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Surface-Associated Host Proteins on Virulent *Treponema pallidum*

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A surface coat of host serum proteins was detected on virulent *Treponema pallidum* by sodium dodecyl sulfate-gel electrophoresis. The loosely associated serum proteins could be removed by repeated washings in a protein-free medium. Washed *T. pallidum* retained the ability to readsorb numerous host proteins from rabbit serum as well as iodinated rabbit or human albumin. In addition, various avidly associated host serum proteins including albumin, α_2-macroglobulin, transferrin, ceruloplasmin, immunoglobulin G, immunoglobulin M, and C3 were identified on the outer envelope of washed treponemes by an immunoadsorbent technique with protein A-bearing staphylococcus. Hyaluronidase treatment did not remove the avidly associated host proteins from the surface of washed treponemes, whereas trypsin treatment resulted in decreased levels of agglutination. Electrophoretic patterns of trypsin-treated treponemes showed that treponemal proteins as well as adsorbed host proteins were released concurrently by protease digestion. Reacquisition studies involving α_2-macroglobulin and transferrin suggested the presence of noncompetitive binding sites for serum proteins on the treponemal outer envelope. Finally, differences among the *T. pallidum* preparations from individual rabbits with respect to incorporation of [^{35}S]methionine, extent of agglutination with antisera, and length of time required for removal of avidly associated host proteins by trypsin treatment indicated biological variability among the treponemal populations.

Despite extensive research efforts, virulent *Treponema pallidum* cannot be cultivated in vitro. Studies on macromolecular synthesis undertaken to elucidate metabolic lesions in virulent treponemes have failed to identify major anabolic and catabolic deficiencies (2, 4, 28, 29, 32). Also, attempts to grow treponemes by coincubation with tissue culture cells have met with only limited success (12, 13, 20, 31). The difficulties encountered in the above studies have necessitated a broader approach to better understand the biology of *T. pallidum* and the pathogenesis of syphilis.

One area of investigation receiving recent attention focuses on the cell surface of *T. pallidum*. A mucopolysaccharide component surrounding virulent treponemes has been reported, although the origin and function of this material remain unresolved (14, 34). Other work on the role of *T. pallidum* surfaces in virulence relate to the attachment and specific orientation of the treponemes to host cell membranes mediated by a receptor-like mechanism on the terminus of the organism (18). Furthermore, *T. pallidum* adheres to rabbit peritoneal macrophages but is not readily ingested even in the presence of convalescent serum from infected rabbits (3, 7). It would appear that characterization of the *T. pallidum* surface, as with studies on certain pathogens (1, 9-11, 33), is essential to define immunological, pathogenic, and physiological properties of the spirochete.

Although it has been proposed that *T. pallidum* is surrounded by a protective, host-derived surface coat associated with the outer envelope (8), no data are available to elucidate the host surface components. In this report, we demonstrate the presence of specific host proteins both loosely and avidly associated with the treponemal surface and discuss the biological implications of these findings.

MATERIALS AND METHODS

Animals. New Zealand white male rabbits weighing approximately 3 to 3.5 kg were kept at 16 to 18°C in isolation cubicles before and during treponemal infection.

Bacteria. The virulent Nichols strain of *T. pallidum* was kindly provided by the Center for Disease Control, Atlanta, Ga. Organisms used for inoculation were preserved in the presence of 10% dimethyl sulfoxide in liquid nitrogen (27) and quickly thawed before inoculation of rabbits (18). Stock vials of *T. pallidum*

were prepared by extracting infected testes in an air atmosphere in phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.6 mM Na_2HPO_4, and 1.5 mM KH_2PO_4) supplemented with 75 mM sodium citrate, pH 7.4, and 10% normal heat-inactivated rabbit serum (NRS).

Extraction of virulent *T. pallidum*. The extraction procedure was previously described (2) except for the following modifications. Minced testicular tissue was shaken in 15 ml of treponemal medium for 15 min at room temperature (RT) under aerobic conditions. The medium containing treponemes was then removed and centrifuged twice at 500 × *g* for 10 min to sediment host cellular components followed by layering of the suspension on a 0.8% Methocel-50% Hypaque gradient (16:10, vol/vol) and centrifugation at 650 × *g* for 20 min. The supernatant containing the treponemes was removed and used for experimentation.

The treponemal medium was composed of 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), 1.8 mM $CaCl_2$, 5.2 mM NH_4Cl, 1.0 mM $MgCl_2$, 27.7 mM glucose, and 2.4 mM sodium pyruvate. The medium was prepared immediately before use, adjusted to pH 7.5, and sterilized by filtration. Reducing agents, 4.5 mM L-cysteine and 6.1 mM sodium thioglycolate, were also prepared fresh as a 10-fold stock solution, pH 7.5, and sterilized by autoclaving. After equilibration to RT, the reducing agents were added to the treponemal medium.

Radiolabeling of *T. pallidum*. After removal of host cellular contamination as described above, the treponemal suspension containing approximately 3 × 10^8 treponemes per ml was radiolabeled with 10 μCi of [^{35}S]methionine (Schwarz-Mann, Orangeburg, N.Y.) per ml. The suspension was allowed to incubate overnight (20 h) at 34°C under aerobic conditions which permitted the retention of 100% motility. Treponemes were pelleted and solubilized in sodium dodecyl sulfate (SDS)-containing buffer before gel electrophoresis, or they were washed numerous times for either electrophoresis or agglutination as described below.

Preparation of staphylococcal adsorbent. *Staphylococcus aureus* used as the immunoadsorbent in the agglutination assay was kindly provided by Edward Hayes, Duke University, Durham, N.C. The organisms were grown in bulk in Trypticase soy broth (Difco Laboratories, Detroit, Mich.) and prepared as described by Kessler (22). After harvesting and processing, the formaldehyde-treated suspension was adjusted to a 10% (vol/vol) concentration, divided into portions, and frozen at −70°C.

Before use in the agglutination assay, *S. aureus* was pelleted in a Beckman Microfuge-B miniature centrifuge, suspended to the original volume in NaCl-EDTA-Tris [NET; 150 mM NaCl, 5 mM disodium ethylenediaminetetraacetate (EDTA), and 50 mM tris(hydroxymethyl)aminomethane (Tris)] buffer, pH 7.4, containing 0.5% (wt/vol) Triton X-100, and incubated for 15 min. Staphylococci were then centrifuged and suspended in NET–0.05% Triton X-100. After 15 min, the bacteria were centrifuged, and the washings were repeated twice more as just described. Finally, the adsorbent was washed five times in NET buffer and resuspended to a 10% (vol/vol) final concentration. The adsorbent was prepared no more than 2 days before use in the agglutination assay.

Agglutination of radiolabeled *T. pallidum*. Fifty microliters of radiolabeled treponemes at 3 × 10^8 per ml previously washed three times was mixed with 20 μl of each antiserum. After 60 min of incubation at RT, the mixture was diluted with 1 ml of NET buffer followed by addition of 50 μl of a 10% (vol/vol) *S. aureus* suspension. After a 15-min incubation, the mixture was centrifuged for 1 min in a Beckman Microfuge-B miniature centrifuge. The supernatant was discarded, and the pellet was washed once in 0.5 ml of NET buffer. Finally, the resuspended pellet was measured for radioactivity. Each experiment was repeated at least three times, and duplicate samples were assayed for each experimental condition.

Sera. Normal goat serum, NRS, and normal sheep serum were used as controls for the agglutination experiments. Each was adsorbed for 30 min at RT against the avirulent Reiter strain, *Treponema phagedenis* biotype Reiter (18), to remove possible cross-reactive antibody against the genus *Treponema*.

All antisera used were from animals hyperimmunized to specific serum proteins. Immunoelectrophoresis of each antiserum was performed by Cappel Laboratories as well as in our laboratory, and each reacted monospecifically with the respective host protein or fresh NRS. Sera against NRS, rabbit serum albumin (RSA), immunoglobulin G (IgG), C3, and transferrin were goat anti-rabbit. Sera against α_2-macroglobulin and ceruloplasmin were sheep anti-human, and serum against IgM was sheep anti-rabbit. α_2-Macroglobulin and transferrin used for reacquisition experiments were of human origin; all other proteins were purified from rabbit serum.

Enzyme treatment. A 1-ml suspension containing 1.4 × 10^9 treponemes, washed three times in PBS or treponemal medium without reducing agents, was treated with 10 mg of trypsin at 37°C for various lengths of time. After treatment, the treponemes were pelleted by centrifugation at 17,000 × *g* for 15 min and resuspended to 1 ml in NET buffer for agglutination or prepared for SDS-gel electrophoresis.

Similarly, treponemes were treated with hyaluronidase (type VI) by using several experimental conditions, including different concentrations of enzyme in PBS at pH 6 or 7. The treponemal suspension was then centrifuged on Methocel-Hypaque as previously described to remove large aggregates of treponemes which formed during treatment and could not be dissociated. The supernatant containing treponemes was then centrifuged at 17,000 × *g* for 10 min, and the pellet was resuspended in PBS for use in the agglutination assay.

SDS-gel electrophoresis and autoradiography. Approximately 1.4 × 10^9 treponemes treated under a variety of test conditions were centrifuged at 17,000 × *g* for 15 min, and the pellets were resuspended in cold PBS. Trichloroacetic acid was added to a 10% final concentration, and incubation continued at 4°C for not less than 4 h. The acid-precipitated material was washed once with cold PBS and dissolved in 200 μl of solubilizing buffer consisting of 62.5 mM Tris-hydrochloride (pH 6.8), 2% mercaptoethanol, 10% glycerol, and 2% SDS. Bromophenol blue was included

as the tracking dye, and the samples were boiled for 3 min. Undissolved residues were removed by centrifugation before application of a 100-μl volume to individual gels. Stacking and separating gels consisted of 3 and 7.5% acrylamide, respectively, and electrophoresis was performed as previously described (19). Gels were stained with Coomassie brilliant blue (Schwarz-Mann), destained, and sliced longitudinally. Gel slices were processed for autoradiography by the dimethyl sulfoxide-diphenyloxazole procedure (6). X-ray film (XOMAT, XRP-5; Eastman Kodak Co., Rochester, N.Y.) was exposed to the gels for various lengths of time at −70°C before development.

Association of iodinated albumin with *T. pallidum*. Lactoperoxidase-mediated iodination of RSA and human serum albumin was performed by a modification of the procedure of Garvey et al. (15). Briefly, 0.5 ml of albumin solution (5 mg), 0.05 ml of lactoperoxidase (50 μg), and 0.01 ml of ^{125}I (500 μCi; New England Nuclear Corp.) were gently mixed. Then 0.05 ml of H_2O_2 freshly prepared (0.01 ml of stock 30% H_2O_2 in 50 ml of PBS) was introduced with constant gentle stirring, and the incubation continued at RT for 15 min. Another 0.05 ml of H_2O_2 was added, and the iodination was terminated with cysteine after an additional 15 min. The mixture (0.66 ml) was then placed on a Sephadex G-25 column (0.5 by 10 cm) previously washed with 25 mg of bovine serum albumin. The void volume was collected and dialyzed for 3 days against several changes of 4 liters of distilled water, using 10,000-dalton-cutoff dialysis tubing. SDS-gel electrophoresis and autoradiography revealed only one radiolabeled protein which comigrated with the respective albumin.

Varying amounts of the iodinated albumin were incubated with a 1 ml of suspension of 1.4×10^9 freshly extracted or washed treponemes. The mixture was then centrifuged at 17,000 × *g* for 15 min, and the edge of the tube was carefully washed with PBI (similar to PBS, with NaCl replaced with NaI) without disrupting the treponemal pellet. The pellet was then resuspended and measured for radioactivity. For the duplicate sample, the treponemal pellet was washed once in PBI before radioactive determination.

Reagents used. All reagents and enzymes were purchased from Sigma Chemical Co. (St. Louis, Mo.) unless otherwise indicated. Acrylamide for SDS-gel electrophoresis was obtained from Bio-Rad Laboratories (Richmond, Calif.). Calcium chloride and NH_4Cl were from Allied Chemicals (Morristown, N.J.), and $MgCl_2$ and sodium citrate were from J. T. Baker Chemical Co. (Phillipsburg, N.J.). Sodium thioglycolate, trichloroacetic acid, dimethyl sulfoxide, diphenyloxazole, and dialysis tubing were purchased from Fisher Scientific Co. (Fair Lawn, N.J.). Lactoperoxidase was obtained from Calbiochem-Behring Corp. (La Jolla, Calif.). Methocel F4M was purchased from Dow Chemical Co. (Midland, Mich.), and Hypaque was from Winthrop Laboratories (New York, N.Y.). All sera, antisera, and serum proteins were acquired from Cappel Laboratories (Cochranville, Pa.).

RESULTS

Loosely associated host proteins on the surface of *T. pallidum*. Figure 1 shows the protein profile and accompanying autoradiogram of *T. pallidum* after SDS-gel electrophoresis. The Coomassie brilliant blue staining pattern of gel A is of freshly extracted treponemes free of host cellular contamination. The corresponding autoradiogram (gel D) suggests that a large number of high-molecular-weight proteins as well as the intensely stained area in the middle of gel A were possibly of host origin. Gel B, therefore, is of treponemes washed once in PBS or protein-free treponemal medium, and gel C is of treponemes washed at least three times. In each case, the autoradiogram was identical to the profile in gel D. Washing the treponemes removed the intensely staining loosely bound proteins and allowed for better resolution of stained treponemal proteins, as could be determined by alignment with the autoradiogram.

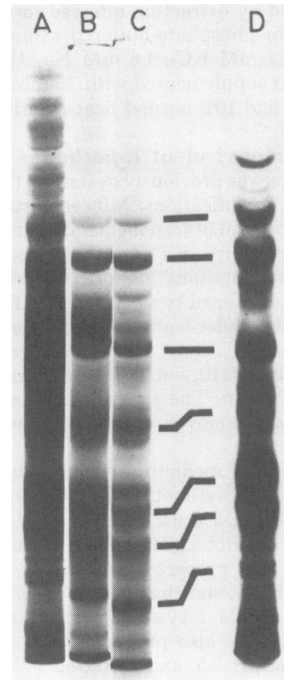

FIG. 1. *Electrophoretic pattern of radiolabeled T. pallidum under various experimental conditions. Gel A is purified treponemes freshly extracted from infected rabbit testes. Gel B is a treponemal sample washed once in PBS, gel C represents treponemes washed at least three times, and major bands are aligned with those on the autoradiogram (gel D). Sample preparation and washings are as described in Materials and Methods.*

Adsorption of host proteins to the treponemal surface. The capacity of *T. pallidum* to reacquire host serum proteins was then examined. Protein profiles of treponemes washed three times and incubated with 10-fold serial dilutions of rabbit serum appear in Fig. 2. Gel A

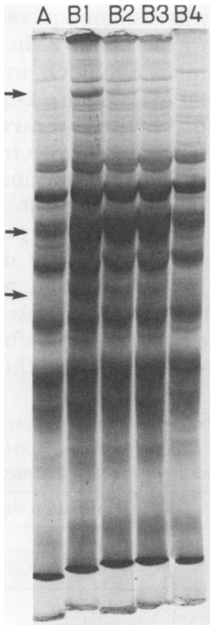

FIG. 2. *Gel electrophoresis of T. pallidum washed three times (gel A) followed by incubation with 10-fold serial dilutions of NRS (gels B1 through B4). Arrows designate major host proteins readily adsorbed to the surface of washed T. pallidum as described in Results.*

shows the protein profile of washed treponemes, whereas the remaining gels demonstrate the reacquisition of three major host proteins. Decreased amounts of serum resulted in concomitant decreased intensity of staining, indicating that the proteins were serum derived. The three proteins comigrated (from top to bottom) with α_2-macroglobulin, RSA, and the heavy chain of IgG, respectively. Removal of these adsorbed proteins could then be accomplished by repeated washing of the treponemes in PBS consistent with the data presented in Fig. 1.

Further support for the ability of washed treponemes to adsorb host proteins was obtained by incubation of *T. pallidum* with iodinated rabbit or human albumin. Both albumin preparations readily adsorbed to the treponemal surface (Table 1). Dilution of iodinated albumin resulted in approximately the same proportionate decrease in the amount of albumin adsorbed. As expected, washing the treponemes after exposure to the iodinated albumin resulted in almost total loss of associated cpm. Freshly extracted, unwashed treponemes were used as controls and adsorbed less than 20% of the total counts per minute for each respective sample (data not shown).

Avidly associated host proteins. To more carefully detect the presence of host proteins on the surface of washed treponemes, it was necessary to develop a highly sensitive method for quantitation of host material. An agglutination technique was used, with protein A-bearing staphylococcus as an adsorbent for IgG antibodies complexed to the treponemal surface. The resultant staphylococcus-antibody-treponeme complex was specific and readily centrifuged under conditions where *T. pallidum* alone was not pelleted. The radioactivity in the resuspended precipitate, therefore, measured the extent of agglutination of [^{35}S]methionine-labeled *T. pallidum* with antiserum against a specific host protein. All values were subtracted from the agglutination obtained with control serum. The latter always represented less than 30% of the total counts per minute detected in test samples.

TABLE 1. *Adsorption of ^{125}I-labeled RSA and ^{125}I-labeled human serum albumin (HSA) to the surface of T. pallidum*

Amt added (μg)[a]	cpm in pellet[b]	
	RSA[c]	HSA[c]
1,000	169,000	44,810
500	49,000	12,930
100	18,430	5,910
50	7,310	1,800
5	920	700

[a] Amount of iodinated albumin incubated with 1.4 × 10^9 treponemes for 15 min as described in Materials and Methods.

[b] Amount of radioactivity in the treponemal pellet after centrifugation of the reaction mixture.

[c] Specific activity: RSA, 8,060 cpm/μg of protein; HSA, 2,594 cpm/μg of protein.

Table 2 presents typical results from one of several agglutination experiments. Agglutination was observed for each protein examined; however, in this experiment, reduced levels for albumin and α_2-macroglobulin were observed. Heat inactivation of the sera did not affect the extent of agglutination. The inability to remove or decrease agglutination by prior repeated washing of the treponemes suggested that these host proteins were firmly attached to the treponemal surface.

Effect of enzyme treatment on avidly associated proteins. To further characterize the avid association between host proteins and the treponemal surface, trypsin treatment of washed *T. pallidum* preparations was monitored (Table 2). Two hours of trypsin treatment was necessary to remove or decrease the extent of agglutination with antisera.

Because a polysaccharide layer has been detected on the treponemal surface by ruthenium red staining (34), the possibility existed that host proteins were entrapped in the mucoid layer.

TABLE 2. *Presence of avidly associated host proteins on the surface of T. pallidum*

Antiserum[a]	Agglutination with antiserum[b]	
	Untreated[c]	Trypsin treated[c]
Anti-NRS	42.3	28.0
Anti-RSA	10.5	0.0
Anti-α_2-macroglobulin	9.5	0.0
Anti-transferrin	35.1	16.8
Anti-ceruloplasmin	39.0	ND[d]
Anti-IgG	37.4	4.4
Anti-IgM	45.7	13.7
Anti-C3	32.2	15.9
	(9,567.0)[e]	(9,650.0)

[a] All antisera were diluted 1:100 in NET buffer before assay.

[b] Agglutination with antiserum against specific host protein was performed as described in Materials and Methods. Values are expressed as percent counts per minute of agglutination with each antiserum subtracted from counts per minute of agglutination with control serum.

[c] Untreated refers to treponemes washed three times in PBS before incubation with antiserum; trypsin-treated indicates washed treponemes incubated with trypsin at 37°C for 120 min as described in Materials and Methods.

[d] ND, Not done.

[e] Numbers in parentheses represent total counts per minute in agglutination reaction mixture.

Therefore, hyaluronidase treatment of the washed treponemes was performed to remove residual mucopolysaccharide. Exposure of treponemes to hyaluronidase had little effect on the association of host proteins with the treponemal surface and subsequent agglutination (Table 3). As expected, treatment with hyaluronidase followed by trypsin reduced agglutination to levels observed with trypsin treatment alone (data not shown).

Biological variability among treponemal preparations. During these studies, differences were observed among treponemal preparations from individual rabbits with respect to the level of [^{35}S]methionine incorporation, the extent of agglutination with specific antisera, and the length of time required for trypsin removal of the avidly associated host proteins. The results in Table 4 reveal fourfold less counts per minute in the reaction mixture of experiment 1 when compared with equal numbers of treponemes in experiments 2 and 3. Second, agglutination with anti-NRS or anti-RSA varied among the preparations; agglutination values were greater in experiment 1 than in experiment 2 or 3. Finally, only 1 h of trypsin treatment was required to decrease the agglutination three- to fourfold in experiment 1, whereas an equivalent reduction required 2 h in experiment 2. In experiment 3, however, a similar reduction in agglutination was not observed even after a 2-h treatment.

Reacquisition of trypsin-released host proteins. Since treatment with trypsin reduced the extent of treponemal agglutination with specific antisera, the ability of the treated treponemes to reacquire these host proteins was examined. Table 5 presents the data from two representative experiments in which washed, trypsin-treated treponemes were exposed to 1 mg of a specific host protein before the agglutination assay. As can be seen, the extent of ag-

TABLE 3. *Presence of avidly associated host proteins on the surface of T. pallidum after hyaluronidase treatment*

Antiserum[a]	Agglutination with antiserum[b]	
	Untreated[c]	Hyaluronidase treated[d]
Anti-NRS	63.3	41.0
Anti-RSA	44.0	24.1
Anti-IgG	71.3	63.7
Anti-C3	47.8	46.2
	(10,250.0)[e]	(6,148.0)

[a-c, e] As described in Table 2, except that each antiserum was diluted 1:50 in NET buffer.

[d] Treponemes treated with 500 μg of hyaluronidase per ml for 120 min at 37°C.

TABLE 4. *Biological variability among T. pallidum preparations*

Antiserum[a]	Agglutination with antiserum[b]		
	Untreated[c]	Trypsin treated[c]	
		60 min	120 min
Expt 1[d]			
Anti-NRS	75.0	14.0	
Anti-RSA	83.0	26.4	
Anti-IgG	14.3	3.4	
	(4,780.0)[e]	(6,014.0)	
Expt 2			
Anti-NRS	68.0	52.8	17.1
Anti-RSA	59.3	55.8	21.7
Anti-IgG	15.0	28.5	2.4
	(21,150.0)	(19,770.0)	(15,050.0)
Expt 3			
Anti-NRS	35.0		45.3
Anti-RSA	39.5		46.6
Anti-IgG	23.4		18.0
	(16,387.0)		(15,631.0)

[a] As described in Table 3.

[b, c, e] As described in Table 2.

[d] Each experiment represents *T. pallidum* extracted from a different rabbit as described in Materials and Methods.

TABLE 5. *Reacquisition of avidly associated host proteins by trypsin-treated treponemes*

Host protein	Antiserum[a]	Agglutination with antiserum[b]		
		Untreated[c]	Trypsin treated[c]	Trypsin treated + host protein[c, d]
Expt 1				
RSA	Anti-RSA	10.6	5.6	20.0
IgG	Anti-IgG	12.2	8.6	37.2
α_2-Macroglobulin	Anti-α_2-macroglobulin	15.8	23.2	53.5
Transferrin	Anti-transferrin	38.6	12.6	29.0
Expt 2				
RSA	Anti-RSA	0.0	0.0	19.1
IgG	Anti-IgG	31.3	14.2	62.5
α_2-Macroglobulin	Anti-α_2-macroglobulin	33.9	15.2	34.5
Transferrin	Anti-transferrin	27.4	19.0	27.9

[a] As described in Table 3.

[b, c] As described in Table 2, except that the amount in the reaction mixture for each treponemal sample was approximately 6,000 cpm.

[d] 1.4×10^9 washed and trypsin-treated treponemes were incubated with 1 mg of each protein for 30 min at RT, centrifuged, and resuspended in NET buffer for agglutination assay.

glutination was always equal to or greater than values obtained from control, non-trypsin-treated treponemes.

Furthermore, competition studies in which two host proteins, α_2-macroglobulin and transferrin, were incubated sequentially with washed, trypsin-treated treponemes indicated that neither protein interfered with the binding capacity of the other individual macromolecule. The data suggest that separate, noncompetitive sites for host proteins existed on the treponemal outer envelope, allowing for reacquisition of individual host proteins to the same extent shown in Table 5. In contrast, studies with combinations using C3 and IgG demonstrated that the absence of IgG from the incubation mixture greatly reduced C3 reacquisition, indicating that C3 was binding to IgG rather than the treponemal surface.

Effect of trypsin treatment on treponemal proteins. Because extended incubation with high levels of trypsin was required to remove avidly associated host proteins from *T. pallidum*, we were concerned that treponemal proteins might also be released during protease treatment. Figure 3 compares the profile of control Coomassie brilliant blue-stained treponemal proteins (gel A) with trypsin-treated (gel B) preparations. Gels C and D represent the respective autoradiograms of *T. pallidum* proteins. Comparison of the stained gels with the accompanying autoradiograms reveals that numerous host-associated (H) and treponemal (T) proteins were released by the treatment. The effect of trypsin on treponemal proteins T1, T2, T3, and T4 could be detected by autoradiography, whereas removal of T5 was only observed

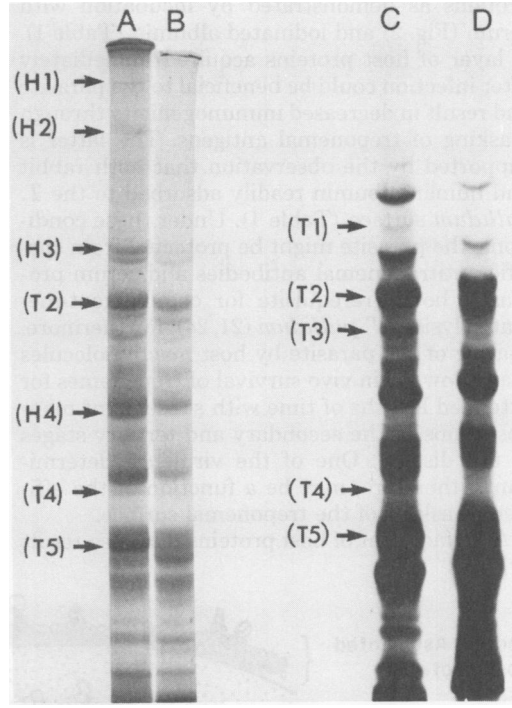

FIG. 3. *Electrophoretic analysis of host (H) and T. pallidum (T) proteins after treatment with trypsin. Gels A and B are Coomassie brilliant blue-stained protein patterns of control and trypsin-treated treponemes, respectively. Gels C and D represent autoradiograms of control (C) and trypsin-treated (D) [^{35}S]methionine-labeled treponemal proteins and are derived from the stained gels. High-molecular-weight treponemal proteins above T1 (gel C) were detected in gel D after extended exposure.*

on the stained protein profile (gels A and B) due to the intensity of the autoradiograms (gels C and D).

DISCUSSION

The data presented here demonstrate the presence of specific host proteins on the surface of *T. pallidum* (Fig. 4). Virulent treponemes had the ability to adsorb a surface coat of host proteins which was readily removed by repeated washings. The interaction between the loosely associated host proteins and the mucoid layer reported by others (34) is not known, although both are removed by washing. Other host proteins were found avidly associated with the treponemal surface and appeared to reside on noncompetitive binding sites on the outer envelope. Removal of these host proteins could only be accomplished by trypsin treatment which resulted in the concomitant release of treponemal proteins.

Washed *T. pallidum* readily adsorbed host proteins as demonstrated by incubation with serum (Fig. 2) and iodinated albumin (Table 1). A layer of host proteins acquired immediately after infection could be beneficial to the parasite and result in decreased immunogenicity through masking of treponemal antigens. The latter is supported by the observation that both rabbit and human albumin readily adsorbed to the *T. pallidum* surface (Table 1). Under these conditions, the parasite might be protected from specific antitreponemal antibodies and serum proteases, both prerequisite for complement-mediated lysis of *T. pallidum* (21, 24). Furthermore, coating of the parasite by host macromolecules may allow for in vivo survival of treponemes for extended lengths of time with subsequent manifestations of the secondary and tertiary stages of the disease. One of the virulence determinants, therefore, may be a function of the efficient masking of the treponemal surface.

A surface coat of host proteins may also result in limited or total absence of antibody-mediated phagocytosis of the treponemes. Since phagocytosis requires access to specific ligands at the surface of the object being ingested (17), masking of the treponemal surface would prevent receptor-ligand interactions essential for internalization. Not surprisingly, therefore, limited ingestion of motile treponemes anchored to the surface of activated macrophages has been reported even in the presence of immune serum (3, 7).

Avidly associated host proteins on *T. pallidum* not demonstrable by Coomassie brilliant blue staining of SDS-gels could be detected by using a highly sensitive, quantitative radioactive agglutination procedure. It is significant that specific host proteins could only be removed by trypsin treatment. Alterations in the electrophoretic protein profile of treponemal proteins that accompanied the decreased agglutination (Fig. 3) strongly suggest that these host proteins are intimately associated with the outer envelope of *T. pallidum*.

Thus, the existence of specific and biologically important host proteins at the treponemal outer envelope (Table 2) might serve several possible roles. Such macromolecules could promote membrane integrity of *T. pallidum* or could be involved in modulating important surface components (16) or masking key treponemal immunogens. Furthermore, the presence of physiologically relevant host proteins such as transferrin and ceruloplasmin may suggest a method for treponemal acquisition of nutrients and cofactors from the environment. The ability of *T. pallidum* to sequester these biologically active proteins in vivo may reflect nutritional or metabolic deficiencies for which the parasite requires the host environment.

It is interesting that no complement-mediated lysis of the unwashed treponemes was observed, even though C3 and IgG were detected on the treponemal outer envelope (Table 2). These ob-

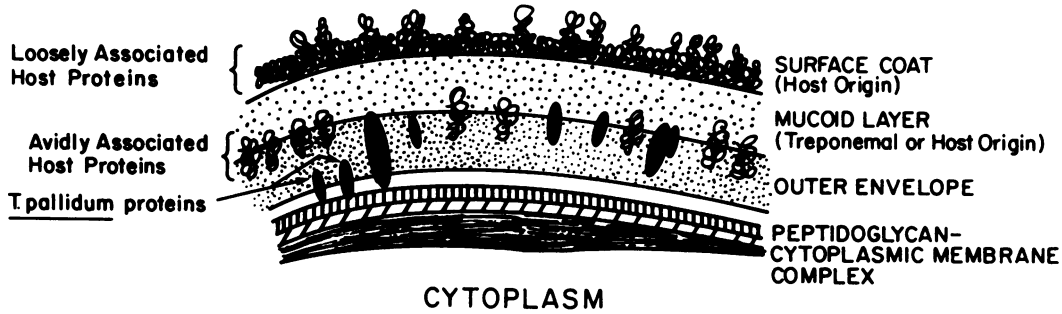

FIG. 4. *Schematic representation of the structural relationship between host serum proteins and T. pallidum.*

servations are consistent with earlier reports indicating that digestion with lysozyme or proteases, or both, must precede complement-mediated treponemal lysis (21, 24). The presence of an outer coat of host proteins as well as a mucoid layer on the treponemal surface might physically prevent additional complement components from reaching the outer envelope to cause membrane perturbations necessary for lysis (23). Alternately, virulent treponemes may be capable of inactivating the remaining complement components.

The presence of host-treponemal protein complexes may induce autoimmune reactions. Attachment of non-parasite-specific IgG and IgM plus complement components to the outer envelope of *T. pallidum* immediately after infection may have serious consequences for the host. In this regard, it has been reported that rheumatoid factor and cryoglobulins are detected in syphilitic sera and may be indicative of autoimmune reactions (25, 30). It has also been proposed that glomerulonephritis in *Trypanosoma rhodesiense*-infected rhesus monkeys is the result of activation of the alternate complement pathway by similar immune complexes (26).

Finally, biological variability of treponemal preparations from individual rabbit extractions was observed throughout these studies (Table 4), implying differences in the metabolic competence and surface composition of *T. pallidum* populations. Several years ago, we reported the separation of two distinct populations of *T. pallidum* by velocity sedimentation in Hypaque (5). The interaction of both loosely and avidly associated host macromolecules with the treponemal surface in a potentially nonrandom manner could explain these results. Furthermore, future investigation of surface-associated host proteins may clarify the complex relationship between virulence and growth of *T. pallidum* and the host's susceptibility to treponemal infection.

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