SINGLE STRESS FIBERS ISOLATED FROM VASCULAR SMOOTH MUSCLE CELLS POSSESS SURPRISINGLY HIGH EXTENSIBILITY

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INTRODUCTION

It has been believed that cytoskeletal proteins polymerizing into filaments or networks provide morphological and mechanical integrity of eukaryotic cells. Stress fibers (SFs), bundles of actin filaments, are found to appear/disappear responding to mechanical stimuli [1]. Therefore, SFs are considered to be one of the major components that sustain mechanical stresses existing in cells. Many investigations about the effects of cytoskeletal proteins on mechanical properties of living cells [2] and polymer gels [3] have been done, however, little is known about the mechanical properties of SFs themselves.

In this study, we measured directly tensile stiffness of single SFs in vascular smooth muscle cells. The SFs were isolated from the cells with chemical treatments and stretched using a pair of cantilevers to obtain the load-elongation curves. The mechanical properties of SFs were compared with those of actin filaments previously reported.

MATERIALS AND METHODS

Isolation of SFs

SFs were isolated from cultured bovine aortic smooth muscle cells according to the previously reported technique [4]. The cells were passaged from 6th to 10th generation. After cells became subconfluent in a 35 mm diameter culture dish, they were washed briefly with PBS. The cells were placed in a low-ionic-strength solution containing 2.5 mM triethanolamine for 20-40 min, a buffer solution containing 0.5% Nonidet P-40 for 5 min, and a buffer solution containing 0.05% Triton X-100 for 5 min. During this process, we shook the culture dish gently on a flat shaking table with ice. After washing the dish with PBS, extracted materials were scraped off and then put through an injection needle of 23-gauge to free SFs from the cell cortex. The isolated SFs were suspended in PBS in a siliconized dish. For visualization, actin filaments were stained with rhodamine-phalloidin. All of the solutions were added by 1 μg/ml leupeptin and 1 μg/ml pepstatin and maintained at 4 ℃.

Tensile test

The dish with the isolated SFs was mounted on the stage of an inverted fluorescence microscope. Under the illumination from a halogen and a mercury lamp, both ends of a single SF were held carefully by two cantilevers of carbon fibers (7 μm in OD). Great care was taken not to apply any tension at the initial state. The cantilevers were manipulated by hydraulic micromanipulators. Epoxy glue was previously coated on the tips of cantilevers so that SFs were rigidly bound to the cantilevers. The two cantilevers were placed parallel and vertical to the axial direction of the SF. Tensile tests were initiated after waiting for about 20 min until the glue sufficiently hardened. The SF was stretched by moving the parallel cantilever toward the outer direction with the step of 2 μm per 5 sec. The displacement of the SF and the deflection of the vertical cantilever were measured from the images obtained through a digital CCD camera at each step. The spring constant of the vertical cantilever was determined from the cross calibration for each experiment to obtain the load-elongation curves. The tensile tests were performed at room temperature of 20℃.

RESULTS AND DISCUSSION

Figure 1 shows fluorescence images of rhodamine-phalloidin stained SFs (a) before and (b) after extraction with Triton X-100 treatment. SFs were confirmed to be extracted because the diffuse staining due to actin filaments unrelated to SFs did not appear after the Triton X-100 treatment. We have already checked that nuclei, microtubules, and cytoplasm were dissolved or removed from cells by labeling them using SYTO13, GFP-tubulin, and Calcein-AM, respectively. In contrast, α-actinin, one of the cross-linking proteins of actin filaments, was observed to localize along the isolated SFs using GFP-α-actinin even after extraction. These observations indicate that SFs could be isolated without any damage.

Figure 2 shows sequential images of the tensile test of a single SF. During the stretching, the single SF was elongated and the vertical cantilever was bent with increasing in stress transfer. The load-elongation curves were examined up to the elongation when breakage or slippage occurs. A typical example of the load-elongation curve was

drawn in Figure 3. In most cases, SFs showed high extensibility together with nonlinear increasing in stiffness. SFs was elongated more than two-fold of the initial length, and such a high extensibility of SFs has never been reported before.

If cross sectional areas of the SFs were calculated assuming that SFs are continuum materials and their cross sections are circle with the diameter of 0.1μm [4], then the initial elastic modulus is 2.7±4.8 MPa (Mean±SD, n=20). This value is much lower than that of synthesized single actin filaments, 1.8 GPa [5]. SFs are composed of linear actin filaments and cross-linking proteins such as α-actinin. It can be speculated, therefore, the bending of α-actinin, which is supposed to be flexible and is connected to two actin filaments vertically at static conditions, is responsible for the low initial elasticity, as schematically shown in Figure 4. In this model, the bending rigidity of α-actinin dominates the tensile properties of SFs at the lower strain range. Meanwhile, the involvement of the intrinsic rigidity of actin filaments will appear at the higher strain range. This mechanical model seems to be rather reasonable taking into account that the scale of elasticity asymptotically approaches to the order of GPa as stretch proceeds.

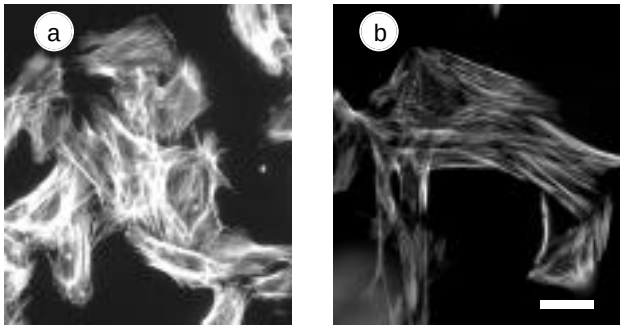

Figure 1 Fluorescence images of stress fibers labeled with Rhodamine-Phalloidin in smooth muscle cells (a) before and (b) after extraction with Triton X-100 treatment. Bar, 50 μm.

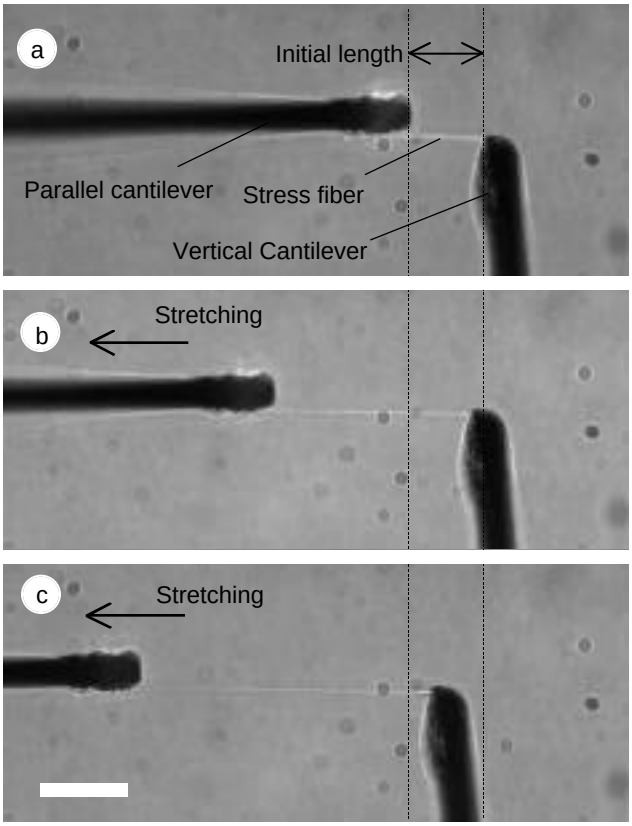

Figure 2. Sequential fluorescence images of a single stress fiber during tensile test. (a) before stretching; (b) during stretching; (c) just before slippage. Bar, 20 μm.

CONCLUSIONS

Tensile stiffness of single SFs isolated from vascular smooth muscle cells was directly measured. The results showed that SFs had high extensibility and the stiffness increased nonlinearly with increasing strain. We proposed the mechanical model of SFs based on the microstructure of them.

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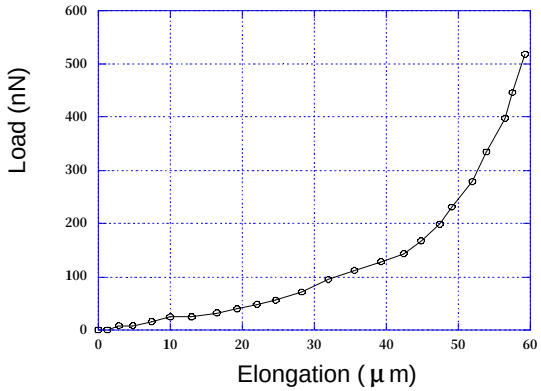

Figure 3. Typical example of load vs. elongation curve (Initial length = 23.6 μm).

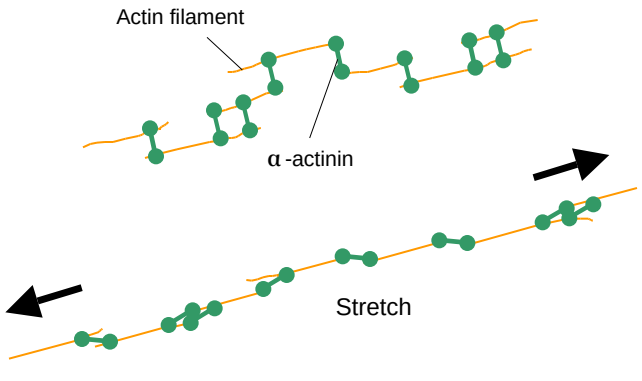

Figure 4. Schematic diagram of mechanical model of stress fiber. (a) under static conditions; (b) under stretched conditions.