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Binding of Streptococcal Lipoteichoic Acid to Fatty Acid-Binding Sites on Human Plasma Fibronectin

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The ability of *Streptococcus pyogenes* lipoteichoic acid and palmitic acid to bind to purified human plasma fibronectin was investigated. Initial studies indicated that intact fibronectin formed soluble complexes with lipoteichoic acid, resulting in a change in the mobility of fibronectin in an electrical field. Fibronectin covalently linked to agarose beads bound radiolabeled lipoteichoic acid in the acylated form but not in the deacylated form. An 18-M excess of fibronectin inhibited binding of lipoteichoic acid to the immobilized protein by 92%. Fibronectin-bound [^{3}H]lipoteichoic acid could be specifically eluted with unlabeled lipoteichoic acid, as well as by fatty acid-free serum albumin. Serum albumin, which is known to contain fatty acid-binding sites capable of binding to the lipid moieties of lipoteichoic acid, inhibited the binding of lipoteichoic acid to fibronectin in a competitive fashion. The fibronectin-bound lipoteichoic acid could be eluted by 50% ethanol and various detergents but not by 1.0 M NaCl, various amino acids, or sugars. Similarly, radiolabeled palmitic acid adsorbed to fibronectin could be eluted with 50% ethanol but not with 1.0 M NaCl. Fibronectin adsorbed to a column of palmityl-Sepharose was eluted with 50% ethanol in 0.5% sodium dodecyl sulfate but not with 1.0 M NaCl or 1% sodium dodecyl sulfate alone. The binding of lipoteichoic acid to fibronectin followed first-order kinetics and was saturable. A Scatchard plot analysis of the binding data indicated a heterogeneity of lipoteichoic acid-binding sites similar to that previously found for serum albumin. Nevertheless, fibronectin contains at least one population of high-affinity binding sites for lipoteichoic acid. The binding affinity ($nKa \simeq 250\ \mu M^{-1}$) is 2 orders of magnitude greater than the binding affinity of serum albumin. These data suggest that human plasma fibronectin contains specific binding sites for fatty acids and that lipoteichoic acid binds to these sites by way of its glycolipid moiety.

Lipoteichoic acid (LTA), an amphipathic molecule found in most gram-positive bacteria, consists of a chain of polyglycerol phosphate covalently linked to a glycolipid at one end. Depending on the species of origin, the polyglycerol phosphate backbone is substituted to varying degrees with glycosyl and alanyl residues (49). The backbone of *Streptococcus pyogenes* LTA is substituted with alanyl but not glycosyl residues (23, 24, 41); the glycolipid moiety consists of glycerophosphoryldiglycosyl diglyceride (10, 42).

LTA has been implicated in a number of inflammatory reactions in experimental animals, including arthritis (28), nephritis (47), and bone resorption (17). LTA causes lymphocytes to undergo mitogenesis (4), stimulates the release of lysosomal enzymes from macrophages (16), and binds to a variety of eucaryotic cells (2–8, 29, 40). In addition, LTA inhibits attachment of streptococci to oral epithelial cells (2, 6, 29), and the number of LTA-binding sites on these cells is related to their binding capacity for streptococci (40). Each of these biological activities depends on the lipid moiety of the LTA molecule. In addition, the polyanionic backbone of LTA forms complexes with cell wall proteins of *Streptococcus pyogenes* (30).

Fibronectin, a large (440,000-dalton) glycoprotein (1, 25, 26, 31, 35), is a soluble constituent of blood plasma and other tissue fluids and an insoluble component of the extracellular matrix of various cells and tissues (9, 21). This molecule is present in stimulated saliva (37) and is associated with human buccal mucosal cells (51). Recently, plasma fibronectin has been found to bind to *Staphylococcus aureus* (20, 27) and to *Streptococcus pyogenes* (38). We have shown that LTA blocks the binding of fibronectin to streptococci (38). Deacylated LTA failed to inhibit the binding of fibronectin to streptococci or to inhibit streptococcus-induced agglutination of

fibronectin-coated latex beads, suggesting that the interaction of LTA and fibronectin is dependent on the intact ester-linked fatty acids of the LTA molecules. In this paper we present evidence that plasma fibronectin possesses fatty acid-binding sites similar to those of serum albumin (13), β-lactoglobulin (43), and the z-protein of heart and liver tissue (11, 48). Furthermore, we show that the binding of LTA to these fatty acid-binding sites is mediated by the lipid moiety of the LTA molecule.

MATERIALS AND METHODS

Materials. [^{3}H]palmitic acid (specific activity, 11.6 Ci/mmol), [^{125}I]fibronectin (specific activity, 23 μCi/μg), and [2-^{3}H]glycerol (specific activity 200 mCi/mmol) were purchased from New England Nuclear Corp., Boston, Mass. Bovine serum albumin (fatty acid free), hyaluronic acid, amino acids (arginine, histidine, tyrosine, tryptophan, phenylalanine), heparin, sugars (α-methyl-D-mannoside, sucrose, maltose, glucose), Tween 20, and palmitic acid were obtained from Sigma Chemical Co., St. Louis, Mo. Triton X-100 and sodium dodecyl sulfate were purchased from Bio-Rad Laboratories, Richmond, Calif.

[^{3}H]LTA (specific activity, 3,000 to 9,000 cpm/μg) was extracted with phenol from the 1RP41 strain of group A streptococci (originally obtained from R. Lancefield, The Rockefeller University, New York, N.Y., and stored frozen at −70°C in Todd-Hewitt broth supplemented with 20% normal rabbit serum) grown in a chemically defined medium (44) supplemented with [2-^{3}H]glycerol and purified on a bovine serum albumin column, as previously described (8, 39). Deacylated [^{3}H]LTA was obtained by NH_4OH hydrolysis of purified [^{3}H]LTA as described previously (29).

Fibronectin was purified by affinity chromatography of human plasma on columns of gelatin-Sepharose, followed by chromatography on columns of arginine-Sepharose, as described by Vuento and Vaheri (46). The purified fibronectin migrated as a typical doublet during 0.1% sodium dodecyl sulfate–5% polyacrylamide gel electrophoresis and migrated as a homogenous protein with an apparent molecular weight of ≃220,000 under reducing conditions. In some experiments, the purified fibronectin was labeled with [^{3}H]formaldehyde by the method of Grinnel (14).

Preparation of fibronectin-Sepharose beads. Purified fibronectin was coupled to cyanogen bromide-activated Sepharose 6-B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) by the procedure of March et al. (22). The amounts of fibronectin cross-linked to several batches of activated beads prepared during the course of our experiments ranged from 50 to 100 μg/ml of packed beads, as determined by automatic amino acid analysis with a Beckman model 121 instrument of 2 ml of packed beads hydrolyzed for 18 h in constantly boiling 6 N HCl (19).

Assays for binding of [^{3}H]LTA to fibronectin. Assays for binding of [^{3}H]LTA to fibronectin were performed by adding different amounts of [^{3}H]LTA to a 50% suspension of fibronectin-Sepharose to a final volume of 0.25 ml in 0.15 M NaCl–0.02 M phosphate (pH 7.4) (NaCl/P_i). At different times, triplicate samples were removed, and the beads were collected by membrane filtration (pore size, 1.0 μm; Nucleopore Corp., Pleasanton, Calif.) under reduced pressure and washed with 10 ml of NaCl/P_i. The membrane filters were then mixed with 10 ml of Scintiverse (Fisher Scientific Co., Fairlawn, N.J.) and counted with a Packard Tri-Carb scintillation counter with an efficiency of 26%. Quenching was monitored as previously described (39). The binding of [^{3}H]LTA to control gelatin-Sepharose beads (see below) was less than 5% of the binding to the fibronectin-Sepharose beads.

Various substances were tested for the ability to elute bound [^{3}H]LTA from fibronectin-Sepharose by first incubating the immobilized fibronectin with [^{3}H]LTA for 30 min, removing the unbound LTA by washing three times with NaCl/P_i, and then adding the substances. After 30 min of incubation at ambient temperature, triplicate samples were removed and washed, and the radioactivity was counted as described above. The amount eluted was expressed as follows: {1 − (counts per minute of [^{3}H]LTA bound in the presence of a test substance/counts per minute of [^{3}H]LTA bound in the presence of NaCl/P_i)} × 100.

Competitive inhibition assays were performed by incubating different concentrations of [^{3}H]LTA with a 50% suspension of fibronectin-Sepharose in the presence or absence of albumin. The mixture was incubated for 60 min at ambient temperature, filtered, washed, and assayed for radioactivity as described above.

Chromatography of [^{3}H]LTA on fibronectin-Sepharose columns. Disposable columns (10 by 0.7 cm; Bio-Rad) were packed with fibronectin-Sepharose to yield a total bed volume of approximately 2.5 ml. [^{3}H]LTA or deacylated [^{3}H]LTA (6 μg) in NaCl/P_i was loaded onto the columns and equilibrated overnight at ambient temperature. The columns were washed consecutively with 2 column volumes of NaCl/P_i, 1.0 M NaCl, and 10 to 50% ethanol in NaCl/P_i. Fractions (0.2 ml) were collected and assayed for radioactivity as described above. Control columns consisted of gelatin rather than fibronectin conjugated to Sepharose.

Chromatography of [^{125}I]fibronectin on palmityl-Sepharose column. [^{3}H]palmitic acid was coupled to AH-Sepharose (Pharmacia) with carbodiimide by the method of Peters et al. (32). Approximately 10 μmol of palmitic acid was bound per ml of packed beads. A column (10 by 0.7 cm; Bio-Rad) was packed with 3 ml of palmityl-Sepharose in NaCl/P_i, 3 μg of [^{125}I]fibronectin was applied to the column, and the column was then washed with various eluting agents as described above. Fractions (0.75 ml) were collected and monitored for eluted [^{125}I]fibronectin with a Packard Autogamma scintillation counter. In some experiments [^{3}H]fibronectin was used, and similar results were obtained.

Binding of [^{3}H]palmitic acid to fibronectin-Sepharose. Distilled water was saturated with [^{3}H]palmitic acid by dissolving [^{3}H]palmitic acid in heptane, adding an equal volume of distilled water, and mixing. The two phases were allowed to separate and equilibrate for 2 days at ambient temperature. A sample from the aqueous phase was removed, applied to a fibronectin-Sepharose column (10 by 0.7 cm; Bio-Rad), and allowed to equilibrate overnight at ambient temperature. The column was then washed with various eluting agents as described above. Fractions (1.0 ml) were

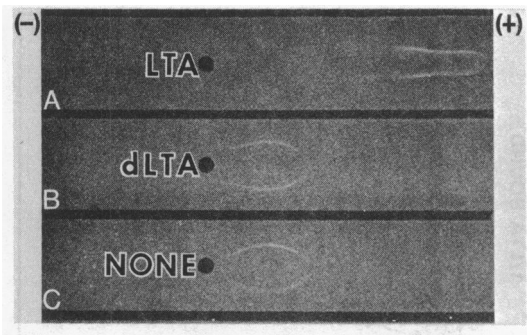

FIG. 1. Immunoelectrophoresis of fibronectin alone (C), fibronectin mixed with LTA (A), and deacylated LTA (dLTA) (B). After electrophoresis, rabbit antifibronectin was placed in each of the troughs, and immunoprecipitin lines were allowed to develop for 25 h in a humid atmosphere at ambient temperature.

collected and assayed for radioactivity as described above.

Immunoelectrophoresis. Fibronectin (500 μg/ml) was mixed with LTA (2 mg/ml), deacylated LTA (2 mg/ml), or NaCl/P_i and incubated for 18 h at ambient temperature. The mixtures were placed in wells on an agarose plate (Immunotec II; Behring Diagnostic, Somerville, N.J.) and electrophoresed for 1.5 h at 140 V in 0.04 M Veronal buffer (pH 8.3). Immunoprecipitin lines were developed by using rabbit antifibronectin.

Antisera. Fibronectin (100 μg in complete Freund adjuvant) was injected subcutaneously into New Zealand White rabbits, and a booster (100 μg in Freund incomplete adjuvant) was injected after 4 weeks. Sera were collected at weekly intervals and stored at 4°C. The specificity of antifibronectin was demonstrated by a single precipitin line when it was tested against human plasma.

RESULTS

Complexing of LTA with fibronectin. LTA mixed with fibronectin markedly changed the mobility of fibronectin in an electrical field, whereas deacylated LTA had no effect (Fig. 1), suggesting that LTA formed complexes with fibronectin through the lipid moiety of the LTA molecule. This idea was further substantiated by mixing fibronectin with radiolabeled LTA, followed by immunoelectrophoresis; assays of 0.5-cm slices of the agar gel showed that the region of maximum radioactivity coincided with the precipitin line produced by antifibronectin (data not shown). These results are similar to the results of previous studies of the interaction of LTA with serum albumin, which is known to possess fatty acid-binding sites (39). To characterize further the binding of LTA to fibronectin, purified human plasma fibronectin was immobilized by covalently linking it to Sepharose beads. When radiolabeled LTA was added to a column of fibronectin-Sepharose, the LTA was retained on the column. When this preparation was applied to a second column of fibronectin-Sepharose, almost all (87%) of the material in the flow-through (first peak) was retained, indicating that the first column was overloaded with LTA. The bound LTA was quantitatively eluted by aqueous solutions of ethanol but not by 1.0 M NaCl (Fig. 2). In contrast, none of the radiolabeled, deacylated LTA added to the column was able to bind to the fibronectin-Sepharose; all of it was eluted in the initial buffer flow-through (Fig. 2) in a manner similar to the manner previously observed in binding studies of LTA to immobilized serum albumin (39). In control experiments in which a gelatin-Sepharose column was used, more than 98% of the applied LTA was recovered in the initial flow-through, indicating that nonspecific binding of LTA to the support medium was negligible. These results further suggested that the lipid moiety was an important determinant in the binding of LTA to fibronectin.

Specificity of the binding of LTA to fibronectin. To determine the specificity of the binding of LTA to fibronectin, various substances were tested for the ability to elute the LTA bound to immobilized fibronectin. More than 85% of the radiolabeled LTA was eluted by unlabeled LTA (Table 1). Serum albumin (14.9 μM), which is known to bind to the lipid moieties of LTA (39), eluted 72% of the fibronectin-bound LTA. Each of the detergents tested eluted more than 92% of

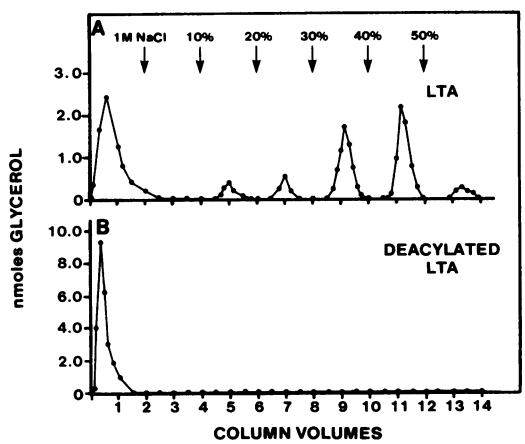

FIG. 2. Elution profile of LTA (A) and deacylated LTA (B) from a fibronectin-Sepharose column. A 6-μg portion of [^{3}H]LTA (A) or deacylated [^{3}H]LTA (B) was loaded onto a fibronectin-Sepahrose column with a bed volume of 2.5 ml and equilibrated overnight at ambient temperature. The column was washed consecutively with 5 ml of NaCl/P_i, 1 M NaCl, and a 5-ml stepped gradient containing 10 to 50% ethanol in NaCl/P_i. Fractions (0.2 ml) were collected and assayed for radioactivity as described in the text.

TABLE 1. Elution of radiolabeled LTA from immobilized fibronectin by various substances

Test substance[a]	% Eluted
$NaCl/P_i$	0
Unlabeled LTA (117.6 μM)	87
Bovine serum albumin (14.9 μM)	72
Fibronectin[c] (2.3 μM)	42
(4.6 μM)	63
Fibronectin-binding substances	
Gelatin (1 mg/ml)	32
Heparin (1 mg/ml)	0
Hyaluronic acid (1 mg/ml)	8
Glucose (2.5%)	8
Arginine (1 mg/ml)	4
Detergents	
Sodium dodecyl sulfate (1%)	92
Tween 20 (1%)	93
Triton X-100 (1%)	95
Zwittergent (1%)	95
Amino acids	
L-Tryptophan (1 mg/ml)	6
L-Phenylalanine (1 mg/ml)	6
L-Tyrosine (1 mg/ml)	0
L-Histidine (1 mg/ml)	0
Sugars	
α-Methyl-D-mannopyranoside (2.5%)	12
Sucrose (2.5%)	0
Maltose (2.5%)	7

[a] Test substances were dissolved in $NaCl/P_i$.

[b] Fibronectin-Sepharose beads with bound [^{3}H]LTA were mixed with test substances. After 30 min, triplicate samples were removed, washed with $NaCl/P_i$, and assayed for radioactivity as described in the text. Percent eluted was calculated as follows: [1-(amount of LTA bound with test substance/amount of LTA bound with $NaCl/P_i$)] × 100.

[c] Fibronectin concentrations in excess of 2 mg/ml often resulted in visible aggregates of fibronectin and therefore were not used.

the LTA. Because Vuento et al. (45) have shown that the nonionic detergents used in our experiments have no effect on the binding of fibronectin to gelatin-Sepharose, the elution of LTA by these detergents appears to be specific for a fatty acid-binding site(s) distinct from the gelatin-binding sites on the fibronectin molecule. Of the known fibronectin-binding substances tested, only gelatin eluted small amounts of LTA (Table 1).

Because we have shown previously that LTA binds by its lipid end to the fatty acid-binding sites of albumin (39), we performed experiments to determine the inhibitory effect of albumin on the binding of LTA to fibronectin. Fibronectin-Sepharose beads were incubated with different concentrations of radiolabeled LTA in the presence of two different concentrations of albumin, and the binding data were analyzed by a double-reciprocal plot. Figure 3 shows that albumin competitively inhibited the binding of LTA to fibronectin, suggesting that albumin competes

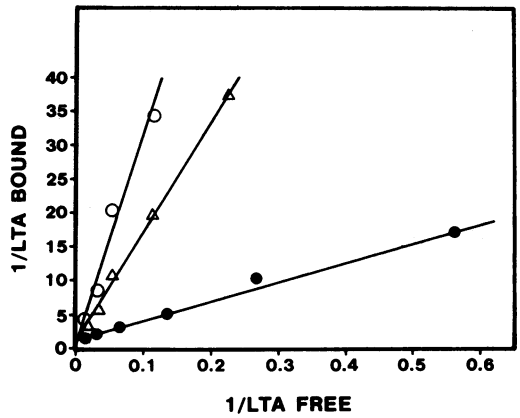

FIG. 3. Competitive inhibition of [^{3}H]LTA binding to fibronectin by albumin. Different amounts of [^{3}H]LTA (3.8 to 25 μg) were added to 0.2 ml of a 50% suspension of fibronectin-Sepharose. $NaCl/P_i$ (●) or bovine serum albumin (250 μg [○] or 50 μg [△]) was added to a final volume of 0.25 ml. Tripicate samples (0.075 ml) were removed, washed with 10 ml of $NaCl/P_i$, and assayed for particle-bound radioactivity as described in the text.

with fibronectin for the lipid end of the LTA molecule. To demonstrate that lipid-binding sites similar to those on the immobilized fibronectin reside on the intact soluble molecule, increasing concentrations of soluble fibronectin were tested for inhibition of the binding of LTA to fibronectin-Sepharose. An 18-M excess of soluble fibronectin inhibited binding by 92% (Fig. 4). Together with previous data, these

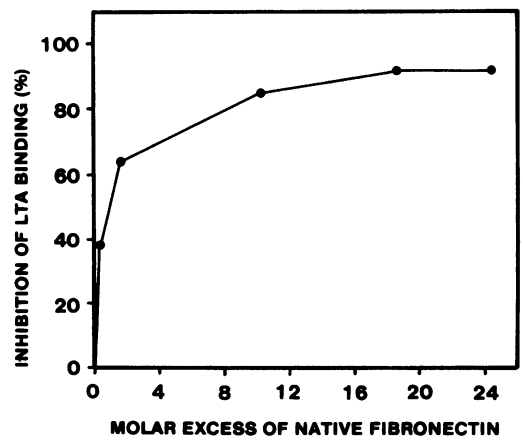

FIG. 4. Inhibition of LTA binding to fibronectin-Sepharose by native fibronectin. [^{3}H]LTA (13 μg) was added to suspensions of fibronectin-Sepharose containing molar excesses of native fibronectin. The mixtures were incubated for 1 h at ambient temperature and washed four times with 10 volumes of $NaCl/P_i$, and triplicate samples were removed and assayed for radioactivity.

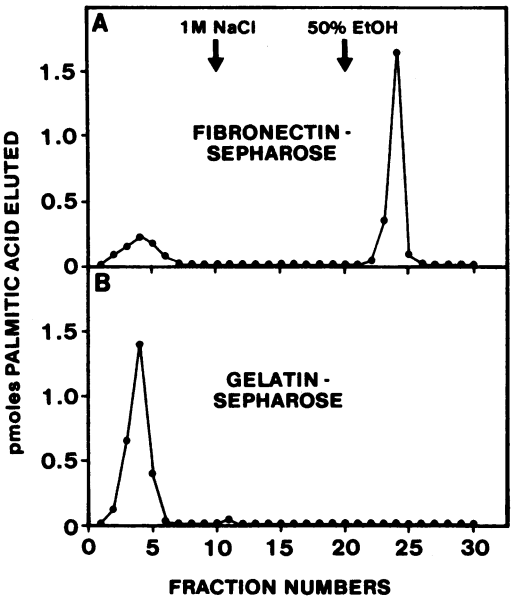

FIG. 5. Profile of [^{3}H]palmitic acid elution from a fibronectin-Sepharose column. Approximately 3 pmol of [^{3}H]palmitic acid in distilled water was added to a fibronectin-Sepharose (A) or gelatin-Sepahrose (B) column with a bed volume of 3 ml and allowed to equilibrate for 18 h at ambient temperature. The column was washed consecutively with 10 ml of NaCl/P_i, 10 ml of 1 M NaCl, and 10 ml of 50% ethanol (EtOH) diluted in NaCl/P_i. Fractions (1 ml) were collected and assayed for radioactivity as described in the text.

results suggest that fibronectin contains a fatty acid-binding site(s) that recognizes the ester-linked fatty acids of LTA.

Binding of palmitic acid to fibronectin. Because of the suggestion that fibronectin contains binding sites for fatty acids, the ability of purified plasma fibronectin to bind radiolabeled palmitic acid (the major fatty acid in LTA) was investigated in two complementary experiments. In the first experiment [^{3}H]palmitic acid was adsorbed to a column of fibronectin-Sepharose, and attempts were then made to desorb the fatty acid with various eluting agents. As Fig. 5 shows, the adsorbed palmitic acid was totally resistant to elution with 1.0 M NaCl, but was quantitatively eluted with 50% ethanol in NaCl/P_i, indicating that the interaction of palmitic acid with fibronectin is hydrophobic. More than 95% of the radiolabeled palmitic acid applied to a control column of gelatin-Sepharose was recovered in the initial flow-through (Fig. 5).

In the second experiment, radiolabeled fibronectin was adsorbed to a column of palmityl-Sepharose. The bound fibronectin could be eluted with 50% ethanol in 0.5% sodium dodecyl sulfate but not with 1.0 M NaCl or with up to 1% sodium dodecyl sulfate alone (Fig. 6). In contrast, all of the fibronectin applied to an octyl-Sepharose column was eluted in the initial flow-through with phosphate-buffered saline. This is consistent with the decreasing affinities of binding of shorter hydrocarbon chains to fatty acid-binding sites, as has been shown for albumin (13).

Affinity of the binding of LTA to fibronectin. The binding of radiolabeled LTA to immobilized fibronectin reached equilibrium after 30 min of incubation (Fig. 7) and was saturable. Since unlabeled LTA eluted most of the labeled LTA from the immobilized fibronectin (Table 1), the values were not corrected for nonspecific binding. Analysis of the binding by a Scatchard plot (Fig. 8) indicated a heterogeneity of binding sites similar to that shown for the binding of LTA to serum albumin (39). Because of the complexity of the Scatchard plot, the affinity constant was estimated from the y intercept, which equals *nKa* (39), where *n* represents the total number of binding sites. A further examination of the plot revealed at least one population of high-affinity binding sites and possibly several others having low affinities for LTA. The *nKa* was approxi-

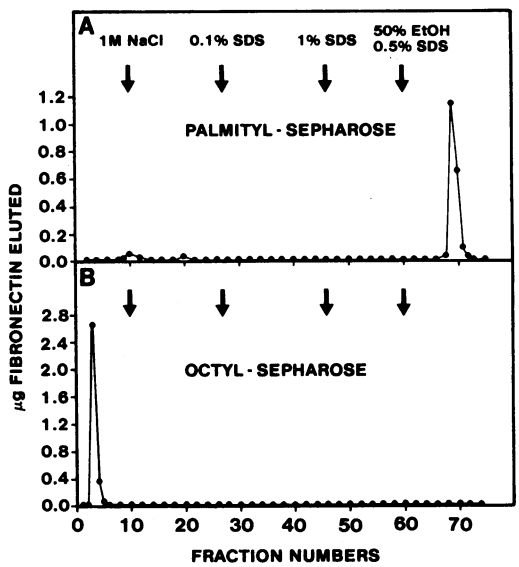

FIG. 6. Elution of [^{125}I]fibronectin from a palmityl-Sepharose column. Approximately 3 μg of [^{125}I]fibronectin was loaded onto a palmityl-Sepharose (A) or octyl-Sepharose (B) column (bed volume, 3 ml) and then washed consecutively with 10 ml of NaCl/P_i, 10 ml of 1 M NaCl, 10 ml of 0.1% sodium dodecyl sulfate (SDS), and 10 ml of 0.5% sodium dodecyl sulfate in 50% ethanol (EtOH). Sodium dodecyl sulfate was diluted in NaCl/P_i. Fractions (0.75 ml) were collected, and radioactivity was counted with a Packard Autogamma scintillation counter.

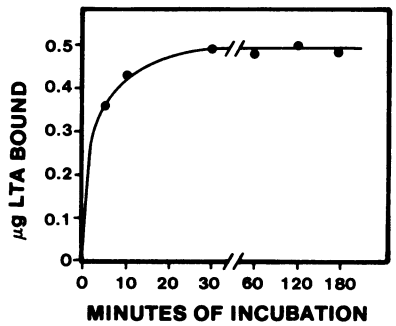

FIG. 7. Time course of [^{3}H]LTA binding to fibronectin. [^{3}H]LTA (25 μg) was added to 0.85 ml of a 50% suspension of fibronectin-Sepharose beads to a total volume of 1 ml. Triplicate samples (0.05 ml) were removed at different times and washed with 10 ml of NaCl/P_i over membrane filters, which were then assayed for radioactivity as described in the text.

mately 250 μM^{-1}, compared with an *nKa* of 0.63 μM^{-1} for the binding of LTA to albumin (39). Thus, the affinity of the binding of LTA to fibronectin is 2 orders of magnitude greater than the binding of LTA to albumin.

DISCUSSION

From our data the following lines of evidence suggest that fibronectin contains a fatty acid-binding site(s) that binds streptococcal LTA: (i) LTA but not deacylated LTA changed the electrophoretic mobility of fibronectin; (ii) LTA bound to fibronectin was eluted by alcohol, detergents, and serum albumin, and serum albumin is known to contain fatty acid-binding sites that recognize the glycolipid of LTA (39) (moreover, albumin competitively inhibited binding of LTA to fibronectin, suggesting a similar binding site on fibronectin); and (iii) immobilized fibronectin was able to bind soluble palmitic acid, and immobilized palmitic acid was able to bind soluble fibronectin. Although there is the possibility that the immobilized fibronectin may have exposed new hydrophobic sites due to conformational changes of the molecule (15), this explanation for the binding of LTA and palmitic acid seems unlikely since soluble fibronectin was able to inhibit more than 90% of the binding of LTA to fibronectin-Sepharose beads.

A Scatchard plot analysis of LTA binding to fibronectin revealed at least one population of high-affinity binding sites. Since fibronectin bound to palmityl-Sepharose but not to octyl-Sepharose, it is possible that the affinity of fibronectin for fatty acids increases with chain length, as has been reported for albumin (13, 34). This notion is strengthened by the observation that LTA was eluted from fibronectin-Sepharose in five peaks by increasing concentrations of ethanol (Fig. 2). Since LTA is known to contain a range of fatty acids (29), these fives peaks may represent different populations of LTA, as determined by the relative lengths of their fatty acids. These findings are consistent with the concept that fibronectin either possesses more than one population of binding sites or posesses a single binding site with differing affinities for different fatty acids.

Our data show that the binding site(s) of LTA on the fibronectin molecule is distinct from the previously defined binding sites for heparin (18), hyaluronic acid (18), glucose (12), and arginine (9). Gelatin only slightly inhibited the binding of LTA to fibronectin, suggesting that the LTA-binding site(s) and thus the fatty acid-binding site(s) on fibronectin may be near but not identical to the collagen-binding region.

Recent studies have shown that fibronectin binds to *Staphylococcus aureus* (20, 27, 33) and to *Streptococcus pyogenes* (38) and that fibronectin mediates attachment of some strains of both organisms to neutrophils (33, 38). In this

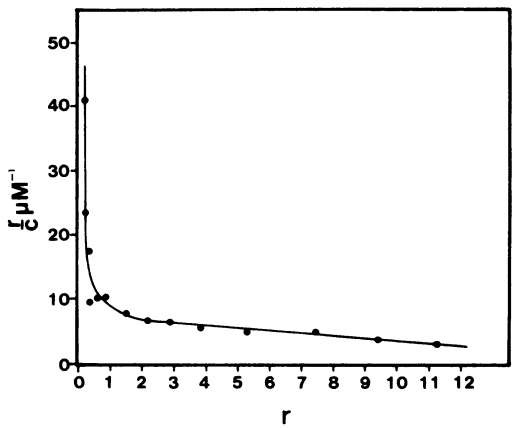

FIG. 8. Scatchard plot (36) of [^{3}H]LTA binding to fibronectin. Different amounts of [^{3}H]LTA (0.025 to 10 μg) were added to 0.2 ml of a 50% suspension of fibronectin-Sepharose to a total volume of 0.25 ml. The mixture was incubated for 60 min at ambient temperature. Triplicate samples (0.075 ml) were removed, filtered, and washed with 10 ml of NaCl/P_i. The radioactivity associated with each filter was assayed as described in the text. The ratio of LTA bound per mole of fibronectin (r) (assuming a molecular weight of 8,500 for LTA and a moelcular weight of 440,000 for fibronectin) to the molar concentraiton of free LTA (c) was plotted as a function of the LTA bound per mole of fibronectin. The intercept with the ordinate corresponds to the number of binding sites per fibronectin molecule (*n*) times the affinity constant (*Ka*). By this analysis, fibronectin appears to have at least one population of binding sites with high affinity for LTA (*nKa*, 250 μM^{-1}).

paper we report that LTA, a surface component of streptococci, binds to fibronectin, suggesting that LTA may be a streptococcal receptor for fibronectin. This hypothesis is supported by the fact that LTA inhibits the binding of fibronectin to streptococci (38).

Although the biological consequence of the binding of LTA to plasma fibronectin is unknown, it may be of particular relevance that LTA has been shown to mediate attachment of *Streptococcus pyogenes* to oropharyngeal cells (2, 6, 29, 40), which in turn are coated with fibronectin (50, 51). Procter et al. (R. A. Procter, D. F. Mosher, and G. Christman, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother, 20th, abstr. no. 643, 1980) suggested that tissue fibronectin may serve as binding sites for gram-positive bacteria and that plasma in circulation could inhibit binding. In addition, we have found that fibronectin inhibits the binding of streptococci to epithelial cells, whereas pretreatment of the epithelial cells with fibronectin enhances streptococcal binding (36a). Thus, fibronectin is a plausible candidate for a receptor in the LTA-mediated binding of *Streptococcus pyogenes* to oropharyngeal epithelial cells. Whether epithelial cell-associated fibronectin plays a broader role in the attachment of other gram-positive bacteria known to contain LTA remains to be determined.

In view of the above described, we conclude that human plasma fibronectin contains a fatty acid-binding site(s) and that streptococcal LTA binds to this site(s) via its fatty acid moiety.

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