

## FIBRONECTIN TETRAPEPTIDE IS TARGET FOR SYPHILIS SPIROCHETE CYTADHERENCE

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Several host serum proteins avidly associate with the outer envelope of the pathogenic spirochete, *Treponema pallidum* (1, 2). The preferential acquisition of serum proteins by this prokaryote, particularly the highly specific binding of the adhesive dimeric glycoprotein fibronectin (Fn), is extremely noteworthy and important to the ability of *T. pallidum* to parasitize cells and tissues (1, 3). Fn-affinity chromatography of a Zwittergent-soluble treponemal detergent extract purified three treponemal outer envelope proteins (mol wt 89,500, 37,000, and 32,000) previously implicated as ligands responsible for cytoadherence of *T. pallidum* to host cells (4). Peptide mapping and immunologic analysis of the treponemal adhesins indicated that a comigrating, functional peptide (12,000 mol wt) was common to each protein (5). For example, antibodies to the crossreactive comigrating treponemal peptide significantly blocked *T. pallidum* adherence to HEp-2 cell monolayers (5). Furthermore, detection of a common, functional domain for each *T. pallidum* ligand (5) was consistent with the existence of a single class of treponemal Fn-binding proteins (3).

Another approach to this study recently involved proteolytic digestion and fractionation of dimeric Fn by standard procedures (6, 7). Heparin, gelatin, and cell-binding domains of Fn were obtained for binding studies (3). Preferential acquisition by live spirochetes of the cell-binding domain was demonstrated, and the purified cell-binding peptide promoted equal levels of treponemal adherence, as did undigested Fn (3).

The hydrophilic sequence arg-gly-asn-ser (RGDS) of the cell-binding domain of each monomer appears to be the critical functional region of the fibronectin molecule, since it promotes attachment of neighboring eukaryotic cells to their extracellular matrices (8–11). For example, this RGDS sequence inhibits the attachment of rat kidney fibroblasts (NRK cells) to Fn-coated surfaces (9, 11), while related peptides altered within the RGD region of the tetrapeptide are ineffective. The following experiments, therefore, were designed to test whether this important RGDS sequence was also recognized by *T. pallidum* for acquisition of Fn cell-binding domain and for parasitism of host cells. In this report, we show the treponemal recognition of the same tetrapeptide (RGDS) that is important for eukaryotic cell–substratum interactions (9–11), and we discuss the uniqueness of the strategy employed by *T. pallidum* for host parasitism.

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### Materials and Methods

Cell-binding domain of Fn monomers was isolated by affinity chromatography of a tryptic digestion of purified human plasma Fn (3). Synthetic heptapeptides gly-arg-gly-asp-ser-pro-cys (GRGDSPC) (12) and three heptapeptides altered in the RGD region, gly-arg-ala-asp-ser-pro-cys (GRADSPC), gly-lys-gly-asp-ser-pro-cys (KGKDSPC), and gly-arg-gly-glu-ser-pro-cys (GRGESPC) were the kind gifts of M. Pierschbacher (La Jolla Cancer Research Institute, La Jolla, CA). GRGDSPC was also purchased from Peninsula Laboratories (Belmont, CA).

Competitive binding experiments were performed as recently reported (3). Briefly,  $7 \times 10^6$  *T. pallidum* organisms harvested as detailed elsewhere (1, 12, 13) were washed twice with phosphate-buffered saline (PBS) and resuspended in microfuge tubes with 1.0 ml of PBS containing saturating levels (48  $\mu$ g) of  $^{125}$ I-labeled cell-binding domain (sp act  $5.8 \times 10^5$  cpm/ $\mu$ g) (3). Increasing amounts of synthetic heptapeptides were added to the reaction as indicated in Fig. 1. After 20 min incubation at 23°C, treponemes were washed twice with PBS and centrifuged for determination of radioactivity.

Inhibition of treponemal attachment to cell monolayer cultures by the synthetic peptides was examined using glass coverslips seeded with human tumor HT1080 cells (3) (American Type Culture Collection, Rockville, MD) or human epithelial HEP-2 cells (1, 2) (American Type Culture Collection) at a density of  $5 \times 10^4$  cells per Leighton tube coverslip. Cells were grown in Dulbecco's minimal essential medium (DMEM) (Gibco Laboratories, Grand Island, NY) containing 10% fetal calf serum (KC Biologicals, Lenexa, KS). Cultures were incubated overnight at 37°C in a humidified air atmosphere of 7.5% CO<sub>2</sub>. 1-ml suspensions of  $7 \times 10^7$  [ $^{35}$ S]methionine-labeled treponemes with or without various concentrations of synthetic peptides were preincubated at room temperature for 20 min before addition of spirochetes to cell monolayers as previously described (3). After incubation at 37°C for 2 h, the coverslips were removed, washed three times with PBS, and counted for radioactivity using scintillation spectroscopy.

### Results and Discussion

Four synthetic Fn heptapeptides were used in competitive binding experiments between *T. pallidum* and  $^{125}$ I-labeled cell-binding domain. As shown in Fig. 1, only GRGDSPC, and not three peptides with modifications in one amino acid of the RGDS sequence, produced a 65% concentration-dependent inhibition of  $^{125}$ I-labeled cell-binding domain acquisition by live *T. pallidum*. Identical results were obtained when experiments were performed in the presence of reducing agents in order to prevent peptide dimerization. These data implicate the GRGDSPC sequence as the Fn site recognized by the syphilis organisms for cytodherence.

Consistent with the competitive binding data (Fig. 1), the RGDS sequence also diminished treponemal parasitism of host cells. Table I shows that preincubation of  $^{35}$ S-labeled treponemes with GRGDSPC before addition to cell monolayers resulted in a similar concentration-dependent reduction of *T. pallidum* adherence. Importantly, two alternative peptides with RAD~~S~~ and KGD~~S~~ sequences instead of RGDS were ineffective in reducing treponemal cytodherence. These data further implicate the RGDS region of the Fn monomers as the principal site of *T. pallidum* cytodherence.

Fibronectin appears to function as an anchoring component for the tip-mediated attachment of virulent treponemes to host cell surfaces (13). This report reinforces our earlier findings that specific outer envelope proteins of *T. pallidum* interact with host Fn in a highly specific, well-defined orientation. Furthermore, these data are consistent with the role of Fn and not other extracellular matrix molecules in *T. pallidum* cytodherence (3).

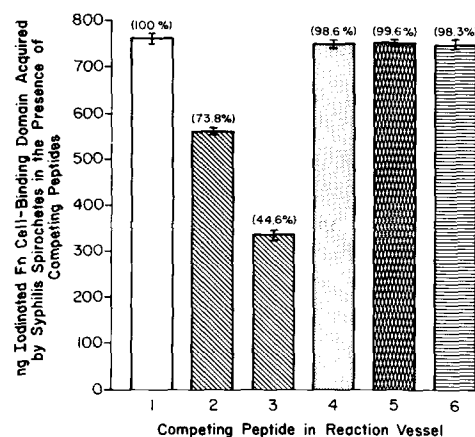


FIGURE 1. Competition for  $^{125}\text{I}$ -labeled cell-binding domain acquisition by *T. pallidum* using GRGDSPC and synthetic peptides altered in the RGD sequence. Treponemes ( $7 \times 10^6$  cells/microfuge tube) were washed with PBS and resuspended in 1.0 ml PBS containing  $48 \mu\text{g}$   $^{125}\text{I}$ -labeled cell-binding domain and synthetic peptides. After a 20 min incubation at  $23^\circ\text{C}$ , spirochetes were washed twice with PBS, and pellets were measured for radioactivity. Numbers refer to the type of competing ligand present in the reaction mixture. Sample 1, PBS control (no competing peptide); 2, 0.5 mg GRGDSPC; 3, 1.0 mg GRGDSPC; 4, 1.0 mg GRGDSPC; 5, 1.0 mg GRADSPC; and 6, 1.0 mg GRGESPC. Numbers in parentheses refer to percent acquisition of cell-binding domain in the presence of competing heptapeptide.

TABLE I  
Inhibition of Syphilis Spirochete Cytoadherence by Fn Cell-binding Domain Synthetic Peptides

Exp.	Treatment reagent	( $\mu\text{g}/\text{ml}$ )	Radiolabel recovered*	
			HT1080	HEp-2
1	DMEM	—	24,718 $\pm$ 2,116 (100)	26,455 $\pm$ 3,218 (100)
	GRGDSPC	50	13,077 $\pm$ 1,919 (53)	13,761 $\pm$ 2,119 (52)
	GRADSPC	50	22,114 $\pm$ 1,602 (90)	24,884 $\pm$ 2,273 (94)
	GKGDSPC	50	23,325 $\pm$ 1,881 (94)	27,043 $\pm$ 2,881 (102)
2	DMEM	—	20,565 $\pm$ 861 (100)	19,561 $\pm$ 580 (100)
	GRGDSPC	25	14,017 $\pm$ 1,050 (68)	12,271 $\pm$ 261 (63)
	GRGDSPC	50	8,019 $\pm$ 164 (39)	8,265 $\pm$ 111 (42)
	GRGDSPC	250	7,749 $\pm$ 128 (38)	7,712 $\pm$ 138 (39)
	GRGDSPC	500	7,318 $\pm$ 96 (36)	7,392 $\pm$ 165 (38)
	GRGDSPC	750	7,386 $\pm$ 186 (36)	7,315 $\pm$ 161 (37)

Cytoadherence assays were performed as previously described (3). Radiolabeled treponemes ( $7 \times 10^7$  cells/reaction volume) were incubated with the indicated synthetic peptide or medium for 30 min at  $34^\circ\text{C}$  before addition to cultured cell monolayers.

\* Each value represents the mean cpm  $\pm$  SD of three separate determinations. Numbers in parentheses give data as percent of control.

Of special significance may be the unique strategy of treponemal parasitism of host cell surfaces. For example, the RGDS sequence of the cell-binding domain, present on a hydrophilic  $\beta$ -turn of each monomer on the dimeric Fn molecule (8, 9, 14), appears to represent the target for the syphilis spirochete. Since the recognition by syphilis bacteria and eukaryotic cells of host Fn (1, 7, 8) appears to involve the same RGDS site, these studies emphasize the possible importance and role of the dimeric nature of Fn, which contains a cell-binding domain within each monomer. This report may lead to an understanding of the special attributes

of this infectious agent, which may be ultimately responsible for the pathobi-  
ochemistry of syphilis. In addition, continued analysis of the interaction between  
treponemal adhesins and Fn may result in alternative approaches for develop-  
ment of rapid diagnostic tests and for control or eradication of this disease.

### Summary

The syphilis bacterium, *Treponema pallidum*, parasitizes host cells through recognition of fibronectin (Fn) on cell surfaces. The active site of the Fn molecule has been identified as a four-amino acid sequence, arg-gly-asp-ser (RGDS), located on each monomer of the cell-binding domain. The synthetic heptapeptide gly-arg-gly-asp-ser-pro-cys (GRGDSPC), with the active site sequence RGDS, specifically competed with <sup>125</sup>I-labeled cell-binding domain acquisition by *T. pallidum*. Additionally, the same heptapeptide with the RGDS sequence diminished treponemal attachment to HEp-2 and HT1080 cell monolayers. Related heptapeptides altered in one key amino acid within the RGDS sequence failed to inhibit Fn cell-binding domain acquisition or parasitism of host cells by *T. pallidum*. The data support the view that *T. pallidum* cytoadherence of host cells is through recognition of the RGDS sequence also important for eukaryotic cell-Fn binding.

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