INFECTION AND IMMUNITY, Nov. 1982, p. 637–644
0019-9567/82/110637-08$02.00/0

Vol. 38, No. 2

Hydrophobic Interactions and the Adherence of *Streptococcus sanguis* to Hydroxylapatite

WARREN E. NESBITT,[1]† RONALD J. DOYLE,[1]* AND K. GRANT TAYLOR[2]

Department of Microbiology and Immunology[1] and Department of Chemistry,[2] Health Sciences Center, University of Louisville, Louisville, Kentucky 40292

Received 23 April 1982/Accepted 1 July 1982

Streptococcus sanguis demonstrated a high affinity for hydrocarbon solvents. When aqueous suspensions of the organism were mixed with either hexadecane or toluene, the cells tended to bind to the nonaqueous solvent. Increases in temperature resulted in a greater affinity of cells for hexadecane. Interaction between the cells and hexadecane was also enhanced by dilute aqueous sodium chloride and by low pH (pH < 5). The results suggest that the cell surface of *S. sanguis* has hydrophobic properties. Isolated cell walls also tended to partition into the nonaqueous solvent. Amino acid analyses of the walls revealed the presence of several amino acids which possess hydrophobic side chains. It is likely that the hydrophobic amino acids associated with the cell wall contribute to the hydrophobicity of intact *S. sanguis*. When the adherence of *S. sanguis* to saliva-coated hydroxylapatite was measured, it was found that hydrophobic bond-disrupting agents, such as the Li^+ cation, the SCN^- anion, and sodium dodecyl sulfate, were capable of inhibiting the cell-hydroxylapatite union. In addition, it was observed that both urea and tetramethylurea were inhibitors of the adherence, although the latter reagent was the superior inhibitor. The results suggest that the adherence of *S. sanguis* to saliva-coated smooth surfaces is at least partially dependent on the formation of hydrophobic bonds between the cell and adsorbed salivary proteins. Hydrophobic bonding may contribute to cooperative interactions involving *S. sanguis* and saliva-coated hydroxylapatite (Nesbitt et al., Infect. Immun. **35**:157–165, 1982).

The genesis of many bacterial diseases occurs when the organisms adhere to host tissues. The adherence is usually specific in that a given bacterium will adhere only to tissues which have receptors capable of forming a productive union with that organism. When the adherence of oral streptococci to hard surfaces of the oral cavity is considered, it appears that *Streptococcus sanguis* has the highest affinity (1, 3, 11). *S. sanguis* has been considered to be important in cariogenesis because it is an early colonizer of freshly cleaned tooth surfaces and because it is frequently isolated from carious lesions (2, 28).

It has been difficult to describe the adherence of *S. sanguis* to saliva-coated smooth surfaces in molecular terms. Several reports suggest that *S. sanguis* has cell surface-associated proteins which may be important in adherence. Rosan and Appelbaum (25) and Liljemark and Schauer (15) have reported that the adherence of *S. sanguis* is destroyed by protease treatment of the cells. Bloomquist et al. (C. G. Bloomquist, W. F. Liljemark, and L. Fenner, J. Dent. Res., **61**:190, 1982) have isolated a protein from cell walls of *S. sanguis* which has the ability to block the adherence of the intact bacteria. Furthermore, Murray et al. (P. A. Murray, M. J. Levine, and L. A. Tabak, J. Dent. Res. **61**:215, 1982) have isolated a cell surface-associated protein from *S. sanguis* which could bind sialic acid residues of salivary mucins. McBride and Gisslow (17) have suggested that *S. sanguis* can bind the sialic acid residues of certain salivary proteins, thereby promoting aggregation of the bacteria. When adherence data are treated by use of Scatchard and Hill plots, curves suggestive of positive cooperativity are observed (20). We have suggested (R. J. Doyle, W. E. Nesbitt, and K. G. Taylor, FEMS Microbiol. Lett., in press) that cell surface-pellicle complexes, possibly involving protein-protein interactions and stabilized by hydrophobic bonds, could account for the adherence of *S. sanguis* to saliva-coated surfaces. In this report, we provide evidence that the cell surface of *S. sanguis* exhibits characteristics of hydrophobicity and that hydrophobic bonds may stabilize the cell-pellicle union.

† Present address: Department of Microbiology, University of Alabama, Birmingham, AL 35294.

(Preliminary results of this work have been presented previously [W. E. Nesbitt, R. J. Doyle, and K. G. Taylor, J. Dent. Res. **61**:215, 1982; and R. J. Doyle, ASM Conference on Bacterial Adhesion in Pathogenesis, Atlanta, Ga., 1981].)

MATERIALS AND METHODS

Bacterial strains and growth conditions. *S. sanguis* Challis was originally obtained from R. Kolstad (Baylor University, Waco, Tex.). *S. sanguis* MJM 19 has been described previously (20). *Streptococcus salivarius* 13419 was obtained from R. Arnold (University of Louisville, Louisville, Ky.). *Streptococcus mutans* 6715 has been described in a previous study (21). The characteristics of *Bacillus subtilis* hpr 10 have been reported previously (13). Stock cultures of all strains of streptococci were maintained on brain heart infusion agar slants (Difco Laboratories, Detroit, Mich.) supplemented with a few milligrams of calcium carbonate. Inoculations into 10 ml of brain heart infusion broth (Difco) were made from the stock cultures. After overnight incubation at 37°C, the contents of the entire culture were transferred into 500 ml of the same medium supplemented with 2 μCi of [^{3}H]thymidine (specific activity, 82 Ci/mmol) (ICN, Irvine, Calif.) per ml. These radioactive cells were used for adherence measurements. The labeled thymidine was deleted from the growth medium when cells were needed for hydrophobicity assays. In some experiments, Todd-Hewitt broth was used as the culture medium. After overnight inoculation, cells were harvested by centrifugation and washed twice in cold distilled-deionized water before suspension in water or in buffered KCl (1.0 mM potassium phosphate buffer [pH 6.0], 50 mM KCl, 1.0 mM $CaCl_2$, and 0.04% [wt/vol] sodium azide) to the appropriate cell density. All experiments were conducted with freshly harvested cells.

Partition assay. Washed cells suspended to a final volume of 3 ml in distilled-deionized water were added to glass culture tubes (12 by 75 mm). The optical density (500-nm wavelength, 1-cm path length) of the cell suspension was adjusted to 0.5 before the addition of the appropriate volume of hexadecane or toluene (26). The contents of each tube were mixed on a Vortex mixer for exactly 30 s. After the contents of the tube had settled for 20 min, a Pasteur pipette was carefully inserted below the nonaqueous phase, and a small sample of the aqueous phase was transferred to a cuvette. Control samples consisted of suspensions of cells without added hydrocarbon. It was necessary to make modifications of the procedures to accommodate changes in ionic strength and hydrogen ion concentration. In these measurements dilutions of salt were added to the cell suspensions to yield the appropriate final solute concentration or pH. A volume of 0.6 ml of hexadecane was then added to all samples. For partition assays in the presence of different hydrogen ion concentrations, cells were suspended in prepared 0.5 M buffer solutions. The buffer substances were chosen to encompass a range of pH values from 3 to 10. Hexadecane (0.6 ml) was added to all samples. Assays conducted under conditions of varying temperature were performed as described above, except all materials were allowed to equilibrate to the appropriate temperature before the assays were conducted.

Adherence assay. The adherence assay used is based upon that originally described by Clark et al. (3). Hydroxylapatite beads (40 ± 2 mg) (BDH Chemicals Ltd., Poole, England) were added to 4-ml plastic scintillation vials (Beckman Instruments Inc., Fullerton, Calif.). After one wash with distilled-deionized water to remove fines, the beads were allowed to equilibrate by constant inversion at a rate of 12 times per min in 1.5 ml of buffered KCl for 2 h. Parafilm-stimulated saliva was collected on ice from a single individual for saliva-coated hydroxylapatite (SHA) bead preparation. The saliva was clarified by centrifugation (at 12,000 × *g* for 10 min) and heated to 60°C for 30 min. After recentrifugation, 0.04% (wt/vol, final concentration) sodium azide was added. After aspiration of the buffer, 1.5 ml of prepared saliva was allowed to mix with the beads for 2 h. The beads were then washed three times with 3.0-ml volumes of KCl buffer to remove unadsorbed saliva. For assays measuring the effects of urea and 1,1,3,3-tetramethylurea (TMU), *S. sanguis* Challis was suspended to a density of 542 μg of cells (dry weight) per ml in buffer. The reagent was diluted in buffer to the appropriate concentration before addition to the cell suspension. The mixture was subsequently added to saliva-coated beads. Similarly, in a direct comparison of the effect of salts and chaotropes on adherence, cells suspended to a density of 236 μg/ml in buffer were mixed with the reagent before addition to saliva-coated beads. All incubations with cells were conducted for a period of 1.5 h. Unadsorbed bacteria and reagents were removed with three washes of buffer. After aspiration of the final buffer wash, the beads were dried at 60°C overnight before being assayed for radioactivity in a scintillation spectrometer. Adventitious adherence to the plastic vials was negligible. All materials for temperature studies were preequilibrated at the appropriate temperature before the assays were conducted.

Cell wall preparation. The procedure used for cell wall preparation has been described previously (21). Cells were ruptured in a French pressure cell (American Instrument Co., Inc., Rockville, Md.) at approximately 20,000 lb/in^2. After differential centrifugation at 39,000 × *g* for 10 min, the walls were extensively washed with water before the extraction procedure to remove loosely bound molecules. Crude walls were subjected to boiling 3% (wt/vol) sodium dodecyl sulfate (SDS) for three 0.5-h intervals. The detergent-treated cells, after being washed in distilled-deionized water several times, were then extracted thrice with 5.0 M lithium chloride for 0.5 h. The walls were again washed twice in water before being stirred overnight in 8.0 M urea. Residual urea was removed from the walls by extensive washing with water before freeze-drying.

Amino acid analyses. Amino acids were analyzed on columns packed with Durrum resin. Wall samples were hydrolyzed in 6 N HCl at 110°C for 24 h. No corrections were made for loss of amino acids during hydrolysis.

Chemicals and reagents. All salts, toluene, and urea were obtained from Fisher Chemical Co., Cincinnati, Ohio. Hexadecane and Tris were purchased from Sigma Chemical Co., St. Louis, Mo. SDS, imidazole, and TMU were products of Aldrich Chemical Co., Milwaukee, Wis. Citric acid was provided by J. T. Baker Chemical Co., Phillipsburg, N.J. Potassium phthalate was purchased from Allied Chemical Co.,

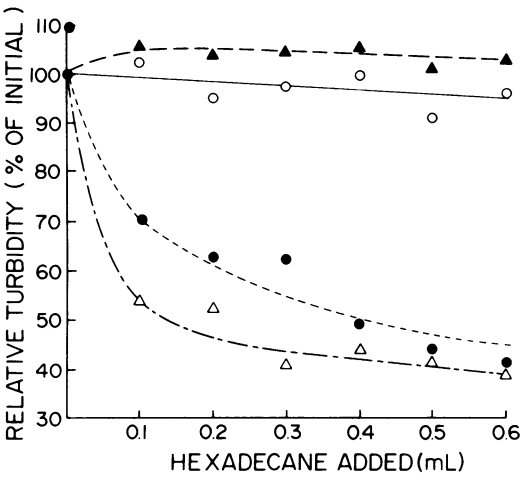

FIG. 1. Partitioning of *S. sanguis*. ●, *S. sanguis*; ○, *S. salivarius*; ▲, *B. subtilis*; △, *S. mutans* 6715.

Morristown, N.J. 2-(*N*-morpholino)ethanesulfonic acid was a product of Calbiochem, San Diego, Calif.

RESULTS

Hydrophobicity of *S. sanguis*. The assumption that hydrophobic-hydrophobic interactions could stabilize the cell-pellicle complex was derived from theoretical considerations of positive cooperativity (4, 20; Doyle et al., in press). Experimental verification for the premise that *S. sanguis* possesses a hydrophobic surface is provided below. The assay described by Rosenberg et al. (26) was used in these studies. When hexadecane or some other water-immiscible solvent is mixed with an aqueous suspension of cells, the cells will exhibit an affinity for one of the two phases upon partitioning. *S. sanguis* tended to bind to the hexadecane phase (Fig. 1). When the results were quantitated by measuring the remaining opacity of the aqueous phase, it was seen that the extent of the adherence of the cells for the hexadecane layer is a function of the hexadecane concentration (Fig. 1). Because saturation appeared to occur at approximately 0.2 ml of hexadecane per ml of cell suspension, we chose this hydrocarbon concentration for subsequent studies. In contrast to the hydrophobicity demonstrated by *S. sanguis*, we noted that *S. salivarius* and *B. subtilis* had little or no tendency to partition into the nonaqueous phase (Fig. 1). Furthermore, when *S. sanguis* MJM 19 (20) and several other clinical isolates of *S. sanguis* were subjected to the assay, results similar to those for *S. sanguis* Challis were obtained (data not shown). Results for *S. mutans* 6715 are also shown in Fig. 1. The data reveal that this organism is at least as hydrophobic as *S. sanguis* Challis. This result for *S. mutans* 6715 is in contrast to our previous claim (Doyle et al., in press), based on the partitioning of clinical isolates of *S. mutans* in hexadecane-water, that *S. mutans* was hydrophilic. Heterogeneity in the hydrophobicity of streptococci has also been observed by Miörner et al. (18). They have observed that the binding of proteins by streptococci can markedly change the isoionic points and surface hydrophobicities of the bacteria.

To confirm the results described above, toluene was substituted for hexadecane in the assay procedures. The data obtained showed that *S. sanguis*, but not *S. salivarius* or *B. subtilis*, could be partitioned into the toluene phase of toluene-water mixtures. Finally, to rule out artifacts caused by possible membrane disruption, *S. sanguis* Challis was radiolabeled with [^{3}H]thymidine and mixed with either hexadecane or toluene (0.2 ml of hydrocarbon per 1.0 ml of cell suspension). The suspension was centrifuged to sediment the cells, and samples were removed from the hydrocarbon and aqueous layers. No radioactivity was found in either phase, indicating that organic solvents did not disrupt the cells.

Enhancement of the hydrophobicity of *S. sanguis* by salt. According to theory, hydrophobic groups can be readily "salted out" with various kinds of salts (16, 24, 29). In fact, one convenient means of determining the hydrophobicity of bacteria is to measure the extent of clumping induced by ammonium sulfate (16). *S. sanguis* was mixed with dilute salt solutions (sodium chloride); hexadecane was added; and the suspensions were blended in a Vortex mixer. After attainment of phase separation, it was found that the sodium chloride enhanced the affinity of *S. sanguis* for the hexadecane phase (Fig. 2). Only small amounts of salt (16) were required to

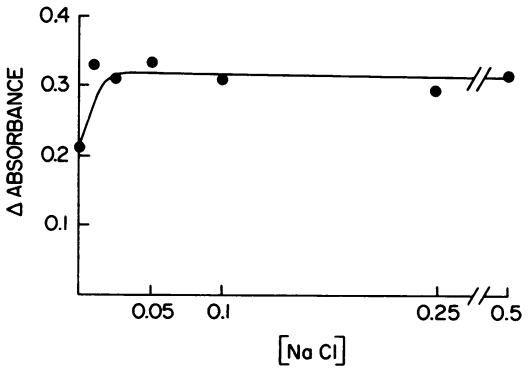

FIG. 2. Effect of salt on phase partitioning of *S. sanguis* with hexadecane. Sodium chloride was added to cells at the indicated concentrations before the assay.

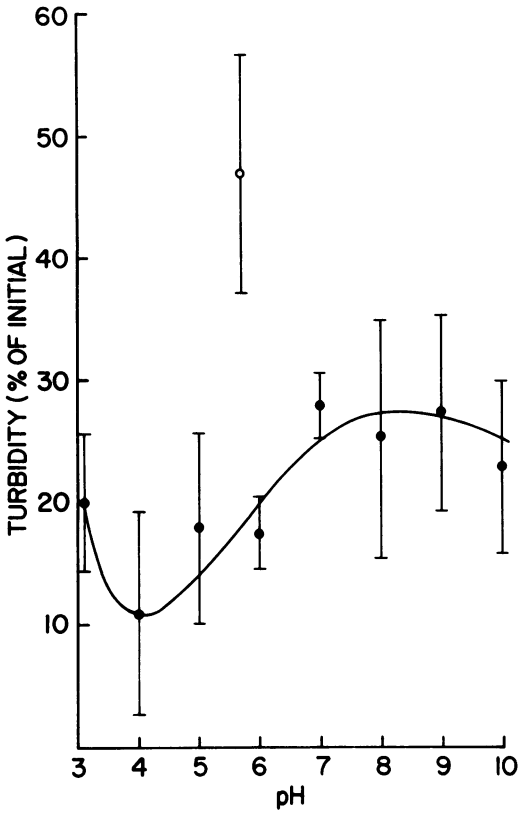

FIG. 3. Effect of pH on phase partitioning of *S. sanguis* with hexadecane. Data points represent the average of four determinations (± standard deviation). O, distilled-deionized water. All buffers were prepared to a 500 mM concentration. Buffers were as follows: pH 3.0, citric acid; pH 4.0, potassium phthalate; pH 5.0, sodium acetate; pH 6.0, 2-(*N*-morpholino)ethanesulfonic acid; pH 7.0, imidazole; pH 8.0, Tris; pH 9.0, sodium carbonate; pH 10.0, sodium carbonate.

achieve maximal expression of hydrophobicity on the cells. The results suggest that the cell surface of *S. sanguis* may have apolar groups that are readily salted out in dilute salt solutions.

Effect of hydrogen ion concentration on hydrophobicity of *S. Sanguis*. It has been well documented that the hydrophobic character of proteins and other amphipathic molecules can be modified by hydrogen ions (16, 19, 24, 29). It would be expected that the most protonated species would be the most hydrophobic form. Various buffers were prepared at 0.5 M concentrations to circumvent the effects of ionic strength (Fig. 2). Cells washed with distilled water were then suspended in the buffers, and the suspensions were subjected to the standard assay procedures. It was observed that a low-pH environment was optimal for partitioning the cells into the hexadecane phase (Fig. 3). The

TABLE 1. Partitioning of *S. sanguis* by a hexadecane-water mixture at various temperature[a]

Temperature (°C)	Turbidity (% of initial)[b]
23	70.5 ± 8.8
37	55.8 ± 9.7
60	48.5 ± 8.3

[a] Cells were grown in Todd-Hewitt broth, harvested by centrifugation, and washed twice in cold distilled-deionized water. The cells were then suspended in water to an absorbance of 0.5.

[b] Values given are averages (± standard deviation) of six separate determinations.

results shown (Fig. 3) suggest, but do not statistically prove, that hydrogen ion concentrations representing pH values between 3 and 4 were required to elicit the maximal expression of hydrophobicity. A distilled water-hexadecane-cell control is also shown (Fig. 3). In all cases, the buffer enhanced the hydrophobic nature of the cell surface of *S. sanguis*.

Adherence and hydrophobicity of *S. sanguis* as a function of temperature. Increased temperatures are used to strengthen hydrophobic bonds. It could be predicted that elevated temperatures would result in an enhancement of the hydrophobic characteristics of *S. sanguis*. In turn, if hydrophobic forces alone are responsible for adherence of the bacteria to SHA, then an increase in the number of cells bound to the hydroxylapatite should accompany an increase in temperature. When the assays were conducted at temperatures above ambient (22 to 23°C), more cells were attracted to the hexadecane (Table 1). It was not practical to conduct the assays at temperatures lower than 22°C because the hexadecane tends to solidify.

Adherence assays were conducted with SHA beads by standard procedures (3, 20). The cell density was adjusted so that the amount bound was not near saturation (20). Adherence was measured at 4, 23, and 37°C. There was no significant change in the bound/unbound ratio over the temperature range studied (Fig. 4). Adherence, therefore, appears to be insensitive to temperature changes, whereas the surface hydrophobicity of *S. sanguis* is increased by an increase in temperature.

Inhibition of adherence by hydrophobic bond-disrupting agents. In assessing how molecules interact to form stable complexes, it is expedient to use inhibiting agents of defined specificity. We reasoned that if agents such as urea could be used to inhibit adherence, then substituted ureas, such as TMU, should be even more effective inhibitors if hydrophobic bonds contribute to the binding between cell and pellicle. When assays were performed in the presence of

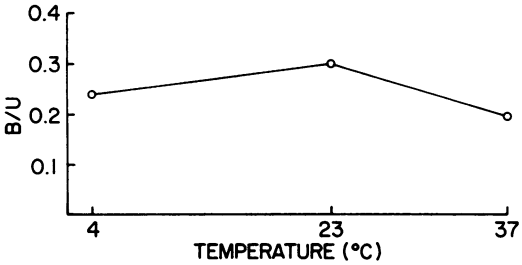

FIG. 4. Effect of temperature on binding of *S. sanguis* to SHA. A cell density corresponding to an optical density of 0.4 was added to the beads, and the adherence assay was performed as described in the text. The number of adherent cells at 23°C represents 3,910 cpm of 13,915 cpm added to the beads. B/U, Bound/unbound ratio.

urea or TMU, it was found that TMU was a much more potent inhibitor of adherence (Fig. 5). For example, at 0.125 M, urea reduced the bound/unbound ratio from 0.43 to approximately 0.30, whereas TMU reduced the ratio to 0.21 (Fig. 5). When higher concentrations of urea or TMU (1 to 2 M) were used, no additional inhibition of adherence was observed.

Some ions, such as Li^+ and SCN^-, are known breakers of hydrophobic interactions, whereas other ions, such as K^+, do not greatly influence hydrophobic bonding (24, 29). Several salts were tested for their ability to prevent the adherence of *S. sanguis* to SHA beads (Table 2). When 1.0 M K^+ ion was used, adherence was actually

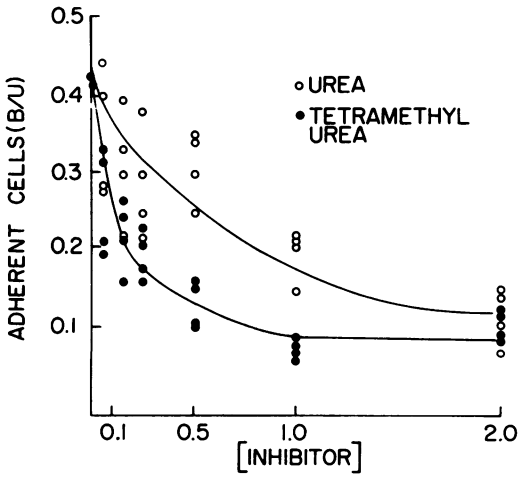

FIG. 5. Effect of denaturants on the adherence of *S. sanguis* to SHA. The amount of the denaturant represents the final concentration used in the adherence assay. The amount bound at a denaturant concentration of zero was 5,718 cpm of 18,815 cpm added to the beads.

TABLE 2. Effect of salts and chaotropes on the adherence of *S. sanguis* to SHA[a]

Inhibitor	B/U	% of control
None (control)	0.48[b]	100
KCl	0.64	133
NaCl	0.32	72
LiCl	0.09	19
NaSCN	0.03	6
Urea	0.27	56
TMU	0.06	13
SDS (1%, wt/vol)	0.02	4

[a] Mixtures of cells suspended to a density of 236 μg/ml in buffer and inhibitor were added to 40 mg of SHA. The final concentration of the inhibitors, with the exception of SDS, was 1.0 M. Controls consisted of cells suspended in buffer without inhibitor. B/U, Bound/unbound ratio.

[b] The control value represents 4,249 cpm bound of 13,261 total cpm added to SHA.

enhanced. In contrast, the Na^+ cation partially inhibited adherence, whereas the Li^+ ion was a highly effective inhibitor. In addition, both SDS and the SCN^- anion were potent inhibitors of adherence. Data are also included for urea and TMU to show their relative effectiveness of inhibition compared with the other chaotropes. There was no evidence to suggest that the inhibitors aggregated the bacteria. The inhibition of adherence is therefore probably not a result of cellular aggregation. We conclude that adherence is highly sensitive to agents capable of disrupting hydrophobic bonding. It must be considered also that other forces, such as ionic or hydrogen bonding, may be destroyed by high concentrations of ions or denaturants.

Amino acid composition of cell walls of *S. sanguis* Challis. Although the foregoing results show that *S. sanguis* possesses hydrophobic properties associated with its cell surface, the origin of the hydrophobic groups remains obscure. In other work, we have observed that cell walls of *S. mutans* contain non-peptidoglycan amino acids (21). The presence of hydrophobic amino acids in cell walls does not necessarily confer a hydrophobic character to a bacterium, but their presence constitutes a natural and convenient parameter for more detailed studies. Suspensions of washed *S. sanguis* Challis were burst in a French press, and the walls were isolated by conventional methods (21). The wall preparations were subjected to multiple treatments with boiling SDS, which were followed by dialysis against 8.0 M urea, to remove adventitiously bound proteins and other molecules. The "cleaned" walls were then subjected to HCl hydrolysis, and the hydrolysate was examined for amino acids. The results (Table 3) reveal that several amino acids not generally regarded as

TABLE 3. Amino acid composition of cell walls of *S. sanguis* Challis[a]

Amino acid	Amt (nmol/mg)
Aspartic acid	80
Threonine	40
Serine	35
Glutamic acid	475
Glycine	50
Alanine	1,530
Cysteine	25
Valine	53
Methionine	5
Isoleucine	25
Leucine	20
Tyrosine	20
Phenylalanine	13
Lysine	490
Histidine	5
Arginine	15

[a] Cells were grown in brain heart infusion broth, harvested, and washed twice in cold distilled-deionized water. The walls were extracted sequentially with SDS, LiCl, and urea as described previously (21).

components of peptidoglycan (14, 21), such as aspartic acid, leucine, isoleucine, tyrosine, phenylalanine, valine, histidine, arginine, serine, threonine, and methionine, were present in the wall preparation. Amino acids such as alanine, lysine, and glutamic acid were present in high quantities and probably originate from the peptidoglycan (8, 14, 21). The results provide evidence to suggest that a polypeptide(s) which contains amino acids possessing hydrophobic side chains is tightly bound to the cell walls of *S. sanguis*.

If the wall-associated and hydrophobic amino acids are involved in the affinity that *S. sanguis* displays for hexadecane, then the isolated walls should partition into the nonaqueous layer. Wall preparations were suspended to 1.0 mg/ml in distilled water and stirred overnight to achieve a homogeneous suspension (5). The walls (optical density of 0.90 at a 500-nm wavelength and a 1-cm path length) were diluted, and hexadecane was added (0.2 ml of hexadecane per ml of aqueous suspension). The suspensions were blended in a Vortex mixer for 2 min, and the phases were allowed to separate. Absorbance measurements were then made on the aqueous layers to quantitate the extent of partitioning of walls between the two solvent phases. The walls displayed an affinity for the hexadecane layer (Table 4). The distribution of walls between the solvents was independent of the amount of wall assayed. When *B. subtilis* 168 walls were subjected to the same partitioning procedures, their distribution was not modified by the hydrocarbon. We conclude that the walls of *S. sanguis* have an affinity for hexadecane.

DISCUSSION

The results from this study support the view that hydrophobic interactions contribute to the adherence of *S. sanguis* to SHA. To account for the observation that adherence involves positive cooperativity (20), it was necessary to predict that hydrophobic interactions were providing a stable environment for other weak interactions, such as ionic or hydrogen bonds (Doyle et al., in press). As a corollary to this reasoning, we predicted that the cell surface of *S. sanguis* should exhibit characteristics of hydrophobicity. The present studies verified that *S. sanguis* has a hydrophobic surface.

The observations that low pH (Fig. 3), elevated temperatures (Table 1), and dilute sodium chloride (Fig. 2) increased the tendency of *S. sanguis* to adhere to hexadecane are consistent with the suggestion that the bacteria assumed an increased hydrophobicity. In a study of the surface hydrophobic characteristics of *Escherichia coli*, it has been noted that low concentrations of the salt ammonium sulfate are effective in eluting only those cells with the most hydrophobic surfaces from hydrophobic gels (16). Conversely, high concentrations of salt are necessary in eluting cells with less hydrophobic surfaces (16). When sodium chloride was added to aqueous cell suspensions of *S. sanguis*, their partitioning in hexadecane was enhanced (Fig. 2). *S. sanguis* partitioned maximally under conditions of low pH (Fig. 3). Stinson et al. (27) have examined the adherence of a number of oral streptococci to glass under conditions of varying pH and have found that maximal adhe-

TABLE 4. Adherence of cell walls to hexadecane[a]

Organism	Amt assayed (μg)	% Decrease in turbidity
S. sanguis	1,000	55
	500	50
	250	55
B. subtilis 168	1,000	0
	500	0
	250	0

[a] Cell walls were prepared according to methods described previously (20, 21). The assay mixtures consisted of 2.0 ml of wall suspension in water and 400 μl of hexadecane. In the absence of hexadecane, *S. sanguis* walls gave an absorbance of 0.9 for 500 μg of wall per ml, whereas *B. subtilis* walls gave an absorbance reading of 0.83. After being blended in a Vortex mixer in the presence of hexadecane, the suspensions were again assayed for turbidity.

sion occurs in a low-pH environment for certain *S. mutans* strains. Because carboxyl groups of amino acids such as aspartic acid and glutamic acid are readily protonated within the pH range at which significant adhesion was observed, Stinson et al. concluded that the involvement of proteins in the adhesion of these organisms was probable (27). It must also be borne in mind that the negative forces present on both bacterial and host surfaces may be minimized by low pH (19), a condition most likely to favor hydrophobic interactions. It is quite probable, however, that protonation of carboxyl groups is responsible for enhancing the hydrophobicity of the surface of *S. sanguis*, thereby allowing an even greater degree of interaction with hexadecane than that observed at higher pH values.

The adherence of *S. sanguis* to SHA must involve several kinds of interactions. If the adherence were purely ionic in nature, then it would be expected that salts should inhibit the binding. This was clearly not the case, as 1.0 M K^+ ion actually enhanced the adherence (Table 2). The K^+ ion, however, has little effect on hydrophobic interactions (24, 29). Other ions, such as Na^+, Li^+, and SCN^-, were inhibitory, although the inhibition induced by Na^+ was marginal. The possibility of lectin-like interactions contributing to the adherence must be considered (9, 10). Salts may or may not inhibit lectin interactions with carbohydrate-containing molecules. Concentrated sodium chloride has no effect on concanavalin A-glycogen complex formation, but dilute salts readily inhibit concanavalin A-teichoic acid interactions (6). This is due to the fact that salts can change the solution structure of the teichoic acids (6). We believe that when interactions involving ionic, dipole-ion, or hydrogen bonds occur between the cell and the pellicle, the interactions will be stable if hydrophobic bonds can also occur (Doyle et al., in press). This view of adherence explains why K^+ ion did not inhibit, whereas Li^+ and SCN^- were effective inhibitors. The effects of urea, TMU, and SDS are also predictable. All of these agents can disrupt macromolecular structures. Urea is less hydrophobic than TMU, and it recently has been reported that the former is the better denaturant of proteins (23), although earlier reports have suggested that TMU is the superior denaturant (7, 12). The results of Orstavik (22), who has shown that the adherence of a streptococcus (designated as OI5) to saliva-coated enamel slabs is sensitive to 2.0 M Na^+, concentrated urea, and Tween 80, may be explained on the basis of the disruption of hydrophobic bonds.

The observation that an increase in temperature increased the hydrophobic tendency of *S. sanguis* yet had little effect on adherence (Table 1 and Fig. 4) also needs explanation. Nonhydrophobic bonds would be weakened at elevated temperatures, whereas hydrophobic bonds would be strengthened. If multiple interactions are required for a productive union between cell and pellicle, then the enhanced hydrophobic effect at higher temperatures may be diminished by the disruption of other bonds.

The origin of the hydrophobicity of *S. sanguis* probably resides in the cell wall of the organism. Hydrophobic amino acids, possibly in covalently bound polypeptide (21), were present in detergent-denaturant-extracted and dialyzed cell walls (Table 3). The walls also displayed an affinity for hexadecane, a quality not inherent in all bacterial walls (Table 4). If cell wall polypeptide is responsible for the affinity of walls for apolar solvents, then the polypeptide must contain a sequence of amino acids which make its hydrophobic properties refractory to the effects of the extraction procedures. The role of "fimbriae," or surface structures emanating from the surfaces, of *S. sanguis* in contributing to the hydrophobicity of the bacterium is not clear. The fimbriae are removed by sudden pressure changes and by chemical reagents, such as urea (25), used in the preparation of the walls. It is possible, however, that both walls and fimbriae contribute to the hydrophobicity of *S. sanguis*.

Our present view of streptococcal adherence is summarized below. Salivary proteins are adsorbed on the hydroxyapatite surface. The proteins may consist of lectin-like molecules or of molecules which may form ionic or ion-dipole complexes with streptococcal surfaces, and they must contain molecules capable of interacting with cells via the hydrophobic effect. The cells must contain molecules on their surfaces complementary to those on the pellicle, but must in addition possess hydrophobic residues. Cells which do not fulfill all of these criteria would interact weakly with the pellicle. Presumably, *S. mutans* falls into this category, even though certain strains possess hydrophobic characteristics. Cells, such as *S. sanguis*, which possess both hydrophobic sites and sites complementary to the pellicle would adhere strongly and probably demonstrate positive cooperativity (20; Doyle et al., in press) in in vitro assays.

ACKNOWLEDGMENT

This work was supported in part by Public Health Service grant NIDR-DE 05102 from the National Institutes of Health.

LITERATURE CITED

1. **Appelbaum, B., E. Golub, S. C. Holt, and B. Rosan.** 1979. In vitro studies of dental plaque formation: adsorption of oral streptococci to hydroxyapatite. Infect. Immun. 25:717–728.
2. **Burnett, G. W., and G. S. Schuster.** 1978. Dental caries, p. 174–212. *In* G. W. Burnett and G. S. Schuster (ed.),

Oral microbiology and infectious disease. The Williams & Wilkins Co., Baltimore.
3. **Clark, W. B., L. L. Bammann, and R. J. Gibbons.** 1978. Comparative estimates of bacterial affinities and adsorption sites on hydroxyapatite surfaces. Infect. Immun. **19**:846–853.
4. **Dahlquist, F. W.** 1978. The meaning of Scatchard and Hill plots. Methods Enzymol. **48**:270–299.
5. **Doyle, R. J., T. H. Matthews, and U. N. Streips.** 1980. Chemical basis for selectivity of metal ions by the *Bacillus subtilis* cell wall. J. Bacteriol. **143**:471–480.
6. **Doyle, R. J., M. L. McDannel, U. N. Streips, D. C. Birdsell, and F. E. Young.** 1974. Polyelectrolyte nature of bacterial teichoic acids. J. Bacteriol. **118**:606–615.
7. **Elbaum, D., E. R. Pandolfelli, and T. T. Herskovits.** 1974. Denaturation of human and *Glycera dibranchiata* hemoglobins by the urea and amide classes of denaturants. Biochemistry **13**:1278–1284.
8. **Ghuysen, J.-M.** 1968. Use of bacteriolytic enzymes in determination of wall structure and their role in cell metabolism. Bacteriol. Rev. **32**:425–464.
9. **Gibbons, R. J., and J. V. Qureshi.** 1978. Selective binding of blood group-reactive salivary mucins by *Streptococcus mutans* and other oral organisms. Infect. Immun. **22**:665–671.
10. **Gibbons, R. J., and J. V. Qureshi.** 1979. Inhibition of adsorption of *Streptococcus mutans* strains to saliva-treated hydroxyapatite by galactose and certain amines. Infect. Immun. **26**:1214–1217.
11. **Gibbons, R. J., and J. van Houte.** 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. **29**:19–44.
12. **Herskovits, T. T., H. Jaillet, and B. Gadegbeku.** 1970. On the structural stability and solvent denaturation of proteins. II. Denaturation by the ureas. J. Biol. Chem. **245**:4544–4550.
13. **Jolliffe, L. K., R. J. Doyle, and U. N. Streips.** 1980. Extracellular proteases modify cell wall turnover in *Bacillus subtilis*. J. Bacteriol. **141**:1199–1208.
14. **Lee, C. J., and T. Y. Liu.** 1977. The autolytic enzyme activity upon pneumococcal cell wall. Int. J. Biochem. **8**:573–580.
15. **Liljemark, W. F., and S. V. Schauer.** 1975. Studies on the bacterial components which bind *Streptococcus sanguis* and *Streptococcus mutans* to hydroxyapatite. Arch. Oral Biol. **20**:609–615.
16. **Lindahl, M., A. Faris, T. Wadstrom, and S. Jherten.** 1981. A new test based on "salting out" to measure relative surface hydrophobicity of bacterial cells. Biochim. Biophys. Acta **677**:471–476.
17. **McBride, B. C., and M. T. Gisslow.** 1977. Role of sialic acid in saliva-induced aggregation of *Streptococcus sanguis*. Infect. Immun. **18**:35–40.
18. **Miörner, H., P.-Å. Albertsson, and G. Kronvall.** 1982. Isolectric points and surface hydrophobicity of gram-positive cocci as determined by cross-partition and hydrophobic affinity partition in aqueous two-phase systems. Infect. Immun. **36**:227–234.
19. **Miyasaki, K. T., and E. Newbrun.** 1981. Effect of pH and some reagents on the sucrose-independent non-specific sorption of the oral bacterium *Streptococcus mutans* to glass. Arch. Oral Biol. **26**:735–743.
20. **Nesbitt, W. E., R. J. Doyle, K. G. Taylor, R. H. Staat, and R. R. Arnold.** 1982. Positive cooperativity in the binding of *Streptococcus sanguis* to hydroxylapatite. Infect. Immun. **35**:157–165.
21. **Nesbitt, W. E., R. H. Staat, B. Rosan, K. G. Taylor, and R. J. Doyle.** 1980. Association of protein with the cell wall of *Streptococcus mutans*. Infect. Immun. **28**:118–126.
22. **Orstavik, D.** 1978. The *in vitro* attachment of an oral streptococcus sp. to the acquired tooth enamel pellicle. Arch. Oral Biol. **23**:167–173.
23. **Pavlic, A., and S. Lapanje.** 1981. Interactions of human serum albumin and some alkylureas. Biochim. Biophys. Acta **669**:60–64.
24. **Robinson, D. R., and W. P. Jencks.** 1965. The effect of concentrated salt solutions on the activity coefficient of acetyltetraglycine ethyl ester. J. Am. Chem. Soc. **87**:2470–2479.
25. **Rosan, B., and B. Appelbaum.** 1982. Surface receptors of selected oral streptococci and their role in adhesion to hydroxyapatite, p. 342–345. *In* D. Schlessinger (ed.), Microbiology—1982. American Society for Microbiology, Washington, D.C.
26. **Rosenberg, M., D. Gutnick, and E. Rosenberg.** 1980. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. FEMS Microbiol. Lett. **9**:29–33.
27. **Stinson, M. W., D. C. Jinks, and J. M. Merrick.** 1981. Adherence of *Streptococcus mutans* and *Streptococcus sanguis* to salivary components bound to glass. Infect. Immun. **32**:583–591.
28. **van Houte, J., R. J. Gibbons, and A. J. Pulkkinen.** 1971. Adherence as an ecological determinant for streptococci in the human mouth. Arch. Oral Biol. **16**:1131–1141.
29. **von Hippel, P. H., V. Peticolas, L. Schach, and L. Karlson.** 1973. Model studies on the effects of neutral salts on the conformational stability of biological macromolecules. I. Ion binding to polyacrylamide and polystyrene columns. Biochemistry **12**:1256–1264.