Mycoplasmas are parasitic bacteria with a small genome and no peptidoglycan layer (27). Several mycoplasma species have a distinct cell polarity characterized by a protruding membrane extension, the attachment organelle (27). They are able to attach to and glide on glass, plastic, and eukaryotic cell surfaces, always moving in the direction of the organelle (19). The gliding mechanism is unknown. Mycoplasmas do not have any appendages such as flagella or pili (19) or any genes obviously related to motility, including motor proteins such as myosin or kinesin (7, 10, 13). However, a transmembrane protein associated with a cytoskeleton-like structure has been shown to be necessary for glass binding in Mycoplasma pneumoniae (22).

Mycoplasma mobile, isolated from a fish gill organ, has a conical cell structure, with the attachment organelle at the apex of the cone. It glides up to four cell lengths per second (2.5 μm/s) in the direction of the apex, which is designated as a head-like structure (30). However, little is known about force generation (28, 29). In this study, to characterize its gliding motility, we attached a bead to the tail end of *M. mobile* and measured the gliding force as a function of speed using viscous flow and an optical tweezer.

**MATERIALS AND METHODS**

**Cultivation.** *M. mobile* strain 163K (ATCC 43663) was grown as described previously (25).

**Measurements of gliding speed.** *M. mobile* cells in a culture at an optical density at 600 nm of 0.03 to 0.1 were incubated with an equal volume of Aluotto medium (25), recovered by centrifugation at 10,000 x g for 4 min at room temperature, and suspended with the original volume of medium. The cells were incubated with an equal volume of the bead suspension at room temperature, the beads were diluted with a 200-fold volume of Aluotto medium (25), recovered by centrifugation at 10,000 x g for 1 min at room temperature, washed twice with a 200-fold volume of the same medium, and then suspended in a 40-fold volume of this medium. *M. mobile* cells in a culture at an optical density at 600 nm of 0.03 to 0.1 were collected by centrifugation at 10,000 x g for 4 min at room temperature and suspended with the original volume of medium. The cells were incubated with an equal volume of the bead suspension at room temperature for 10 min. For the flow experiments, we used a flow chamber similar to the one described previously (2) but made from brass and gold plated. Its windows were cleaned with saturated ethanol KOH (4). The cell-bead mixture was allowed to settle for 10 min onto the bottom window of the chamber, and unattached beads were washed away by flow at 67 μm/s for 2 min, generated by suction with a mechanical syringe pump (model 22; Harvard Apparatus) (see the next paragraph). For the optical tweezer experiments, the mixture was inserted into a tunnel between a glass slide and a coverslip raised by two strips of double-sided tape, two layers thick. After 20 min of incubation, excess beads were washed away with 200 μl of medium, and the ends of the tunnel were sealed with Apiezon grease.

**Force-velocity measurements by fluid flow.** Cells attached to a bead in a flow chamber were observed with an inverted microscope (Diaphot 200; Nikon) equipped with a 100× oil-immersion objective (Plan Fluor, 1.3 NA). The temperature of the objective was controlled with a Peltier device similar to the one described previously (18). The movement of cells with beads was recorded with a Hi-8 recorder, and frames were digitized at 2 fps. The gliding velocity relative to the glass surface was calculated from the displacement of the center of mass of Mycoplasmas gliding on the coverslip were observed with a 40× phase-contrast objective and recorded on Hi-8 videotape. Tapes were digitized at 2 frames per second (fps) on a G3 Power Macintosh (Apple Computer, Cupertino, Calif.) equipped with an LG-3 video capture board (Scion Corp., Frederick, Md.) using Scion Image software (version 1.62c). The center of mass of cells were measured using Scion Image software, and gliding speeds of cells were calculated from their displacements. The temperature was varied from 25°C to higher (41°C) and lower (11.5°C) temperatures. At the end of each run, the temperature was returned to 25°C to confirm that the gliding speed at that temperature remained unchanged. For each cell, 4 s of continuous gliding was analyzed. Ten cells were analyzed at each temperature.

**Attachment of beads.** Rabbit polyclonal antiserum against whole *M. mobile* cells was prepared according to the method in reference 32. It was purified on a protein A column and contained immunoglobulin G (0.7 mg/ml). Suspensions of polystyrene beads (2.2-μm diameter for experiments with fluid flow and 1.1-μm diameter for experiments with optical tweezers) conjugated with protein A (1% solid; Bang's Laboratories, Fishers, Ind.) were mixed with the same volume of the antiserum and a 10-fold volume of phosphate-buffered saline. After 10 min of incubation at room temperature, the beads were diluted with a 200-fold volume of Aluotto medium (25), recovered by centrifugation at 10,000 x g for 1 min at room temperature, washed twice with a 200-fold volume of the same medium, and then suspended in a 40-fold volume of this medium. *M. mobile* cells in a culture at an optical density at 600 nm of 0.03 to 0.1 were collected by centrifugation at 10,000 x g for 4 min at room temperature and suspended with the original volume of medium. The cells were incubated with an equal volume of the bead suspension at room temperature for 10 min. For the flow experiments, we used a flow chamber similar to the one described previously (2) but made from brass and gold plated. Its windows were cleaned with saturated ethanol KOH (4). The cell-bead mixture was allowed to settle for 10 min onto the bottom window of the chamber, and unattached beads were washed away by flow at 67 μm/s for 2 min, generated by suction with a mechanical syringe pump (model 22; Harvard Apparatus) (see the next paragraph). For the optical tweezer experiments, the mixture was inserted into a tunnel between a glass slide and a coverslip raised by two strips of double-sided tape, two layers thick. After 20 min of incubation, excess beads were washed away with 200 μl of medium, and the ends of the tunnel were sealed with Apiezon grease.

**Measurements of gliding speed.** A polystyrene bead was attached to the tail of *M. mobile* using a polyclonal antibody raised against whole *M. mobile* cells. Cells attached to beads glided at the same speed as cells without beads. When liquid flow was applied in a flow chamber, cells reoriented and moved upstream with reduced speeds. Forces generated by cells at various gliding speeds were calculated by multiplying their estimated frictional drag coefficients with their velocities relative to the liquid. The gliding speed decreased linearly with force. At zero speed, the force measurements extrapolated to 2.3 and 3.3 μm/s at 22.5 and 27.5°C, respectively—the same speeds as those observed for free gliding cells. Cells attached to beads were also trapped by an optical tweezer, and the stall force was measured to be 26 to 28 pN (17.5 to 27.5°C). The gliding speed depended on temperature, but the maximum force did not, suggesting that the mechanism is composed of at least two steps, one that generates force and another that allows displacement. Other implications of these results are discussed.
The force vectors were calculated. The speed relative to the fluid and the size of the object. Therefore, we modified the other two factors, namely, the speed relative to the fluid and the size of the object.

*M. mobile* cells were attached to 2.2-μm-diameter beads by a polyclonal antibody raised against surface molecules (Fig. 2). Cells with beads glided at the same speed as cells without beads. Cells attached to beads were exposed to viscous flow in a flow chamber. *M. mobile* was oriented by the flow (31) and glided upstream (Fig. 3). This orientation facilitated measurements of force as a function of velocity from Stokes’ law. Gliding speeds relative to the glass were measured under various flow rates. Speed decreased inversely with the force (Fig. 4). Extrapolation of the data indicated a zero-speed force of 26 pN at both 22.5 and 27.5°C, and zero-force speeds of 2.3 and 3.3 μm/s at 22.5 and 27.5°C, respectively. We could not get data near stall, because at high flow rates all cells detached from the surface when their gliding speed was reduced to about one-third of its unloaded value. Also, it was difficult to get data at

### RESULTS

**Temperature dependence of gliding speed.** Gliding speed was measured over a wide temperature range (Fig. 1). Speed increased linearly with temperature from 0.46 μm/s at 11.5°C to 4.0 μm/s at 36.5°C. At temperatures higher than 36.5°C adhesion to the glass surface was reduced; at 41.0°C cells no longer adhered.

**Force-velocity measurement by fluid flow.** We measured the force-velocity relationship of *M. mobile* gliding using viscous fluid flow. *M. mobile* can glide without apparent reduction of speed while pulling an erythrocyte, a load estimated to be equivalent to a force of 0.17 pN (28, 29). Therefore, we had to apply higher loads to produce a wider range of speeds. The viscous drag on an object moving in a fluid is proportional to the size of the object, its speed relative to the fluid, and the viscosity of the fluid. We found that adding viscous agents such as Ficoll (3) interferes with gliding motility. Therefore, we modified the other two factors, namely, the speed relative to the fluid and the size of the object. *M. mobile* cells were attached to 2.2-μm-diameter beads by a polyclonal antibody raised against surface molecules (Fig. 2). Cells with beads glided at the same speed as cells without beads. Cells attached to beads were exposed to viscous flow in a flow chamber. *M. mobile* was oriented by the flow (31) and glided upstream (Fig. 3). This orientation facilitated measurements of force as a function of velocity from Stokes’ law. Gliding speeds relative to the glass were measured under various flow rates. Speed decreased inversely with the force (Fig. 4). Extrapolation of the data indicated a zero-speed force of 26 pN at both 22.5 and 27.5°C, and zero-force speeds of 2.3 and 3.3 μm/s at 22.5 and 27.5°C, respectively. We could not get data near stall, because at high flow rates all cells detached from the surface when their gliding speed was reduced to about one-third of its unloaded value. Also, it was difficult to get data at

![FIG. 1. Temperature dependence of gliding speed. The gliding speed at various temperatures was analyzed by video microscopy. Movement was measured every 0.5 s, and the average speed was calculated for 4-s intervals. Ten cells were analyzed for each point. The bars show standard deviations for the cell population.](http://jb.asm.org/)

![FIG. 2. Images of attached beads. (A) Schematic illustration of a cell attached to a bead. An *M. mobile* cell was attached to a polystyrene bead coated with an antibody and protein A. Beads with a 2.2-μm diameter were retarded by viscous flow, and beads with a 1.1-μm diameter were held by optical tweezers. (B) A video image of one 2.2-μm-diameter bead attached to an *M. mobile* cell in a flow experiment.](http://jb.asm.org/)
temperatures below 17.5°C, because most cells detached at flow rates (67 μm/s) too low to orient the cells upstream.

**Stall force measurements by optical tweezer.** To measure the stall force directly, we used an optical tweezer to apply forces to beads (diameter, 1.1 μm) attached to *M. mobile*. The force exerted by the cell pulled the bead from the trap center. The trapping force was linearly proportional to the distance from the trap center, so by measuring this displacement, the force could be calculated (Fig. 5). Some cells repeatedly moved away from the center of the trap, stalled for a moment (velocity = 0 μm/s), and then came off the glass (as in Fig. 5). For these cells, multiple stall force measurements were taken and averaged. Other cells detached from the glass before stalling, so their stall forces could not be measured. The average value of stall force was calculated to be 26 to 28 pN at all three temperatures, which is consistent with the value obtained by extrapolating the results of the flow experiments (Fig. 4). Specifically, the mean stall force (in piconewtons ± standard deviation) was 25.9 ± 6.8 (*n* = 19), 27.9 ± 6.0 (*n* = 23), and 27.5 ± 8.4 (*n* = 13), at 27.5, 22.5, and 17.5°C, respectively.

**DISCUSSION**

The gliding speed increased linearly with temperature, as seen in bacterial flagellar motility (23). The fraction of cells bound to glass was much reduced at 36.5°C, but cells that did bind glided more rapidly. This suggests that binding and force generation involve two different molecular mechanisms. The temperature range at which cells can glide is higher than that for growth (4 to 30°C [20]), which is not surprising, since many more processes are involved in growth than in motility.

The polystyrene beads were attached by using a polyclonal antibody raised against the whole cell. The antibody recognizes at least 40 mycoplasma proteins and has some inhibitory effect on glass binding if it is added at concentrations higher than 0.05 mg/ml. However, no inhibitory effect was observed in the present experiments, because the antibody was adsorbed to beads that were resuspended in antibody-free medium and only single beads were attached to the cell. Immunolabeling of living cells using a fluorescent dye showed distribution of target proteins over the entire cell surface (data not shown), but the beads seemed to bind mainly to the tail (as indicated schemat-
ically in Fig. 2A). This observation might be explained by one of the following three possibilities: (i) beads attached to the tail probably interfere less with binding and gliding than those attached to other parts of the cell, (ii) antibodies against molecules located at the tail might bind to their target molecules more tightly, or (iii) the beads move to the tail due to viscous drag as the cell glides.

When fast flows were applied to beads attached to gliding cells, the cell bodies could be seen to stretch (data not shown). Stretching of cells also was observed in the laser trap experiment. This elasticity prevented the detection of steps (if they exist) and the analysis of speed in these experiments. However, the stretching of cells did not seem to damage motility. When the applied forces were removed, cells often continued to move at their initial speeds.

The gliding speed decreased continuously with force (Fig. 4). All mycoplasmas found so far are parasitic and live mainly in tissues of animals or plants and sometimes in host cells (27). M. mobile was isolated from a gill organ of a freshwater fish (20). The wide range of the velocity-force relationship suggests that the cells can glide in a variety of environments that require increased thrust.

Binding to glass during gliding requires a 349-kDa acidic protein that is localized at the neck region of cells (our unpublished data). The protein molecule has an extracellular domain with at least one transmembrane domain near its N terminus. M. pneumoniae, a human pathogen, also has a polarized cell morphology, and it also glides in the direction of its attachment organelle (19, 22). The gliding mechanism of M. pneumoniae is believed to be similar to that of M. mobile, although the speed is 10 times slower (19). The adhesion molecule of M. pneumoniae, designated P1, is a 170-kDa protein containing at least three transmembrane domains with most of the protein outside of the cell (26). P1 protein is located mainly at the attachment organelle with some accessory cytadherence proteins (1, 8, 14, 33). The mutant lacking P1 protein cannot bind to glass (26) and consequently cannot glide.

M. mobile bound to glass and moved, even when its speed was reduced to one-third of its unloaded value (Fig. 4). One or more copies of the 349-kDa protein responsible for glass binding must remain bound to the substrate at all times; otherwise, M. mobile cells would be carried away by the flow. However, cells stalled by the optical tweezer frequently released from the surface, and at high flow rates gliding M. mobile cells were carried away when exerting 35% less force than at stall (Fig. 4). This suggests that the strength of binding to glass fluctuates during gliding even at stall. Force generation probably involves multiple binding events, each occurring with finite probability.

The extrapolated speed at zero force was similar to the free gliding speed (Fig. 4). Evidently, the free gliding speed does not depend on an equilibrium between force generation and drag on the cell body but rather reflects the rate-limiting step of the gliding machinery.

The stall force measured by the optical tweezer agreed with the value obtained by extrapolation in the flow experiment. This suggests that the velocity in the flow experiment might have decreased linearly to zero speed if the cells were not carried away by the liquid flow. The stall force is five to seven times that of myosin and kinesin (9, 34) and comparable to that generated by RNA polymerase (36) or a force-generating element of the flagellar motor (6). Of course, more than one force-generating molecule might be active at the same time. In any event, this rather large stall force suggests that the 349-kDa protein is supported by a scaffold. Although mycoplasmas lack a peptidoglycan layer, many species have cell shapes other than spherical, such as conical, filamentous, and spiral. These special shapes are believed to be maintained by filamentous protein structures (35). Filamentous structures of M. pneumoniae can be seen in a Triton-insoluble fraction (11, 24). Indeed, some P1 protein is contained in this structure (15), suggesting
that the adhesion protein is supported by filamentous scaffolds. Cytaadherence mutants of \textit{M. pneumoniae} are known to have abnormal cell morphology (21, 33) and all nonbinding and nongliding mutants of \textit{M. mobile} isolated so far also have an abnormal cell morphology (25). Evidently, cytoskeletal and cytaadherence molecules are closely linked; defects in one system can perturb the other. The gliding speed depended on temperature, but the maximum force did not. This suggests that the gliding mechanism is composed of at least two steps, one that generates force and another that allows displacement. At stall (near equilibrium), the energy available to power an elementary step does not depend on temperature; however, when cells are moving, the stepping rate does depend on temperature. The same thing is true for ATP-driven motor proteins (16, 17).

\section*{ACKNOWLEDGMENTS}
We thank R. Rosengarten and C. Citti of the Veterinary University of Vienna, Austria, for their help in making the polyclonal antibody. This work was supported by the Rowland Institute for Science and partly by a Grant-in-Aid for Scientific Research (A) from the Ministry of Education, Science, Sports, and Culture (Japan).

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