Forisomes: Mechano-Proteins that Exert Force in Contraction and Expansion

Michael Knoblauch*, Gundula A. Noll**, Dirk Prüfer*, Hannah Jaag*, Maria E. Fontanellaz*, Ingrid Schneider-Hüther**, Aart J. E. van Bel**, Winfried S. Peters**

*Fraunhofer Institut für Molekularbiologie und Angewandte Ökologie, Auf dem Aberg 1, D-57392 Schmallenberg-Grafschaft, Germany, michael.knoblauch@bot1.bio.uni-giessen.de

**Institut für Allgemeine Botanik, Justus Liebig-Universität, Senckenbergstr. 17-21, D-35390 Gießen, Germany

ABSTRACT

Forisomes are elongate protein bodies of up to 30 µm length from cells of the sieve tube network of higher plants. In this natural microfluidics system, they act as reversible stopcocks by undergoing rapid conformational changes which involve more than three-fold increases in volume. The conformational switch is controlled by Ca^{2+} with a threshhold concentration in the nM range. We here report recent progress in our attempts to define the technological potential of this novel biological actuator.

Keywords: biomimetic actuator, contractile protein, forisome, intracellular Ca^{2+}, microfluidics

1 INTRODUCTION

Sieve elements are highly specialized cells in the phloem of vascular plants. They form a continuous microtube system enabling pressure driven long distance transport of photo-assimilates [1]. Unique elongate protein bodies of up to 30 µm length occur in the sieve elements of legumes (Fabaceae). We showed that these Forisomes act as molecular stopcocks [2]. Under normal physiological conditions, they exist in a condensed state, characterized by a highly ordered fibrillar ultrastructure. At increased intracellular Ca^{2+}, the protein bodies enter a dispersed state, forming plugs in the sieve tube. The reaction is fully reversible.

2 FORISOME CONTRACTION *IN VITRO*

We scrutinized the Ca^{2+} response *in vitro*, using forisomes isolated from broad bean (*Vicia faba*) sieve elements [3]. Surprisingly, forisomes did not only swell radially in response to Ca^{2+}, but also contracted longitudinally. In contraction, forisomes shortened by 29.4 ± 6.7 % and increased in diameter (measured across their center) by 119.5 ± 47.9 % (means ± s.d., n = 15). The response to $Ca^{2+}$was fully reversible by transfer to Ca^{2+}-free solutions. Contraction-expansion cycles could be repeated at least 50 times in the same forisome without decrease in responsiveness. Contraction seemed to follow an all-or-none law: either it occurred to the full extent or not at all. The threshhold concentration for Ca^{2+}-induced contraction was below 100 nM.

3 GENERATION OF FORCE

In forisomes that were attached to one glas pipette tip at each end, contraction to the normal extent could still be induced reversibly by increased Ca^{2+}. As a result, the pipettes became bent (Fig. 1). Thus, contracting forisomes develop significant mechanical forces.

4 pH EFFECTS

In Ca^{2+}-free buffers, forisomes did not react to changes of pH between 5.0 and 9.5. When the pH was increased beyond 9.6, the protein bodies started to swell radially and contracted gradually and reversibly in a pH-dependent fashion. At pH 10.6, contraction reached the degree normally evoked by Ca^{2+} at the physiological pH 7.3.

5 FORISOME CONTRACTION CONTROLLED BY ELECTROTITRATION

Considering technical applicability, it would appear desirable if forisome action could be controlled electrically. We used diffusional electrotitration [3] to drive pH-dependent contraction and expansion in isolated forisomes. Provided an appropriate pH range was covered by the electrically induced proton gradients, forisomes showed pH responses as discussed above (Fig. 2). Voltage pulsed at varying frequency led to corresponding oscillations of the state of the forisome, demonstrating in principle that forisome action can be controlled electrically. At least 4,000 electrically induced contraction–expansion cycles could be observed in single forisomes.

6 OUTLOOK

Present research in our labs and those of our collaborators centers on the mechanism of forisome contraction [4],

on the characterisation of forisome molecular structure, on the development of integrated leak-free microfluidic systems in which forisomes serve as reversible switches, and on the construction of prototypic micro-devices in which forisomes act as actuators.

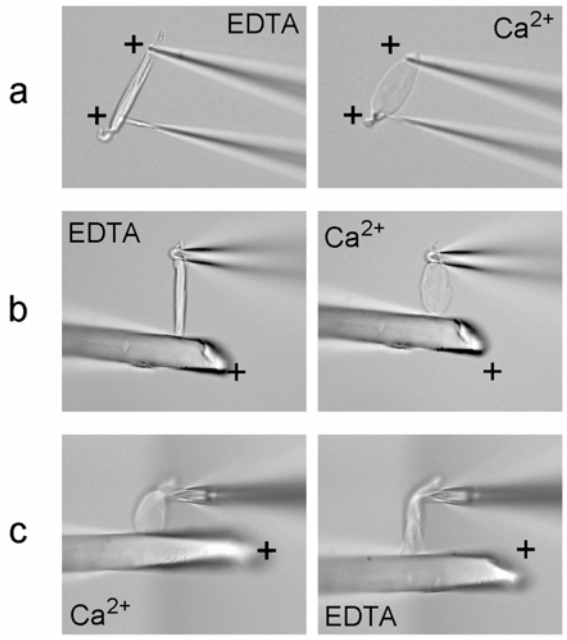

Figure 1: Demonstration of mechanical force exerted by forisomes

a, Forisome fixed between two glass pipette tips in the expanded state (left). Application of Ca^{2+} caused longitudinal contraction by some 30%, resulting in bending of the pipettes (right; black crosses are at identical positions in both images, to indicate the relative movement of the pipette tips).The response was fully reversible.The length of the expanded forisome (left) was 21µm. Repetitions of the experiment in five different forisomes yielded similar results. **b**, Determination of pulling force in a forisome fixed in the expanded state between the tip of a rigid glass pipette (which enters the visual field from the right) and a flexible glass fibre (9µm diameter; entering from the left). On addition of Ca^{2+}, the forisome pulls the fibre towards the pipette (right image). **c**, Analogous experiment to **b**; this time, a forisome is fixed in the contracted state (left). Addition of EDTA chelator causes it to expand, pushing the flexible fibre away from the rigid pipette (right). Black crosses in the pairs of images in **b** and **c** are at identical positions relative to the tip of the rigid glass pipette, respectively. Note that the amount of displacement of the fibre tip is similar in **b** and **c**, indicating that similar forces (about 0.1µN, in this case) are exerted by pulling and pushing forisomes.

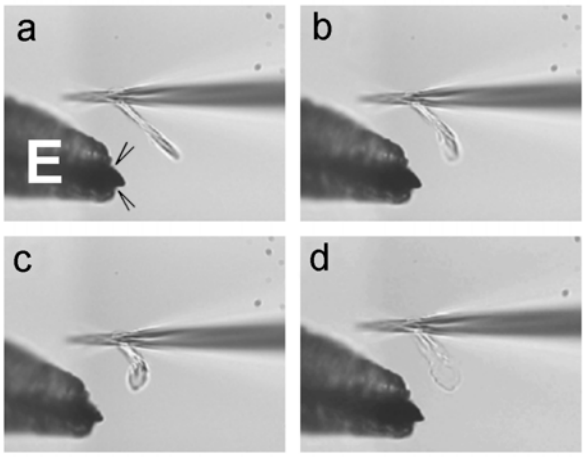

Figure 2: Forisome swelling and curvature induced by electrotitration.

The images show a forisome of 23 µm length in the expanded state (**a**) stuck to a glass pipette, and a shielded platin microelectrode (E; the unshielded, conducting tip is the pointed protrusion between the two arrowheads) driven as the cathode to induce zones of increased pH in its vicinity. The anode was placed 50 µm away and is not visible here. The pH of the weakly buffered (0.4 mM Tris/HCl) medium was 7.3. **a**, No voltage applied.**b**–**d**, Reactions to 950 µs pulses of 6.2 V applied at increasing frequencies. **b**, 5 Hz; slight curvature of the weakly swollen forisome. **c**, 8 Hz; strong curvature (roughly 60°) exhibited by the significantly contracted forisome. **d**, 20 Hz; the forisome contracts longitudinally and expands radially in response to the zone of increased pH that develops around the electrode.

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