Characterization of the Interaction between Fibronectin and *Treponema pallidum*

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**Abstract.** The interaction between *Treponema pallidum* and rabbit plasma fibronectin was characterized. Fibronectin was isolated from rabbit plasma and radioiodinated by the lactoperoxidase method. Fibronectin bound to the surface of *T. pallidum*, reaching saturation at approximately 54 µg/ml. The association affinity constant was 2.85 × 10⁷ M⁻¹, much lower than that of *Staphylococcus aureus* (5.6 × 10⁹ M⁻¹). Fibronectin binding plateaued within 15 min at 20° and 37°C, with some repletion at 37°C by 30 min. Little fibronectin bound to *T. pallidum* at 4°C. The greatest amount of fibronectin was bound at the lowest pH tested (pH 6.0); the poorest binding was at pH 7.5. Approximately 90% of the binding was reversible in the presence of excess unlabeled fibronectin. The data indicate a more dynamic and weaker interaction between *T. pallidum* and fibronectin than that seen with *S. aureus*.

Adherence to host cells has been implicated as a primary mechanism of pathogenicity in a wide variety of infectious diseases [1, 17]. *Treponema pallidum* readily attaches to mammalian cells in vitro [11, 14], providing a model system for studying the pathogenesis of syphilis. *Treponema pallidum* adherence to mammalian cells has major implications for the two most significant areas of recent research in syphilis: the development of an in vitro cultivation system [6, 7] and the potential importance of adhesins as target antigens for cloning in the developing of a vaccine against syphilis. The possible importance of attachment in the pathogenesis of syphilis has been demonstrated by the fact that syphilitic immune serum blocks attachment of treponemes to mammalian cells in vitro [10, 14], and this blocking effect correlates better with immunity to superinfection than other antibody responses such as the appearance of treponemacidal antibodies [28].

Two host cell macromolecules have been identified as possible receptors for *T. pallidum*. Early work stressed the possible importance of hyaluronic acid accumulation in syphilitic chancrens [25, 26], and Fitzgerald and co-workers suggested that hyaluronic acid may be a treponemal receptor for treponemal adherence [8]. Later work has also implicated the connective tissue glycoprotein fibronectin in treponemal adherence [22]. Fibronectin mediates a wide variety of attachment phenomena in eucaryotes [16, 24] and is known to bind avidly to *Staphylococcus aureus* [18, 23]; the binding of fibronectin to *S. aureus* appears to correlate with pathogenicity also [27]. Because of the potential importance of the *T. pallidum* to fibronectin for the pathogenesis of syphilis, we have investigated the chemical characteristics of this interaction.

**Materials and Methods**

**Source of Treponema pallidum.** Treponema pallidum were grown in the testes of adult New Zealand rabbits (approximately 3 kg in weight). Rabbits were killed by the intracardiac injection of 1.5 ml of T61 euthanasia solution (America Hoechst Corporation, Somerville, NJ). The testes were aseptically removed, minced, and extracted in FTA-ABS buffer (130 mM NaCl, 5 mM Na₂HPO₄, and 1.5 mM KH₂PO₄, pH 7.2) [21] for 10–15 min. Eucaryotic cells and tissue debris were removed by centrifugation twice at 1000 g for 10 min, and the treponemes were then isolated from the remaining host serum proteins by Percoll density-gradient centrifugation [12]. The isolated *T. pallidum* generally made a visible band in the Percoll gradient and were carefully removed with a Pasteur pipette. The Percoll was then removed by centrifugation at 100,000 g for 1 h in an SW 27 swinging-bucket rotor in an L-8 Beckman ultracentrifuge. The treponemes were a fleecy pellet on top of the Percoll. In early experiments, after the two low-speed centrifugations, the extracted treponemes were subjected to centrifugation at 12,000 g in an SS34 Sorvall centrifuge head and resuspended in FTA-
blue staining and autoradiography, was the only labeled protein present when run on 5%–20% gradient SDS–polyacrylamide gels. The 5%–20% gradient gels were run with the buffers and conditions as per Laemmli [19]. Specific activity of the purified iodinated fibronectin was typically 1.5 × 10^{12} cpm/mmol.

**Assay for binding of fibronectin to *Treponema pallidum***. The assay for binding of fibronectin was a modified version of that used by Proctor et al. [23]. The reactions were carried out in duplicate in 0.4-ml volumes in Eppendorf 1.9-ml microfuge tubes. All experiments were performed 3–6 times. The binding buffer consisted of 20 mM HEPES (N-2-hydroxy ethyl-piperazine-N-2-ethane sulfonic acid) containing 2 mg/ml ovalbumin at pH 7.4 (unless otherwise stated). In some experiments, 0.05% Tween 20 was included in the reaction mixture and gave excellent results in reducing nonspecifically bound fibronectin. Because of frequent loss of pelleted *T. pallidum* when the supernatant was drawn off in the presence of Tween 20, this method was not generally used in the following experiments. *Treponema pallidum* were used at 2 × 10^{8}/ml and fibronectin was present at a concentration of 8 μg/ml in experiments other than those in which the number of *T. pallidum* binding sites were being determined. A level of 8 μg/ml of fibronectin was chosen because this concentration gave reliable results, and the kinetics of binding were slow enough to allow for sampling without the requirement of centrifuging through oil. The treponemes were pelleted by centrifugation in an Eppendorf model 5412 centrifuge, the supernatant was removed, and the bottom of the tube was cut off and counted in a gamma counter. Blank tubes containing no treponemes were used to determine the background, which was subtracted to give the actual amount of fibronectin bound to *T. pallidum*.

In experiments designed to test the effect of temperature on fibronectin binding to *T. pallidum*, all reagents and the treponemes were brought to the required temperature before mixing (the ovalbumin buffer was incubated in the Eppendorf centrifuge tubes to block binding sites on the plastic). In the pH experiments, a single batch of buffer was split into aliquots, and each aliquot was brought to the desired pH. When reversibility was being tested, the treponemes were incubated with 125I-fibronectin (8 μg/ml) for 30 min and then exposed to 300 μg/ml of unlabeled fibronectin for 1 h. This level was sufficient to remove any passively or reversibly bound fibronectin, since tubes without treponemes showed levels of radioactivity similar to tubes that contained only buffer after this regime. Further details of protocols used in each of these experiments are given in the figures.

**Source of materials**. Cyanogen-bromide-activated Sepharose for making the gelatin affinity column was purchased from Pharmacia Chemicals (Piscataway, NJ) or from Sigma Chemical Company (St. Louis, MO). Ovalbumin, HEPES buffer, and other chemicals were of reagent grade or better and were purchased from Sigma Chemical Company Carrier-free Na125I was purchased from Amersham (Chicago, IL) and had an activity of 2250 mCi/mmol.

**Results**

Saturation-binding kinetics were determined for fibronectin. Fibronectin bound rapidly to *Treponema pallidum*, but required a much higher concentration before saturation occurred when compared with...