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Elastic properties of the forisome

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Abstract. Forisomes are elongate Ca^{2+}-responsive contractile protein bodies and act as flow blocking gates within the phloem of legumes. Because an understanding of their mechanical properties *in vitro* underpins understanding of their physiology *in vivo*, we undertook, using a microcantilever method, microscopic tensile tests (incremental stress-relaxation measurements) on forisomes from *Canavalia gladiata* (Jacq.) DC Akanata Mame and *Vicia faba* L. Witkiem Major. Viscoelastic properties of forisomes in their longitudinal direction were investigated before and after Ca^{2+}-induced contraction, but in the radial direction only before contraction. Forisomes showed mechanical properties typical of a biological material with a unidirectional fibrous structure, i.e. the modulus of elasticity in the direction of their fibers is much greater than in the radial direction. Creep data were collected in all tensile tests and fit with a three parameter viscoelastic model. The pre-contraction longitudinal elastic moduli of the forisomes were not differentiable between the two species (*V. faba*, 660 ± 360 kPa; *C. gladiata*, 600 ± 360 kPa). Both species showed a direction-dependent mechanical response: the elastic modulus was dramatically smaller in the radial direction than in the longitudinal direction, suggesting a weak protein cross-linking amongst longitudinal protein fibers. Activation of forisomes decreased forisome stiffness longitudinally, as evidenced by the loss of toe-region in the stress strain curve, suggesting that the forisome may have dispersed or disordered its protein structure in a controlled fashion. Contractile forces generated by single forisomes undergoing activation were also measured for *V. faba* (510 ± 390 nN) and *C. gladiata* (570 ± 310 nN).

Additional keywords: microcantilever, P-protein, sieve element, spasmoneme, viscoelasticity.

Introduction

Forisomes are P-protein crystalloids contained within the sieve elements of the phloem of legumes. ATP-independent contractility of the forisome, triggered by an increase of cytosolic Ca^{2+}, allows forisomes to halt nutrient transport within the microfluidic network of the phloem when tissue has been damaged. Forisome contraction is anisotropic and rapid, yielding (in times under a second) a dramatic transition from a relaxed conformation of relatively minor disturbance of flow to a contracted conformation entirely filling the cross-section of a sieve element (Peters *et al*. 2007*a*).

Because kinetic studies of conformational change have shown a polyphasic kinetic response to chemical stimulation (Peters *et al*. 2007*a*), it would seem that the mechanism for forisome shape change is at least two-fold. Moreover, water uptake and supramolecular reorganisation are thought to drive the chemical response (Pickard *et al*. 2006). Damaged forisomes change shape isotropically, apparently due to the disruption of molecular coupling that is required to generate anisotropic shape change in healthy forisomes (Knoblauch *et al*. 2003). The mechanical response of forisomes may yield further insight into their intriguing structure and contractility mechanism. Detailed mechanical exploration of the forisome has yet to be undertaken; and this paper probed the mechanical properties of forisomes for the first time. To study the mechanical properties of the forisome, microcantilever tensile experiments were performed on forisomes extracted from two species, *Vicia faba* L. Witkiem Major and *Canavalia gladiata* (Jacq.) DC.

Microcantilever techniques are one of the oldest experimental methods used for probing mechanical properties of whole cells (Felder and Elson 1990; Van Vliet *et al*. 2003). These techniques employ small cantilevers to probe forces and deformations of small biological objects. Deformations of the biological object and the cantilever are recorded with a camera under the microscope and are measured through image analysis and interpreted through cantilever theory. These methods are well suited for exploring the mechanical response of forisomes, which have micron dimensions similar to many biological entities, such as smooth muscle cells (Nagayama *et al*. 2006) and contractile spasmonemes (Moriyama *et al*. 1999) both studied previously with microcantilever experimental methods.

Closely related to the methods employed in this work are tensile studies performed on the effects of actin filaments on the mechanical properties of murine smooth muscle

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