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Albumin as a Blocking Agent in Studies of Streptococcal Adsorption to Experimental Salivary Pellicles

R. J. GIBBONS* AND I. ETHERDEN
Forsyth Dental Center, Boston, Massachussetts 02115

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A procedure involving blocking with bovine albumin was useful for differentiating between streptococcal interactions with components of experimental salivary pellicles similar to those which form on teeth and potential interactions with uncoated areas of mineral.

Dental caries and periodontal diseases are caused by bacterial accumulations called dental plaques, which develop on the teeth. Consequently, considerable effort has been devoted to studying the mechanisms by which bacteria attach to teeth and other surfaces of the mouth. Teeth are covered by a membranous film, termed the "acquired pellicle," which is thought to be formed by the selective adsorption of components from oral fluids to the hydroxyapatite (HA) mineral of enamel (5, 9). Components of saliva, crevicular fluid, and bacteria likely contribute to pellicle formation. The attachment of bacteria to the tooth surface, therefore, is thought to involve interactions between surface components of the microorganism and macromolecular constituents composing the pellicle (5, 9, 13). Although bacteria can adsorb to uncoated HA, this is thought to be of little in vivo significance because the mineral of enamel is almost always covered by pellicle components in the mouth (5, 13).

Several in vitro models have been used to mimic bacterial attachment to teeth (5, 8). Powdered enamel (10), various HA preparations (2, 3), dentine (12), and glass (14) have been exposed to saliva to form a film of adsorbed components, and bacterial attachment to such experimental pellicles can be quantitated by the use of radiolabeled bacteria. Commercially available calcium phosphate beads (spheroidal HA beads; BDH, Poole, England) have gained wide acceptance as an adsorbent because they have a low surface area and can easily be separated from unattached bacteria (2, 8, 13). These assays have significantly contributed to our understanding of the mechanisms of bacterial attachment to teeth. However, their use for delineating the nature of salivary and other macromolecules present in pellicles, which serve as bacterial receptors, has been hindered because many bacteria adsorb as well or better to untreated HA (2, 10, 13, 14). Therefore, before pellicle receptors for bacteria can be identified and characterized, an in vitro assay must be available which can clearly distinguish between bacterial attachment to pellicle components and bacterial attachment to any uncovered areas of the mineral surface. Most adhesion studies have assumed that there are no free HA sites available when the beads are treated with unfractionated saliva (4, 8), and several observations support this assumption (2, 8). However, it is particularly important to establish that there are no naked HA sites present when using low cell concentrations (4) or when preparing pellicles from various purified components or saliva fractions (14). We recently observed that a strain of *Streptococcus sanguis* attaches poorly and with very low affinity to albumin-treated HA (7), and this suggested that albumin might be used as a blocking agent to coat any naked regions of the HA substratum that might be available for bacterial attachment.

Initial experiments compared the effects of pretreating HA beads with various concentrations of albumin on the adsorption of *S. sanguis* C5 and *Streptococcus mutans* JBP. Both organisms were obtained from the culture collection of the Forsyth Dental Center. They were grown in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) and supplemented with 4 μCi of [^{3}H]thymidine (New England Nuclear Corp., Boston, Mass.) per ml at 35°C under an atmosphere of 80% N_2–10% H_2–10% CO_2 as previously described (6). Cells from overnight cultures were washed three times and suspended in 0.05 M KCl containing 1 mM phosphate (pH 6.0), 1 mM $CaCl_2$ and 0.1 mM $MgCl_2$ (buffered KCl). Attachment of the ^{3}H-labeled bacteria to the HA beads, which had been treated with various concentrations of albumin, was then determined. Briefly, reaction mixtures (125 μl) were prepared which contained 5 mg of HA beads and 2.5×10^6 ^{3}H-labeled streptococcal cells suspended in buffered KCl (6). The mixtures were continuously inverted (6 rpm) at room temperature for 60 min, and then the beads were washed three times with buffered KCl to remove unattached organisms. The number of bacteria attached to the HA beads was determined by scintillation counting as previously described (6).

Pretreatment of the HA beads with albumin sharply reduced the numbers of *S. sanguis* C5 and *S. mutans* JPB cells which attached (Fig. 1). Saturation of the HA appeared to occur with albumin concentrations of approximately 2 to 5 mg/ml. These findings are consistent with reports of others (11).

To determine if albumin could be used as a blocking agent in studies of streptococcal adsorption to experimental pellicles, samples of HA beads were treated with either buffered KCl or with clarified, unheated, whole saliva for a period of 1 h to form an experimental pellicle. Unstimulated whole saliva was collected in containers, chilled in ice, clarified by centrifugation (10,000 × *g* for 10 min), and frozen until used. The blocking procedure employed consisted of secondarily treating the HA beads or experimental salivary pellicles with 2 mg of bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) per ml in buffered KCl for 30 min. The beads were then permitted to settle, and the liquor was removed. ^{3}H-labeled, washed streptococcal cells suspended in buffered KCl containing 2 mg bovine albumin per ml were then added. Control preparations consisted of beads which were pretreated with buffered KCl instead of albumin; these

* Corresponding author.

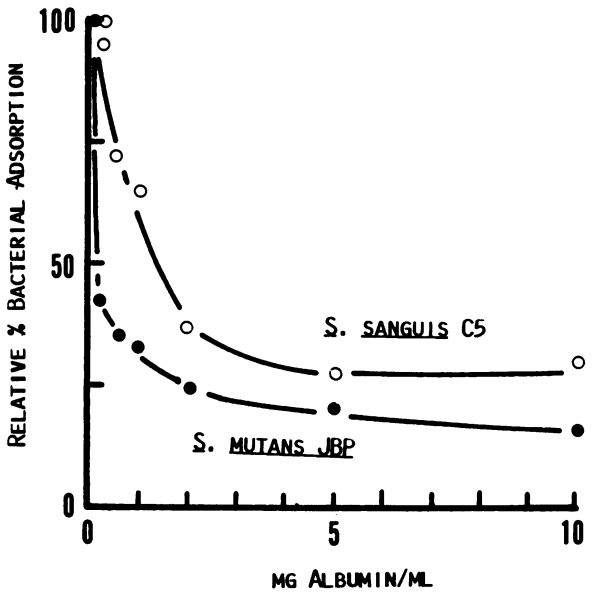

FIG. 1. Adsorption of *S. sanguis* C5 and *S. mutans* JBP to HA beads pretreated with various concentrations of bovine albumin. Reaction mixtures (125 μl) contained 5 mg of HA beads and 2.5 × 10^6 ^{3}H-labeled streptococci suspended in buffered KCl. The number of streptococci which adsorbed to albumin-treated HA is expressed as a percentage of that of buffer-treated HA.

were incubated with ^{3}H-labeled streptococcal cells suspended in buffered KCl without albumin. The mixtures were continuously inverted at room temperature for 60 min as described above, and after washing, the number of bacteria which attached to the HA beads was determined. In the absence of albumin blocking, similar or lower numbers of *S. sanguis* C5 and *S. mutans* JBP cells generally attached to saliva-treated HA beads than to buffer-treated HA (Table 1). These observations are consistent with reports of previous investigators (1, 2, 4, 8, 13) and illustrate that it is not possible to determine from such experiments alone whether the streptococci have interacted with pellicle components or with naked regions of the HA surface (Table 1). However, when albumin blocking was performed, the number of *S. sanguis* and *S. mutans* cells which adsorbed to buffer-treated HA was greatly reduced, while the number which adsorbed to saliva-treated beads was not greatly affected (Table 1). This indicates that albumin blocking does not significantly mask or cover pellicle components involved in attachment of the bacteria, although it strongly reduces binding to HA surfaces which have not been pretreated with saliva.

To determine if albumin blocking could be used to quantitate the contribution of adsorbed salivary components in bacterial attachment, experimental pellicles were prepared from dilutions of whole saliva. The number of *S. sanguis* C5 cells which adsorbed to such pellicles with and without albumin blocking was then determined. In this experiment, reaction mixtures (125 μl) contained 5 × 10^6 streptococcal cells. *S. sanguis* C5 cells adsorbed in lower numbers to saliva-treated HA than to buffer-treated HA (Table 2). The numbers of bacteria which adsorbed to saliva-treated HA were similar in the presence or absence of albumin blocking when undiluted saliva or saliva which had been diluted 1:25 (Table 2) was used. Further dilution of saliva resulted in lower numbers of *S. sanguis* C5 cells attaching to saliva-treated HA when albumin blocking was performed; at a saliva dilution of 1:625, the number of streptococci which attached began to approach the low number which attached to buffer-treated HA (Table 2). However, in the absence of albumin blocking, increased numbers of streptococcal cells attached to HA treated with diluted saliva (1:125 or 1:625), presumably because they were binding to uncoated mineral (Table 2). These data illustrate the value of albumin blocking in delineating the contribution of bacterial interactions with salivary components in attachment to experimental pellicles on HA surfaces. In effect, albumin blocking establishes as a base line the weak interaction of the streptococci with albumin; higher-affinity interactions between the bacteria and salivary components, therefore, can be readily discerned. In the absence of albumin blocking, it is often not possible to discern whether the organisms are interacting with adsorbed pellicle components or with naked regions on the HA. Since albumin blocking did not greatly affect the number of streptococci which adsorbed to HA treated with undiluted saliva (Tables 1 and 2), it seems clear that naked areas do not occur to a significant extent in such preparations. Comparisons of the properties of bacterial adsorption

TABLE 2. Effect of albumin blocking on adsorption of *S. sanguis* C5 cells to HA beads treated with various dilutions of whole saliva

HA beads treated with:	No. ± SE[a] (10^5) of streptococci adsorbed/5 mg of HA[a] treated with:	
	Albumin blocking	Buffered KCl
Buffered KCl	0.6 ± 0.2	28.2 ± 2.4
Undiluted saliva	14.4 ± 0.9	14.1 ± 1.0
Saliva diluted 1:5	14.6 ± 1.0	13.3 ± 1.1
Saliva diluted 1:25	14.2 ± 0.6	15.3 ± 1.3
Saliva diluted 1:125	7.3 ± 0.6	27.8 ± 1.1
Saliva diluted 1:625	2.3 ± 0.4	29.2 ± 1.0

[a] Reaction mixtures (125 μl) contained 5 mg of HA beads and 5.0 × 10^6 ^{3}H-labeled streptococci in buffered KCl.

TABLE 1. Effect of albumin blocking on adsorption of *S. sanguis* C5 and *S. mutans* JBP to untreated and saliva-treated HA beads

HA treatment	Blocked with albumin	*S. sanguis* C5		*S. mutans* JBP	
		No. ± SE[a] (10^5) adsorbed	Relative %	No. ± SE[a] (10^5) adsorbed	Relative %
Buffered KCl	−	13.3 ± 0.3	108	5.1 ± 0.2	116
Whole saliva	−	12.1 ± 0.5	100	4.4 ± 0.1	100
Buffered KCl	+	2.0 ± 0.2	17	0.8 ± 0.2	18
Whole saliva	+	10.3 ± 0.2	85	4.3 ± 0.2	98

[a] Reaction mixtures (125 μl) contained 5 mg of HA beads and 2.5 × 10^6 ^{3}H-labeled streptococci in buffered KCl.

to saliva-treated HA and to untreated HA have led to a similar conclusion (8). In other experiments (manuscript in preparation), albumin blocking proved to be highly useful in the identification of specific salivary components which promote bacterial attachment to experimental pellicles.

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