_2$-microglobulin. The receptors for the protein on all strains were extremely temperature sensitive. After treatment at 55°C for 10 min, the affinity of all strains for aggregated $\beta_2$-microglobulin was totally lost (Fig. 5C).

## DISCUSSION

Aggregated $\beta_2$-microglobulin has shown binding to strains of oral streptococci in previous studies (8). In this study, a larger number of oral streptococci were tested for binding of aggregated $\beta_2$-microglobulin under different buffer conditions with special consideration of possibly important factors in the oral cavity. Bacterial attachment in the oral cavity depends on a wide variety of interactions (9, 10). The importance of any single factor in this complex system of interactions is difficult to evaluate. As compared with serum and other body fluids, saliva is considerably different in its composition and provides an extraordinary environment for bacteria. For example, there is a lower total protein content, hypotonicity, and variations of pH (23). Also, direct and indirect dietary effects on the saliva add factors to the environment of oral bacteria.

When studying binding of aggregated $\beta_2$-microglobulin in 0.15 M PBSAT, 36% of the oral bacterial strains showed affinity for the protein. When comparing groups or serotypes of oral bacteria with binding of aggregated $\beta_2$-microglobulin, no correlations were seen. When saliva rather than 0.15 M PBS was used as the buffer, in assays for uptake of aggregated $\beta_2$-microglobulin, an increase of binding between protein and bacteria was seen (Fig. 1). Comparing oral bacteria and the $\beta_2$-microglobulin-positive group A *Streptococcus* strain AR-1, the increase in affinity of oral streptococci was more than twice the increase for strain AR-1. This phenomenon can be caused by several factors in saliva such as pH, salt concentration, and salivary proteins.

A change in salt concentration of the buffers used affected binding characteristics of different strains in different ways (Fig. 4). Oral streptococci had distinct uptake maxima of aggregated $\beta_2$-microglobulin at low salt concentrations, and the group A *Streptococcus* strain had a maximum at a higher salt concentration. The maxima for the oral bacteria were close to the salt concentration of saliva, and the maximum of the group A *Streptococcus* strain was close to the salt concentration in serum. Also, there seemed to be a strict species-correlated salt-dependent uptake pattern for the positive strains. Strains of *S. salivarius* all showed a high uptake of aggregated $\beta_2$-microglobulin in 0.066 M PBS, whereas only 2 of the 11 strains were positive in 0.15 M PBS. The different salt concentration-dependent uptake patterns of the oral and the group A streptococci might reflect their choice of natural habitats.

Wide pH variations are not uncommon in saliva, and in dental plaque the pH can be less than 5, particularly after sucrose intake (14). In the test systems presented above, an increase in hydrogen ion concentration below pH 6 in the buffers used significantly increased the affinity between aggregated $\beta_2$-microglobulin and the bacteria tested (Fig. 3). Native $\beta_2$-microglobulin has an isoelectric point at pH 5.4 to 5.7 (21). Below this pH the protein has a positive net charge, whereas most oral bacteria have negative net surface charges (20). Electrostatic forces might, therefore, explain the high nonspecific affinity at low pH. At a pH above the isoelectric point of the protein, affinity between bacteria and protein is dependent upon specific structures with receptor-like characteristics on bacterial surfaces.

Receptors for aggregated human $\beta_2$-microglobulin were first detected in group A, C, and G streptococci (16). These receptors are relatively heat stable in contrast to the extreme heat sensitivity seen for receptors on oral streptococci. The receptors on oral streptococci were sensitive to pepsin and formaldehyde treatment, indicating a protein component in the receptor structure. The difference in salt concentration-dependent uptake of aggregated human $\beta_2$-microglobulin and the earlier described difference in trypsin digestion sensitivity (8, 16) underline major structural differences between receptors for aggregated $\beta_2$-microglobulin in oral streptococci and in group A, C, and G streptococci.

Determination of binding constants between *S. mutans* strain ME1 and aggregated $\beta_2$-microglobulin at pH 7.2 revealed binding structures on bacteria with heterogeneous affinity for the protein. The apparent number of binding structures on each bacterium was low for both the high- and the low-affinity types of receptors. Monomeric $\beta_2$-microglobulin did not bind to oral bacteria in the test system. Group A, C, and G streptococci are highly dependent upon the size of aggregates of $\beta_2$-microglobulin for binding of the protein. Aggregates of a molecular weight of

less than 100,000 show only slight affinity for bacteria (16). Aggregates of human $\alpha_1$-microglobulin did not show affinity for oral bacteria in control experiments, indicating that the binding of aggregated $\beta_2$-microglobulin is of a specific character. It is clear from these data that each apparent receptor structure is a measure of a strong synergistic effect obtained through multipoint attachment. When aggregates of the protein are used in binding studies, the primary low-affinity receptors on bacteria together mimic a high-affinity receptor.

The dependency of multipoint attachment for binding of $\beta_2$-microglobulin is of interest considering possible functions in vivo. $\beta_2$-Microglobulin is present on the cell membranes of all nucleated cells, including the epithelial cells of the oral cavity. The arrangement of $\beta_2$-microglobulin on the cell membrane would strongly favor multiple binding, thereby minimizing the effects of soluble $\beta_2$-microglobulin in saliva. This aspect would indicate biological significance of bacterial receptors for $\beta_2$-microglobulin arranged in a macromolecular way on epithelial cell surfaces.

## LITERATURE CITED


1. **Avrameas, S.** 1969. Coupling of enzymes to proteins with glutaraldehyde. Use of conjugates for the detection of antigens and antibodies. Immunochemistry **6**:43–52.
2. **Berggård, B., L. Björck, R. Cigén, and L. Lögdberg.** 1980. $\beta_2$-Microglobulin. Scand. J. Clin. Lab. Invest. **140**: Suppl. 154.
3. **Berggård, I., and A. G. Bearn.** 1968. Isolation and properties of a low molecular weight $\beta_2$-microglobulin occurring in human biological fluids. J. Biol. Chem. **243**:4095–4103.
4. **Bratthall, D.** 1972. Immunofluorescent identification of *Streptococcus mutans*. Odontol. Revy **23**:181–196.
5. **Carlsson, J.** 1967. Presence of various types of non-haemolytic streptococci in dental plaque and in other sites of the oral cavity in man. Odontol. Revy **18**:55–74.
6. **Carlsson, J.** 1968. A numerical taxonomic study of human oral streptococci. Odontol. Revy **19**:137–160.
7. **Ekström, B., and I. Berggård.** 1977. Human $\alpha_1$-microglobulin: purification procedure, chemical and physicochemical properties. J. Biol. Chem. **252**:8048–8057.
8. **Ericson, D., D. Bratthall, L. Björck, E. Myhre, and G. Kronvall.** 1979. Interactions between human serum proteins and oral streptococci reveal occurrence of receptors for aggregated $\beta_2$-microglobulin. Infect. Immun. **25**:279–283.
9. **Gibbons, R. J., and J. van Houte.** 1971. Selective bacterial adherence to oral epithelial surfaces and its role as an ecological determinant. Infect. Immun. **3**:567–573.
10. **Gibbons, R. J., and J. van Houte.** 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. **29**:19–44.
11. **Gibbons, R. J., J. van Houte, and W. F. Liljemark.** 1972. Parameters that effect the adherence of *Streptococcus salivarius* to oral epithelial surfaces. J. Dent. Res. **51**:424–435.
12. **Hay, D. J., R. J. Gibbons, and D. M. Spinell.** 1971. Characteristics of some high molecular weight constituents with bacterial aggregating activity from whole saliva and dental plaque. Caries Res. **5**:111–123.
13. **Hunter, W. M., and F. C. Greenwood.** 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (London) **194**:495–496.
14. **Imfeld, Th.** 1977. Evaluation of the cariogenicity of confectionary by intra-oral wire-telemetry. Schweiz. Monatsschr. Zahnheilkd. **87**:437–464.
15. **Jablon, J. M., and D. D. Zinner.** 1966. Differentiation of cariogenic streptococci by fluorescent antibody. J. Bacteriol. **92**:1590–1596.
16. **Kronvall, G., E. B. Myhre, L. Björck, and I. Berggård.** 1978. Binding of aggregated human $\beta_2$-microglobulin to surface protein structures in group A, C, and G streptococci. Infect. Immun. **22**:136–142.
17. **Liljemark, W. F., S. V. Schauer, and C. G. Bloomquist.** 1978. Compounds which affect the adherence of *Streptococcus sanguis* and *Streptococcus mutans* to hydroxyapatite. J. Dent. Res. **57**:373–379.
18. **Magnusson, I., and Th. Ericson.** 1976. Effect of salivary agglutinins on reactions between hydroxyapatite and a serotype c strain of *Streptococcus mutans*. Caries Res. **10**:273–286.
19. **Magnusson, I., Th. Ericson, and K. Pruitt.** 1976. Effect of salivary agglutinins on bacterial colonization of tooth surfaces. Caries Res. **10**:113–122.
20. **Olsson, J., and P.-O. Glantz.** 1977. Effect of pH and counter ions on the zeta-potential of oral streptococci. Arch. Oral Biol. **22**:461–466.
21. **Poulik, M. D.** 1976. $\beta_2$-microglobulin: present status, p. 155. *In* G. A. Jamieson and T. J. Greenwalt (ed.), Trace components of plasma: isolation and clinical significance, vol. 5. Alan R. Liss, New York.
22. **Scatchard, G.** 1949. The attraction of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. **51**:660–672.
23. **Shannon, I. L., R. P. Suddick, and F. J. Dowd, Jr.** 1974. Saliva: composition and secretion. Monogr. Oral Sci. **2**:3–42.
24. **Westergren, G.** 1978. Transformation of *Streptococcus sanguis* to a rough colonial morphology with an increased ability to adhere. Arch. Oral Biol. **23**:887–891.
25. **Williams, R., and R. J. Gibbons.** 1972. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. Science **177**:697–699.
26. **Williams, R. C., and R. J. Gibbons.** 1975. Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. Infect. Immun. **11**:711–718.