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K M Peterson and J F Alderete
Infect. Immun. **1983, 40(2):640.**

INFECTION AND IMMUNITY, May 1983, p. 640–646
0019-9567/83/050640-07$02.00/0

Vol. 40, No. 2

Acquisition of α_1-Antitrypsin by a Pathogenic Strain of *Trichomonas vaginalis*

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Received 18 October 1982/Accepted 15 February 1983

The interaction of α_1-Antitrypsin, the major serine protease inhibitor in plasma, with pathogenic *Trichomonas vaginalis* and the acquisition by trichomonads of this host protein from normal human plasma were investigated. α_1-Antitrypsin acquired by intact parasites could not be removed by repeated washings in phosphate-buffered saline. Saturation kinetics were observed after incubation of glutaraldehyde-fixed organisms with ^{125}I-labeled α_1-antitrypsin. Evidence suggesting that specific parasite membrane sites were responsible for trichomonal acquisition of α_1-antitrypsin was obtained through competitive binding experiments using purified preparations of homologous versus heterologous plasma proteins. No evidence of degradation of bound antitrypsin by live parasites was observed. The avid binding of α_1-antitrypsin by pathogenic *T. vaginalis* after incubation in normal human plasma was demonstrated by using sensitive electrophoretic and immunodetection techniques. Radioimmunoprecipitation of intrinsically labeled, detergent-solubilized extracts of *T. vaginalis* incubated with monospecific antisera against α_1-antitrypsin and other human plasma proteins revealed the inability of parasites to biosynthesize any substance cross-reactive with host plasma proteins. Finally, *T. vaginalis* organisms pretreated with α_1-antitrypsin inhibited trypsin caseinase activity in an in vitro assay. The implications of these observations are discussed.

Trichomoniasis caused by *Trichomonas vaginalis* is the most common of all sexually transmitted diseases and is responsible for significant morbidity in both men and women (18, 33). Trichomonal vaginitis is characterized by pronounced inflammation of the vagina, severe discomfort, foul-smelling discharge, and tissue cytopathology (17, 18, 33). Although most men remain asymptomatic with this infection, the overall clinical picture and symptomology remain controversial (18). The recent isolation of drug-resistant strains (26), the trends toward increased numbers of sexually transmitted diseases, and the tremendous emotional and economic burden imposed on our society by this parasite (33) reinforce the need for continued studies aimed at dissecting numerous aspects of the biology of this microorganism in order to enhance our understanding of potential mechanisms of disease pathogenesis.

The presence of host-derived macromolecules on the surfaces of microorganisms is receiving increased attention for numerous reasons. For example, biological mimicry by the parasite, through either acquisition of host substances (4, 7, 11, 13, 19, 20, 28, 31, 32, 37) or biosynthesis of hostlike molecules (3, 6, 16, 23, 24, 27, 32), may represent mechanisms for microbial modification of host defense systems. Accumulation of nutrients, sequestration of substances such as hormones, and microbial adherence to cell surfaces (9, 14, 20, 31) may result from binding of plasma or tissue components by microbial membranes.

The biological relevance of trichomonal acquisition of host proteins remains speculative. We have previously demonstrated the ability of *T. vaginalis* to bind numerous plasma proteins both loosely and avidly (28). Because knowledge of any potential biological function(s) of acquired host macromolecules bound to *T. vaginalis* surfaces would be extremely important to our understanding of this host-parasite interaction, we decided to examine in greater depth the binding to intact pathogenic *T. vaginalis* of α_1-antitrypsin (28), a protein protease inhibitor found in plasma (22). The possible relevance of parasite binding of this protease inhibitor in terms of trichomonal disease pathogenesis is discussed.

MATERIALS AND METHODS

Growth of *T. vaginalis*. *T. vaginalis* 286 (28) was cultured in vitro in the Trypticase-yeast extract maltose medium of Diamond (10), as previously described.

Radioiodination of α_1-antitrypsin. Lactoperoxidase-catalyzed radioiodination of α_1-antitrypsin (Sigma Chemical Co., St. Louis, Mo.) was performed by a modification of the procedure of Garvey et al. (15). Briefly, 1 ml of an α_1-antitrypsin protein solution prepared 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) was gently mixed with 100 μl of 1-mg/ml lactoperoxidase (Calbiochem, La Jolla, Calif.) and 10 μl containing 1 mCi of $Na^{125}I$ (Amersham Corp., Arlington Heights, Ill.). To this was added 100 μl of freshly prepared H_2O_2 (Sigma) (10 μl of stock 30% H_2O_2 in 50 ml of cold PBS). After a 15-min incubation at room temperature with gentle stirring, another 100 μl of H_2O_2 was added. The iodination was terminated with cysteine after an additional 15 min. The ^{125}I-labeled α_1-antitrypsin preparation was processed by chromatography using a Sephadex G-25 column (1 by 10 cm; Pharmacia, Piscataway, N.J.). Efficiency of radioiodination was evaluated by trichloroacetic acid (TCA) precipitation (15) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography (21, 28). The specific activity for radioiodinated α_1-antitrypsin was 60,000 cpm/μg of protein unless otherwise stated. Protein determinations were routinely performed with Bradford reagents (Bio-Rad Laboratories, Richmond, Calif.) (5).

Avid binding of radioiodinated proteins to intact trichomonads. A 10-ml volume, representing approximately 2×10^7 extensively washed *T. vaginalis* organisms (28), was fixed by the addition of 150 μl of a 50% solution of glutaraldehyde (Eastman Organic Chemicals, Rochester, N.Y.). The trichomonal suspension was then gently stirred for 1 h at 4°C. After fixation, the trichomonads were washed twice with NaCl-EDTA-Tris buffer (NET; 150 mM NaCl, 5 mM disodium EDTA, and 50 mM Tris; buffer pH 7.4), followed by incubation of the fixed organisms with 1% ovalbumin (Sigma) in NET for 30 min at room temperature. Finally, the parasites were pelleted, washed again at least three times in NET, and suspended to a density of 2×10^7 organisms per ml for binding studies as outlined below. All glassware used in the fixation steps was siliconized with Sigmacote (Sigma).

A 100-μl volume containing 2×10^6 fixed organisms was added to siliconized microfuge tubes pretreated with 1% horse serum. Subsequent addition of various amounts of radioiodinated α_1-antitrypsin in NET to a final volume of 500 μl was followed by incubation for 30 min at 37°C. In competition studies, increasing concentrations of α_1-antitrypsin or 100 μg of albumin, transferrin, and immunoglobulin G (IgG), used singly or in combination, were added to the fixed organisms for 10 min before the addition of 25 μg of ^{125}I-labeled α_1-antitrypsin. After a 30-min incubation period, the parasites were pelleted for 2 min in a Beckman Microfuge B with a Dayton variable transformer at setting 40. The supernatant was discarded, and the trichomonads were washed two times further in NET. Finally, the trichomonads were transferred to another microfuge tube and pelleted. Fixed parasites were suspended in solubilizing buffer (60.5 mM Tris-hydrochloride [pH 6.8], 2% β-mercaptoethanol, 10% glycerol, 2% SDS, and 0.1% bromophenol blue) and processed as described below. Live trichomonads were used similarly in binding studies, except all washes were performed in PBS and were then subjected to TCA precipitation (28) of total proteins for subsequent SDS-PAGE.

SDS-PAGE and immunodetection of electrophoretically blotted proteins. Elution from the surface of glutaraldehyde-treated parasites of any avidly associated proteins from plasma or α_1-antitrypsin preparations was accomplished by suspending pelleted, fixed organisms with 200 μl of solubilizing buffer. After boiling for 3 min, the suspension was stirred vigorously in a Vortex mixer, and this procedure was repeated once more. Less than 15% of all radioactivity remained on fixed trichomonads that were pelleted by centrifugation by this method. Solubilized radioiodinated proteins in the supernatant were then loaded onto individual wells of 3 and 7.5% acrylamide stacking and separating slab gels, respectively (21), for SDS-PAGE. Electrophoresis was carried out in a 16-cm slab gel apparatus (Bio-Rad Laboratories) as previously described (28). Gels were stained with Coomassie brilliant blue (Sigma), destained, dried, and exposed to X-ray film (X-Omat, AR5; Eastman Kodak) for various lengths of time at room temperature before development.

To determine whether α_1-antitrypsin was acquired from unlabeled normal human plasma, electrophoretic blotting onto nitrocellulose (34) of any plasma proteins acquired by fixed *T. vaginalis* was accomplished after SDS-PAGE as described above. Additionally, total TCA-precipitated trichomonal proteins (1, 28) from live organisms incubated with unlabeled normal human plasma (NHP) (28) were electrophoresed and blotted for analysis of acquired, avidly bound host plasma α_1-antitrypsin. The numbers of live trichomonads and experimental conditions used for incubation with NHP or pure α_1-antitrypsin were identical to those described for fixed organisms. Electrophoretic transfer of most or all proteins from the acrylamide gels was performed by a modification of the method of Towbin et al. (34) with a Trans Blot Cell (Bio-Rad Laboratories). Blotting was carried out at 300 mA for 12 h in a solution consisting of 25 mM Tris base, 192 mM glycine, and 20% methanol (pH 8.3). The nitrocellulose blots were then removed and incubated with 5% bovine serum albumin (BSA; Sigma), prepared in Tris-NaCl (TN; 50 mM Tris and 154 mM NaCl) buffer (pH 7.5), for 1 h at 37°C to block any nonspecific binding sites. Finally, 0.5 ml of the respective control sera or of monospecific hyperimmune goat antiserum to human α_1-antitrypsin (Cappel Laboratories, Cochranville, Pa.) in 50 ml of BSA-TN buffer was added to the blots. After a 2-h incubation with gentle rocking at room temperature, the blots were washed well three times with 100 ml of TN–0.05% Nonidet P-40 (Particle Datalabs, Elmhurst, Ill.) for 30 min each, followed by three washes with TN buffer alone. The blots were then incubated with 50 ml of TN–5% BSA containing 100 μl of ^{125}I-protein A (specific activity, 1.2×10^7 cpm/μg) radiolabeled by a modified chloramine T-mediated iodination (12). The nitrocellulose blots were dried in warm air and then exposed to X-ray film for autoradiography. Acrylamide gels from TCA-precipitated organisms were always stained after blotting to insure that efficient transfer of proteins had occurred.

RIP and serum reagents. To determine whether biosynthesis of hostlike molecules by *T. vaginalis* was responsible for the observed results, radioimmunoprecipitation (RIP) experiments using detergent-solubi-

lized preparations (1) of intrinsically labeled trichomonads (28) were performed. The generation of control sera and antisera against *T. vaginalis* 286 in immunized New Zealand white rabbits is described in detail elsewhere (1). These sera have been extensively evaluated for reactivity against specific trichomonal proteins. Approximately 51,000 cpm of detergent-solubilized, radiolabeled *T. vaginalis* was incubated with 50 μl of the respective serum reagent. Quadruplicate samples were processed (1), and individual supernatants from each pair of samples were electrophoresed as described above. The other samples were used for counting on a Beckman LS-230 liquid scintillation counter. A 50% precipitation of radioactivity was obtained, using immunized rabbit sera as a positive control. In contrast, normal rabbit serum and the sera listed below immunoprecipitated less than 0.5% of radioactivity.

Commercially available normal goat serum and normal rabbit serum (Cappel Laboratories) were also used as controls for these RIP experiments. All antisera 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 antiserum reacted monospecifically with its respective host protein or fresh plasma. Sera against whole human plasma and transferrin were generated in rabbits. Sera against α_1-antitrypsin, fibrinogen, ceruloplasmin, fibronectin, α_2-macroglobulin, IgM, IgG, albumin, and β-subunits of chorionic gonadotropin were obtained from goats.

Protease inhibition assay. Inhibition of trypsin by trichomonads was evaluated by using published assays with casein as substrate (2). Washed, motile trichomonads were suspended in 1 ml of PBS containing 10 mg of α_1-antitrypsin and incubated for 30 min at 37°C. After two washings with PBS, 5×10^7 pelleted trichomonads were suspended in a 100-μl volume containing 1 μg of trypsin and incubated for an additional 30 min at room temperature. Parasites were then removed by centrifugation, and 100 μl of supernatant was incubated with 850 μl of 6-mg/ml casein for 1 h at 37°C with occasional shaking. Precipitation of undigested casein substrate was then accomplished by the addition of TCA (5% final concentration), and the supernatant was examined spectrophotometrically at 280 nm. Endogenous parasite protease activity (8) was also determined by using live motile parasites handled identically but without trypsin. Trichomonal caseinase activity in the presence or absence of exogenous α_1-antitrypsin never exceeded 20% of that observed for trypsin only. In addition, this parasite caseinase activity (8) was not inhibited by levels of α_1-antitrypsin shown to be in excess for the same proteolytic activity as trypsin. Individual samples of trypsin and casein processed as described above resulted in no detectable release of amino acids.

RESULTS

Acquisition of α_1-antitrypsin. Initial studies designed to implicate parasite surface structures in the binding of plasma proteins through the use of surface-modifying reagents were ineffective (28). Furthermore, trichomonads appears to be more refractory to treatment with trypsin, a serine protease, than with other nonspecific proteolytic enzymes. Based on our earlier studies demonstrating the presence of α_1-antitrypsin on *T. vaginalis* surfaces (28), the interaction of this effective protease inhibitor with intact organisms was examined.

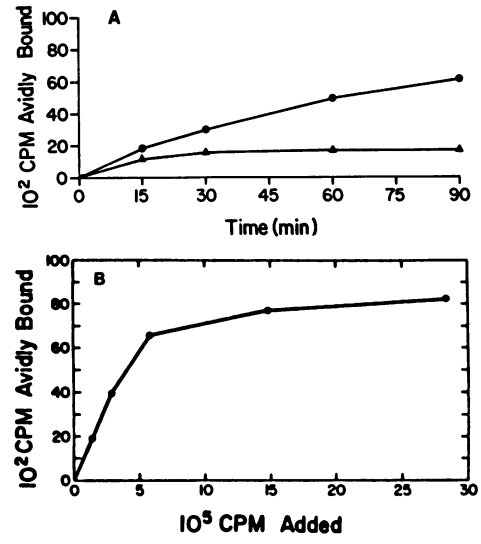

FIG. 1. (A) Representative binding kinetics of ^{125}I-labeled α_1-antitrypsin incubated with pathogenic *T. vaginalis* 286. Acquisition at 37°C by motile trichomonads (●) was compared to binding by live parasites at 4°C (▲). (B) Representative saturation binding curve of α_1-antitrypsin by fixed *T. vaginalis* strain 286. Glutaraldehyde-fixed organisms (2×10^6) were incubated at 37°C with increasing amounts of ^{125}I-labeled α_1-antitrypsin (abscissa), and the cpm eluted from trichomonads (ordinate) was recorded as described in the text. Each point represents the average of three samples from a typical experiment.

The binding of ^{125}I-labeled α_1-antitrypsin to *T. vaginalis* was evaluated at 4 and 37°C, using live trichomonads incubated for various lengths of time. Acquisition of α_1-antitrypsin by motile trichomonads incubated at 4°C was reduced as compared to that at 37°C (Fig. 1). Maximal binding achieved at 90 min represented approximately 100 ng of bound α_1-antitrypsin under these conditions. Also, because trichomonal processing of surface-oriented α_1-antitrypsin might be occurring under these conditions, we incubated trichomonads over extended periods after exposure to ^{125}I-labeled α_1-antitrypsin. Electrophoretic analysis and immunodetection studies (as shown later), however, gave no evidence for degradation of α_1-antitrypsin.

To determine whether ligand-receptor interactions were responsible for the binding of α_1-antitrypsin, we attempted to demonstrate saturation by using live organisms with increasing

TABLE 1. Acquisition of ^{125}I-labeled α_1-antitrypsin by *T. vaginalis* in the presence of unlabeled plasma proteins

Unlabeled protein[a] in reaction vessel (μg)	cpm avidly bound[b] (% of control)
PBS	7,530 (100)
α_1-Antitrypsin (25)	5,050 (67)
α_1-Antitrypsin (50)	1,710 (23)
α_1-Antitrypsin (100)	50 (1)
Albumin (100)	7,680 (100)
Transferrin (100)	7,420 (99)
IgG (100)	7,260 (97)

[a] Unlabeled proteins were incubated with 2×10^6 trichomonads for 20 min before the addition of saturating amounts (25 μg) of radioiodinated α_1-antitrypsin. The final reaction volume in microfuge tubes was 200 μl.

[b] Avidly bound counts were determined as described in the text.

amounts of antitrypsin. Saturation with motile trichomonads under these assay conditions could not be readily achieved in a manner analogous to that shown in Fig. 1. Therefore, we attempted to obtain saturation by using glutaraldehyde-fixed parasites. The ability to readily attain saturable binding with fixed *T. vaginalis* is shown in Fig. 1B, and the amount of antitrypsin bound under these conditions was about 120 ng, a value similar to that obtained by using live parasites and saturating levels of α_1-antitrypsin. These initial data suggest that the live parasites may be actively modulating their membranes and preventing establishment of fixed numbers of receptors for saturation with α_1-antitrypsin.

Experiments demonstrating specific acquisition of α_1-antitrypsin by fixed *T. vaginalis* were then attempted. As can be seen from a representative competition experiment shown in Table 1, the addition of increasing amounts of unlabeled α_1-antitrypsin to the reaction mixture before the incubation of fixed trichomonads with radioiodinated α_1-antitrypsin resulted in decreased levels of bound radioactivity as compared to PBS controls. The levels of radioiodinated α_1-antitrypsin bound to *T. vaginalis* as measured in the PBS control represented about 120 ng. In contrast, no detectable decrease of bound ^{125}I-labeled α_1-antitrypsin was observed when high levels (100 μg) of albumin, transferrin, or IgG (heavy chain has a molecular weight similar to α_1-antitrypsin) were employed.

Elution of ^{125}I-labeled α_1-antitrypsin avidly associated with fixed trichomonads was accomplished by using an SDS-mercaptoethanol detergent system, and only one major band with a molecular weight of 54,000, along with contaminating albumin and another plasma protein, was observed (Fig. 2, lane A). This pattern correlated with a duplicate sample of 100 ng of pure α_1-antitrypsin electrophoresed separately (data not shown) and is in agreement with the levels reported in Table 1 for identical numbers of organisms. The ability of unlabeled α_1-antitrypsin (100 μg) to compete effectively and almost totally with binding of saturating amounts of radioiodinated α_1-antitrypsin is shown in lane B of Fig. 2. Again, this profile is consistent with competition experiments for similar values used, as presented in Table 1. The specificity of α_1-antitrypsin–trichomonal membrane interactions was confirmed by a lack of competition when high levels of albumin (100 μg), transferrin (100 μg), or IgG (100 μg) were used either singly or in combination (Fig. 2, lane C). The amount of radioactivity recovered from fixed trichomonads under these conditions, as seen in lanes A and C

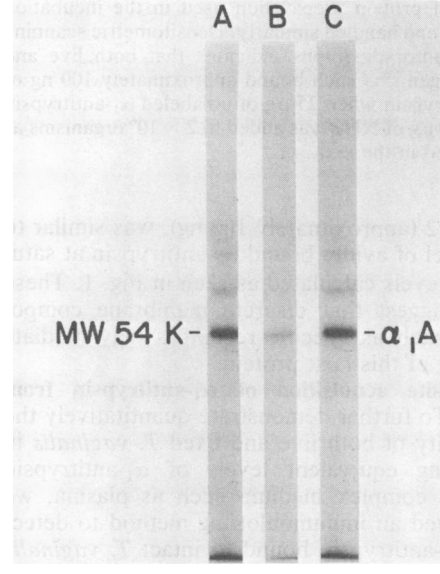

FIG. 2. SDS-PAGE autoradiography of radioiodinated α_1-antitrypsin (α_1-A) eluted from glutaraldehyde-fixed *T. vaginalis* 286. Lane A, α_1-Antitrypsin band with a molecular weight of 54,000 eluted after incubation of trichomonads with 25 μg of ^{125}I-labeled commercially available preparation. This profile was equivalent to a control protein gel pattern of 100 ng of radioiodinated α_1-antitrypsin that was used in the acquisition experiments. Lane B, Competition of 25 μg of ^{125}I-labeled α_1-antitrypsin incubated with trichomonads in the presence of 100 μg of unlabeled α_1-antitrypsin. The intensity of the band represented less than 10% of that seen in lane A. Lane C demonstrates the lack of competition of radioiodinated α_1-antitrypsin binding in the presence of albumin (100 μg), transferrin (100 μg), and IgG (100 μg). These heterologous proteins were added either singly or in combination to the reaction mixture.

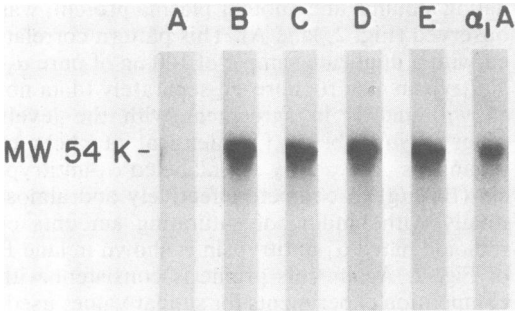

FIG. 3. Electrophoretic transfer and immunodetection of unlabeled α_1-antitrypsin acquired by live (lane B and C) and fixed (lanes D and E) *T. vaginalis* incubated with preparations of commercially purified α_1-antitrypsin (lanes B and D) or with NHP (lanes C and E). Monospecific antiserum against α_1-antitrypsin and incubation of blots with ^{125}I-labeled protein A were used for localization of α_1-antitrypsin as described in the text. Lane A shows the lack of nonspecific protein A binding to control nitrocellulose sheets handled identically. α_1-A refers to a control sample of 50 ng of protein preparation used in the incubation mixture and handled similarly. Densitometric scanning of the autoradiographs indicates that both live and fixed organisms each bound approximately 100 ng of α_1-antitrypsin when 25 μg of unlabeled α_1-antitrypsin or 1,000 μg of NHP was added to 2×10^6 organisms as described in the text.

of Fig. 2 (approximately 100 ng), was similar to the level of avidly bound α_1-antitrypsin at saturation levels calculated as seen in Fig. 1. These data suggest that discrete membrane components such as specific receptors may mediate binding of this host protein.

Parasite acquisition of α_1-antitrypsin from NHP. To further demonstrate quantitatively the similarity of both live and fixed *T. vaginalis* in acquiring equivalent levels of α_1-antitrypsin from a complex medium such as plasma, we employed an immunoblotting method to detect any α_1-antitrypsin bound to intact *T. vaginalis* surfaces (Fig. 3). The immunodetection of α_1-antitrypsin was accomplished by using monospecific hyperimmune antiserum incubated with nitrocellulose blots of total TCA-precipitated proteins obtained after incubation of live, motile organisms with either saturating amounts of α_1-antitrypsin (25 μg) (Fig. 3, lane B) or NHP (1,000 μg) (lane C). Alternately, avidly adsorbed α_1-antitrypsin (lane D) or NHP proteins (lane E) eluted from fixed trichomonads were electrophoresed and processed similarly. These data demonstrate the ability of pathogenic *T. vaginalis* to acquire this distinct protein from whole plasma. The specificity of these procedures was apparent by the absence of ^{125}I-labeled protein A binding to any other protein bands in the blotted protein profiles (Fig. 3, lane A). Again, as with Fig. 2, the amount of unlabeled α_1-antitrypsin (100 ng) bound to live or fixed trichomonads under these conditions was in agreement with the expected values obtained from saturation binding studies (Fig. 1B).

Trichomonal biosynthesis of α_1-antitrypsin and other hostlike molecules. To eliminate the possibility of *T. vaginalis* biosynthesis of an α_1-antitrypsin-like protein, highly sensitive and specific RIP procedures (1) were used. Only anti-*T. vaginalis* serum from immunized rabbits immunoprecipitated intrinsically labeled, Zwittergent-solubilized parasite proteins (1) as compared to control serum or sera against various host plasma proteins (see above) or whole plasma. SDS-PAGE–fluorography of all samples did not detect any protein bands, which confirms the lack of cross-reactivity between any trichomonal protein and either human α_1-antitrypsin or other host macromolecules.

Inhibition of trypsin activity by α_1-antitrypsin on live trichomonads. To test the ability of *T. vaginalis*-bound α_1-antitrypsin to inactivate proteases in an aqueous environment, we incubated α_1-antitrypsin-coated organisms with trypsin and assessed the inactivation of trypsin by using a caseinase assay (2). The extent of inhibition was related to the numbers of pretreated parasites and to the amount of α_1-antitrypsin used in the preincubation mixture (Table 2). The absence of trichomonads from the assay system yielded greater absorbancy values at 280 nm, indicative of aromatic amino acids released by trypsin digestion of the substrate (2). In different experiments, simultaneous addition of α_1-antitrypsin-treated parasites, trypsin, and casein yielded similar results (data not shown).

DISCUSSION

Our initial attempt to understand the interaction of trichomonads with host products was to study the association between plasma or tissue components and parasite surfaces (28). It is possible that parasites capable of acquiring biologically relevant host macromolecules may take advantage of their functional properties as an aid to survival in an otherwise unfavorable environment.

The interaction of commercially available radioiodinated α_1-antitrypsin with the surface of *T. vaginalis* was examined. The avid binding of ^{125}I-labeled α_1-antitrypsin to membranes of live trichomonads was evident from the fact that repeated washing in PBS did not remove it. Studies of binding properties (Fig. 1) and of competition with homologous and heterologous plasma protein preparations (Table 1 and Fig. 2) established the specificity of α_1-antitrypsin acquisition by *T. vaginalis* and implied that membrane sites (receptors) mediate this interaction.

TABLE 2. Inhibition of trypsin-mediated digestion of casein by α_1-antitrypsin on the surface of *T. vaginalis*[a]

Expt	No. of trichomonads in reaction mixture	Preincubation with α_1-antitrypsin[b] (concn, mg/ml)	Trypsin digestion index[c] (OD_{280})
1	0		0.57 ± 0.02
	5×10^7	10	0.40 ± 0.01 (30%)[d]
	10×10^7	10	0.33 ± 0.02 (44%)
2	0		0.62 ± 0.02
	5×10^7		0.51 ± 0.01 (18%)
	5×10^7	10	0.40 ± 0.03 (36%)
	5×10^7	20	0.34 ± 0.02 (46%)

[a] The assay for trypsin digestion of casein substrate was as follows: 1 μg of trypsin in 100 μl of PBS was added to 0.85 ml of PBS containing 6 mg of casein and incubated for 1 h at 37°C. TCA was added to a 5% final concentration; the mixture was centrifuged, and the supernatant was examined spectrophotometrically. Trypsin action was measured by the absorbance of released amino acids at 280 nm (OD_{280}).

[b] Washed trichomonads were pretreated with α_1-antitrypsin as indicated and resuspended in 100 μl of PBS containing 1 μg of trypsin. After incubation for 30 min at room temperature, parasites were removed, and the supernatant was added to the casein substrate as described above. Three micrograms of α_1-antitrypsin will inhibit 1 μg of trypsin.

[c] Final values were obtained by subtracting from each experimental (OD_{280}) value the parasite protease activity detected by incubation of live motile parasites alone with casein substrate. This protease activity never exceeded 20% of that observed for trypsin only.

[d] Numbers in parentheses represent the extent of inhibition as compared to control values.

That similar trichomonal membrane components in both live and fixed organisms were involved in α_1-antitrypsin binding was demonstrated by the recovery of identical levels of α_1-antitrypsin from live and fixed organisms incubated with pure α_1-antitrypsin or NHP (Fig. 3). In addition, the absence of alterations in bound radioiodinated α_1-antitrypsin protein patterns for live trichomonads incubated for extended periods (data not shown) indicated that internalization and degradation of this host molecule does not occur. Support for the idea of a surface orientation of α_1-antitrypsin on both live and fixed parasites was obtained by the ability to remove bound α_1-antitrypsin by brief pronase treatment.

Numerous procaryotic and eucaryotic microorganisms possess the ability to biosynthesize hostlike molecules such as blood group antigens and hormones (3, 6, 7, 23, 32). Additionally, *Ascaris lumbricoides*, an intestinal roundworm, produces three soluble proteins with properties analogous to host α_1-antitrypsin (16). RIP allowed us not only to establish the inability of trichomonads to produce a substance cross-reactive with α_1-antitrypsin (Table 1), but also to screen further for parasite production of a spectrum of other important plasma proteins. No biosynthesis of cross-reactive plasma proteins by these pathogenic trichomonads was evident under these experimental conditions.

α_1-Antitrypsin is a serum glycoprotein which inhibits a variety of proteolytic enzymes such as trypsin, chymotrypsin, pancreatic elastase, and the neutral proteases of leukocytes (25). Its biological role appears to be the prevention of indiscriminate degradation of tissue by endogenous and exogenous proteases (35). Thus, the ability of *T. vaginalis* to bind large amounts of α_1-antitrypsin might afford the parasite similar protection from host fluid (vaginal and plasma) or cellular (leukocyte) degradative substances. This possibility appears reasonable in light of observations by some investigators (29) of the high polymorphonuclear leukocyte-to-trichomonad ratio in the vaginal secretions of infected individuals. The ability of trichomonads coated with α_1-antitrypsin to inhibit trypsin activity in an aqueous environment was also demonstrated in a caseinase assay (2) (Table 2). Finally, calculations based on saturation data by *T. vaginalis* indicate that late-log organisms each possess up to 4×10^5 "receptors" for α_1-antitrypsin. These data are consistent with literature on eucaryotic cell interactions with other plasma proteins (30, 36).

In summary, we have attempted to characterize further the acquisition of a plasma protein previously found to bind avidly to *T. vaginalis* (28). We recognize the need to temper our interpretations of the in vivo biological function of this host protein-parasite interaction. It was difficult to address this important issue because of our inability to grow *T. vaginalis* in the absence of serum or plasma. Additionally, the inability to recover from infected individuals or inoculated animals (17) numbers of organisms sufficient for experimentation continues to impede in vivo studies by us and others. Therefore, answers to these questions await development of an animal model with a urogenital-vaginal infection equivalent to human trichomoniasis. We hope these results will nonetheless ultimately enhance our understanding of the overall process leading to trichomonal disease pathogenesis.

ACKNOWLEDGMENTS

This work was supported by an institutional research grant from UTHSCSA and by Public Health Service grant AI-18768 from the National Institutes of Health to J.F.A.

We extend special thanks to Grace Wagner for her patient and expert secretarial assistance. The helpful discussions with Joel B. Baseman and Robert Thornburg are acknowledged.

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