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Measurement of mechanical forces generated by plant P-protein aggregates (forisomes)

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Abstract Mechanical forces generated by forisomes were measured using a microfabricated polymer cantilever sensor. The forces were simultaneously measured in both the longitudinal and radial directions. Sensors were fabricated from polystyrene using the sacrificial layer micromolding process. The sensor response was simulated using finite element analysis. Forces in the longitudinal direction ranged from 84 to 136 nN and forces in the radial direction were 22–61 nN. This device offers a new approach to measuring small magnitude biological forces. In addition, the ability to accurately measure forces generated by forisomes is an important step toward their implementation as functional structures in microdevices.

Keywords Bio-MEMS · Force measurement · P-protein aggregates · Soft lithography

Introduction

Forisomes are small chemomechanically active P-protein aggregates uniquely found in the sieve tubes of legumes (*Fabaceae*). A motor protein can convert chemical free energy in to mechanical work as described by Urry (1993). Unlike most motor proteins or protein aggregates, forisomes do not rely on adenosine triphosphate (ATP) to supply the energy for actuation (Knoblauch et al. 2003). Instead they contract or expand in response to changes in specific ion concentrations or pH. Given their unique actuation mechanism and ability to produce large conformational changes, forisomes have been suggested for use as micro-actuators, drug delivery devices, and micro-valves (Knoblauch et al. 2003, 2004).

During forisome contraction, free chemical reaction energy it converted to longitudinal and radial mechanical work and volume change. Upon exposure to Ca(II), Sr(II) and Ba(II) ions or during pH shifts from 7.3 to 4.5 or 7.3 to 11.0, the protein aggregates contract by 10–40% of their original length and increase their diameter by as much as 180% (Schwan et al. 2007a). The stability of the energy conversion depends on the dissolved oxygen concentration and can be improved considerably by working under anaerobic conditions.

The focus of this work is the development of a sensor with the ability to measure the forces generated by forisomes in both the longitudinal and radial directions simultaneously. Previous work from Knoblauch et al. (2003) and our own experiments (Schwan et al. 2007b) show that the longitudinal contraction forces are between 50 and 120 nN. However, there is a need for a more refined device for measuring these forces in both the longitudinal and radial directions at the same time. Our approach to measuring forisome forces was the development of a microfabricated polymer cantilever sensor. The use of a polymer material with low elastic modulus provides relatively large and measurable beam deflections in response to relatively low magnitude forces.

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Materials and methods

Device design

The layout of the device is shown in Fig. 1. The structure consists of four cantilever probes, each 250 μm in length and 5 μm wide. This thickness of the beams, which was determined by the fabrication process parameters described later, was 2.3 μm. Two of the probes are designed to measure the force in the longitudinal direction of the forisome, and the spacing of the probes is 30 μm to match the typical geometry of the forisome in the extended confirmation. The other two probes measure force in the radial direction and have a spacing of approximately 3 μm. The device is fixed to the substrate at the edges of the structure and the cantilevers are suspended approximately 1 μm above the substrate using a water soluble poly(vinyl alcohol) (PVA) sacrificial layer that is dissolved prior to testing.

Finite element simulations

The behavior of the device was simulated using finite element analysis (ANSYS ED 10.0). A single cantilever beam was analyzed by applying a fixed support at the base of the beam. Forces ranging of 0–300 nN were applied to a 3 × 3 μm surface at the free end of the beam. An elastic modulus of 3.2 GPa (Brandrup 1999) and Poisson's ratio of 0.325 (Boundy et al. 1952) were used for the structural material (polystyrene). It was assumed that the mechanical properties of the material were similar to the bulk properties as reported in the literature. Previous research on the mechanical properties of polystyrene measured using nanoindentation indicates that this was a reasonable assumption (Palacio et al. 2007).

Device fabrication

Devices were fabricated using sacrificial layer micromolding. This process is a soft lithography (Xia and Whitesides 1998) based micromolding technique that is capable of producing suspended polymer structures such as cantilevers.

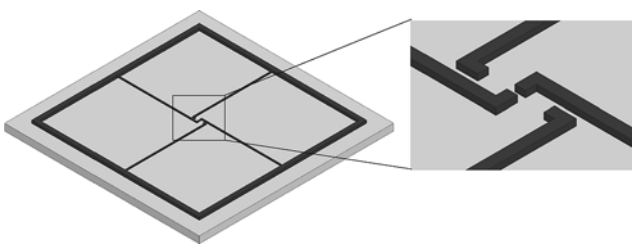

Fig. 1 Schematic diagram of the forisome force sensor. The *inset* shows the measurement region of the device. The long direction of the forisome is placed between the two cantilevers with the larger (30 μm) spacing

The details of the process are described elsewhere (Ferrell et al. 2007). Briefly, a water soluble poly(vinyl alcohol) (PVA, Sigma-Aldrich, average MW 30,000–70,000, 87–90% hydrolyzed) sacrificial layer was fabricated via photolithography and reactive ion etching. The structural portion of the device was defined in SU8 2005 (MicroChem Co.) photoresist using standard photolithography. The structure was then transferred into poly(dimethylsiloxane) (PDMS, T-2, Dow Corning). After curing, the PDMS mold was spin coated at 3,000 rpm with a 5% (wt/wt) solution of polystyrene (melt flow index 4.0, Simga-Aldrich) dissolved in anisole (Simga-Aldrich). The resulting film coated both the recessed and raised features of the mold. To remove the material from the raised features of the mold, the surface was brought into contact with a glass slide heated to 200°C. This removed the polymer from the top surface of the mold, resulting in a mold that was selectively filled with polystyrene in the recessed features. The selectively coated mold was then aligned and brought into contact with the patterned sacrificial layer. Heat (125°C) and pressure (~0.35 MPa) were used to transfer the structure onto the sacrificial layer. During testing, a water-based conditioning solution was introduced into the measurement chamber. This solution dissolved the sacrificial layer and released the cantilever beams. The thickness of the beams was characterized using atomic force microscopy (AFM, Veeco Dimension 3100) in tapping mode.

Forisome preparation

The forisomes tested in this work were isolated from *Vicia faba*. They had original lengths between 30 and 50 μm and diameters between 2 and 3 μm. Forisomes were prepared by mechanically isolating phloem tissue from the stems of plants that were 4- to 6-week old (Schwan et al. 2007a). The rind between the first and seventh internodes of the plants was excised and put into a Ca^{2+}-free solution containing 10 mM EDTA, 0.1 M KCl, and 10 mM Tris adjusted to pH 7.3. The separated and extraneous pre-dried phloem tissue was powdered under liquid nitrogen and subsequently suspended in a Ca^{2+}-free solution. The suspension was filtered through a nylon sieve with a mesh size of 55 μm and transferred to a storage chamber in the measurement setup (Schwan et al. 2007a). To maintain anaerobic conditions, 10 mM glucose was added to the suspension followed by enzymatic oxidation of the glucose with 39 unit/mL glucose oxidase (Sigma-Aldrich) and 70 unit/mL catalase (Sigma-Aldrich).

Measurement setup

The storage chamber was connected by a transfer channel to the measurement chamber. The flow and exchange of

conditioning and rinsing solutions were adjusted by computer-controlled piston pumps in the measurement chamber. Two plunger pumps were connected to stock solutions, adjusting the concentrations of Ca^{2+} ions and EDTA to switch forisomes between the calcium-loaded and calcium-free states.

The force measurement chip was fixed in the measurement chamber. The forisomes were transferred from the storage chamber and attached to the devices using a micropipette attached to a three-axis micromanipulator. With the exception of the pumps, the entire measurement setup was mounted on an actively damped optical bench. During Ca^{2+} induced actuation of the forisomes, images of the devices were captured on a CCD camera attached to the microscope. The displacement of each probe was analysed using a digital greyscale image correlation program (ARAMIS).

Results and discussion

AFM characterization of the beams gave a thickness value of 2.3 ± 0.1 µm ($n = 5$). The results from the finite element simulations with this beam thickness were used to determine the deflection per unit force for the beam and gave a value of 0.064 µm/nN. Results are similar to the numerical solution obtained using linear elastic beam bending equations with a point load applied to the end of the beam (Hibbeler 2000). Slight differences are attributed to the geometry of the end of the beam and the distributed loading condition of the simulation. Stress and strain analyses also indicated that the beam response is well within the linear elastic regime for the expected force range, even for conservative estimates of polystyrene's yield behavior (Saq'an et al. 2004, Palacio et al. 2007). Yield of the beams would not be expected until well into the µN force range.

Figure 2 shows optical micrographs of the forisome (a) before and (b) after actuation with 10 mM Ca^{2+}. The images show that the forisome contraction resulted in significant bending of the cantilevers in both the longitudinal and radial directions. The net displacement of both of the beams in a given axis was used to calculate the corresponding force according to the finite element simulation.

Figure 3 shows an SEM micrograph of the sensor with a forisome attached. The forisome was placed with the long axis between the cantilevers with the larger gap spacing. No additional adhesives were used to attach the forisome to the sensor. The natural adhesion of the forisome to polystyrene was sufficient to maintain contact between the forisome and the sensor during the measurements. The SEM image was taken after the device was used and dried. The label A in the image shows damage to the device caused by the micropipette tip. The label B points out that the cantilevers in the images were attached to the substrate. This was

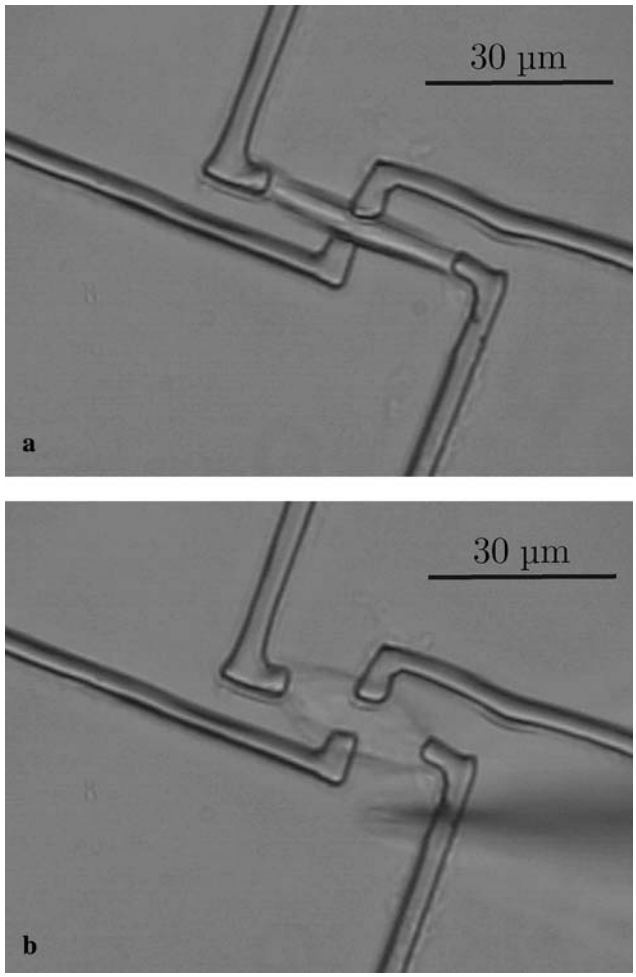

Fig. 2 Optical micrographs of the forisome on the sensor (**a**) in Ca^{2+} free solution and (**b**) in 10 mM Ca^{2+} solution

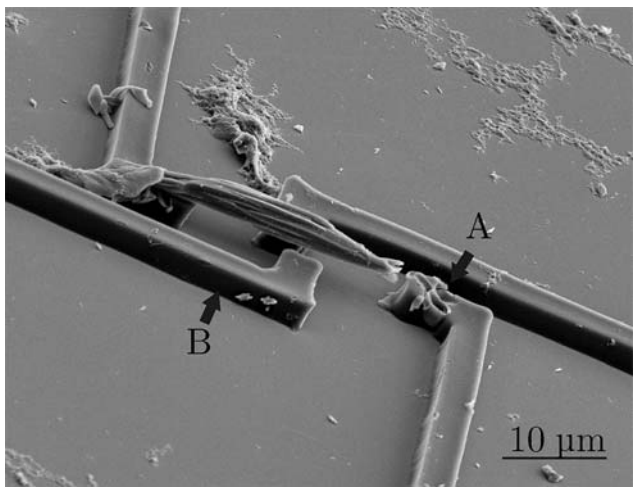

Fig. 3 SEM micrograph of the force sensor with a forisome placed on the device. The label *A* points out damage to the cantilever caused by the micropipette tip; *B* shows that the device is attached to the substrate. This is a result of stiction during the drying process and does not affect the function of the device in aqueous environments

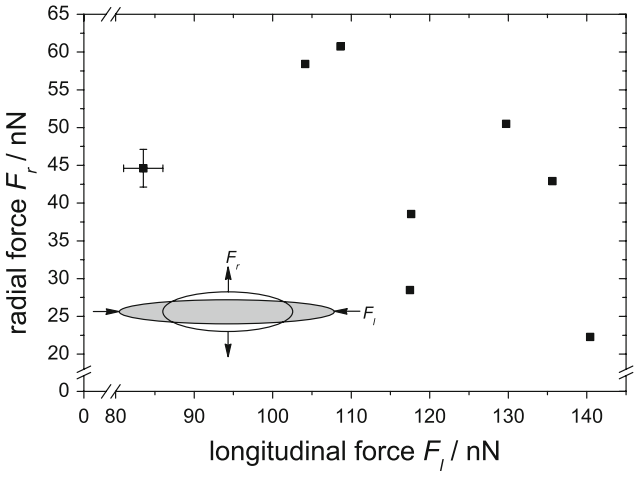

Fig. 4 Plot of the longitudinal versus radial force for eight different forisomes. As expected, the forces in the radial direction are lower than those in the longitudinal direction

a result of stiction of the device during the drying process. The cantilevers remained suspended while in an aqueous environment.

The resulting longitudinal and radial forces were simultaneously measured for each of eight different forisomes. The longitudinal force is plotted versus the radial force in Fig. 4. The forces ranged from 84 to 136 nN with an average force of 120 ± 20 nN (±SD) in the longitudinal direction of the forisomes. Forces in the radial direction were 22–61 nN with an average force of 40 ± 10 nN. The variation in the force magnitude in a given axis is primarily attributed to differences in the geometry and behavior of the individual forisomes. It is suspected that the inhomogeneity between the longitudinal and radial forces is due to preferential orientation of the proteins in the long axis of the forisomes. The results for the longitudinal force magnitudes are in qualitative agreement with previous estimations. This is the first time that the radial forces generated by forisomes have been measured. While the forces are smaller relative to the longitudinal force, these forces are sufficiently large to be functionally incorporated into a microscale actuator or valve system.

Conclusions

In summary, we were able to design and fabricate a polymer cantilever sensor for measuring forisome forces. Measurements were performed to determine both the radial and longitudinal forces generated by forisomes in response to stimulation by Ca^{2+}. Forces ranged from 84 to 136 nN (120 ± 20 nN) in the longitudinal direction and 22 to 61 nN (40 ± 10 nN) in the radial direction. The ability to quantify the bi-directional forces generated by forisomes is an important step in applying these materials as functional components in microdevices.

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