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Model Delineating the Effects of a Salivary Pellicle on the Adsorption of *Streptococcus miteor* onto Hydroxyapatite

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A model describing the adsorption isotherms for *Streptococcus miteor* strain 26 to untreated and saliva-treated hydroxyapatite was developed. The strengths of the adsorption bonds in the two systems were similar; however, the presence of selectively adsorbed salivary components increased the number of available binding sites fourfold.

Teeth are covered by an acquired pellicle that is formed by a selective adsorption of salivary components, at least in its early stages of development (5, 9). The presence of adsorbed salivary material on enamel or hydroxyapatite (HA) surfaces has been shown to alter the selectivity of bacterial sorption to such surfaces. In the case of strains of *Streptococcus miteor*, a numerically prominent organism in human dental plaque (3), the number of buffer-suspended cells that sorb to saliva-treated HA surfaces is increased compared to untreated HA (1, 4, 7). However, it is not known if the adsorbed salivary components promote a stronger adsorption bond between the organism and the surface or whether they provide an increased number of receptor or binding sites for this species. To distinguish between these possibilities, we adopted an adsorption model derived from the following considerations. The model assumes that there are a finite number of identical sites for attachment per unit surface area (or per unit weight) of the HA adsorbent. It further assumes that the bacterial cells approach the surface without steric hindrance and that the adsorption process is reversible, up to and at the equilibrium state. Under such conditions, the rate at which streptococcal cells occupy adsorption sites, R_a, would be proportional to the concentration of free cells in suspension and proportional to the number of unoccupied binding sites on the HA adsorbent at any given time. For a fixed weight of HA, the following equation can be written:

$$R_a = K_1C(N - Q) \quad (1)$$

in which C is the concentration of free cells at any given time, and N and Q are the maximum number of receptor or binding sites on HA and the number of cells adsorbed on to the total mass (weight) of HA, respectively; the proportionality constant (K_1) is the specific rate constant for the adsorption process. Since the adsorption is considered reversible, some adsorbed cells will desorb in the process of reaching an equilibrium between free cells in the liquid phase and the cells sorbed onto the HA. Considering the rate constant for desorption (K_2), the rate of desorption (R_d) at any given time would be given by:

$$R_d = K_2Q \quad (2)$$

At equilibrium, R_a must be equal to R_d. Therefore, combining equations (1) and (2):

$$K_1C(N - Q) = K_2 Q \quad \text{or} \quad (3)$$

$$\frac{K_1CN}{Q} = K_2 + K_1C \quad \text{or} \quad (4)$$

$$\frac{C}{Q} = \frac{1}{KN} + \frac{C}{N} \quad (5)$$

In equation (5), it is apparent that C refers to the concentration of free cells at equilibrium, and the parameter K is defined as equal to K_1/K_2. Since this quotient is the ratio of the two rate constants for sorption and desorption, respectively, the higher the value of K, the longer the bacterial cell will remain on the adsorption site, i.e., the stronger the adsorption bond or sorption affinity the cell has for the absorbent. As indicated by equation 5 the units of K are those of a reciprocal concentration, i.e., milliliters per cell.

Equation (5) describes the model used in the present study. If the model describes adequately the adsorption of cells of *S. miteor* to the HA adsorbents studied, then a plot of the quantity C/Q versus C should result in a straight line, and the adsorption parameters K and N can be calculated from the slope and intercept of this line.

The model was experimentally tested in the following manner. *S. miteor* strain 26, an iso-

late of human dental plaque, has been described previously (1, 3). Twice-washed suspensions of the organism in 0.01 M phosphate-buffered saline (pH 7) were prepared from overnight Trypticase soy broth (BBL) cultures. The suspensions were vigorously forced back and forth through a 27-gauge syringe needle to break up streptococcal chains and then standardized by optical density at 550 nm. The number of bacteria in the standardized suspensions was calculated from a standard curve relating optical density to bacterial cell number as determined by direct microscopic count.

HA powder of large particle size was prepared according to a procedure previously described (6). Suspensions of the powder in phosphate-buffered saline were permitted to settle several times to remove "fines." Samples of the HA were treated with clarified whole human saliva as described previously (1), using a ratio of 2 ml of saliva per mg of HA. Samples of suspensions containing 5 mg of either untreated or saliva-treated HA were added to tubes, and the material was permitted to settle overnight. The supernatant liquors were then removed, and 2 ml of streptococcal cell suspension was added to each tube. The streptococcal concentrations tested covered the range of 2.5×10^7 to 2.8×10^8/ml; these were not corrected for negligible amounts of water incorporated in the HA. The 2-ml mixtures were incubated in a shaking water bath at 37°C for 1 h; previous experiments indicated that this time period was sufficient to reach an adsorption equilibrium. After incubation, the mixtures were permitted to stand for 10 min at room temperature to enable the HA and adsorbed streptococci to settle, and a 1-ml amount of each supernatant liquor containing unadsorbed organisms was removed. A 0.1-ml portion of 0.1 M ethylenediaminetetraacetic acid (pH 8) was added to each sample and to control bacterial suspensions incubated in the absence of HA. Experimental and control suspensions were permitted to stand overnight at room temperature to allow sufficient time for HA fines to dissolve. The suspensions were then mixed on a Vortex mixer, and the number of unadsorbed organisms was determined by optical density at 550 nm; the values were corrected for small decreases that occurred in the optical density of control suspensions. All experiments were performed in duplicate.

A graphic plot of the number of streptococcal cells that sorbed to the 5-mg samples of untreated or saliva-treated HA powder versus the streptococcal concentration initially available is illustrated in Fig. 1. As noted previ-

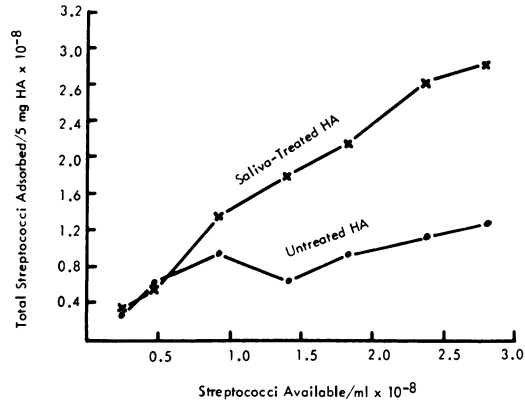

FIG. 1. *Adsorption of S. miteor strain 26 to untreated and to saliva-treated HA powder.*

ously (1, 4), a higher percentage of *S. miteor* cells adsorbed to saliva-treated HA than to untreated HA powder. Plots of equation 5 using the experimental data obtained are illustrated in Fig. 2; the lines drawn were calculated by the method of least squares. It is apparent from Fig. 2 that the model fits the experimental data to a satisfactory degree, having correlation coefficients of 0.95 for untreated HA powder and 0.92 for saliva-treated HA (Table 1). The values for the adsorption parameters K and N, calculated from the values of the slope and intercept of the straight lines, are shown in Table 1. The affinity constants for the two systems were similar; however, the number of adsorption sites for *S. miteor* strain 26 was found to be approximately fourfold greater on the saliva-treated HA powder.

It is evident, therefore, that the reason more cells of *S. miteor* sorb to saliva-treated HA surfaces than to untreated HA is because the layer of selectively adsorbed salivary components increases the number of binding sites available for this organism. However, the strength of the adsorption bonds is similar in both systems. The increase in the number of binding sites on saliva-treated HA indicates that *S. miteor* is interacting with salivary molecules adsorbed on the HA surface. Previous studies have suggested that the initial sorption of *S. mutans* cells to the teeth of rats (11) or to HA surfaces in vitro (W. Clark, R. J. Gibbons, and Z. Skobe, 52nd Annu. Meet. Int. Assoc. Dent. Res., abstr. 749, p. 242, 1974) appears to be reversible. The close agreement of the experimentally derived data with the mathematical model also suggests that the sorption of this organism to both untreated and saliva-treated HA was reversible, at least for the short time periods required to establish equilibrium.

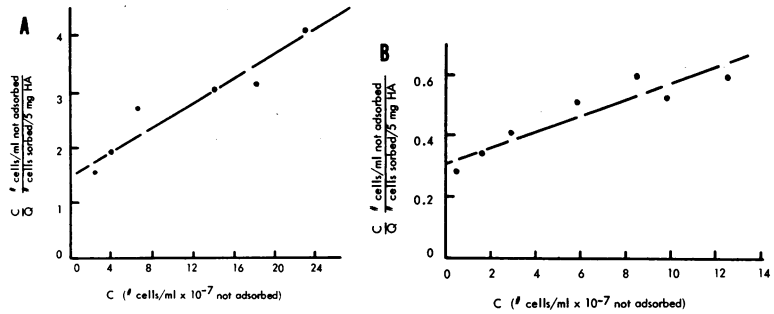

FIG. 2. *(A) Graphic plot of C/Q versus C for the adsorption of S. miteor strain 26 to untreated HA powder. (B) Graphic plot of C/Q versus C for the adsorption of S. miteor strain 26 to saliva-treated HA powder. See text for details for (A) and (B).*

TABLE 1. *Parameters for the adsorption of S. miteor strain 26 onto HA and saliva-treated HA*

Parameter	Untreated HA	Saliva-treated HA
N, Maximun no. of adsorption sites per 5 mg of HA	1.0×10^8	3.9×10^8
K, Affinity constant (ml/cell),	6.1×10^{-9}	8.3×10^{-9}
Correlation coefficient for data in Fig. 2	0.95	0.92

The model used essentially describes a Langmuir adsorption isotherm often used in studies of molecular adsorption. Previous investigators (2, 8; M. Kresak, E. C. Moreno, R. T. Zahradnik, and D. I. Hay, 54th Annu. Meet. Int. Assoc. Dent. Res., abstr. 116, p. 1390, 1976) have shown that this model frequently fits experimental data for the adsorption of macromolecules; it has also been reported as being applicable to the adsorption of bacteria onto clay used in water purification (10). The present investigation indicates that this model is also useful for studying the adsorption of bacteria to apatite surfaces mimicking teeth. The model provides a simple means for differentiating the adsorption affinities of various bacteria. It has the advantage that it permits distinctions to be made between the strength of the adsorption bond and the number of adsorption sites. Coating the HA with purified salivary components should enable a quantitative assessment to be made of the interactions of different bacterial species with the component. Limitations of the model are that it requires that a significant percentage of the bacterial cells initially available be adsorbed and that a constant amount of HA must be used. If the amount of HA is not constant, the data must be normalized with respect to the weights of HA used. Moreover, if different preparations of HA are used in comparative studies, the data must be normalized with respect to the surface area of each preparation.

In the present experiments, the bacteria were suspended in buffer, whereas in vivo, bacterial cells available for adsorbing to teeth are suspended in saliva. Components of saliva have been shown to bind to the surfaces of various bacteria and inhibit their sorption to similar or related molecules representing the binding sites on teeth (7; Clark et al., 52nd Annu. Meet. Int. Assoc. Dent. Res., abstr. 749, p. 242, 1974) or on epithelial cells (12). Consequently, these considerations must be taken into account before it is possible to relate the information obtained from an in vitro model to the in vivo situation.

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