ASSAY AND QUANTIFICATION OF ACTIN IN DEVELOPING COTTON FIBERS

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Abstract

Actin is the structural unit of microfilaments and is an important cytoskeletal protein. Two anti-actin antibodies, mAb3H11 and JLA20, were used to detect actin levels in various cotton plant tissues. Actin levels were estimated using SDS-PAGE and chemiluminescence western blotting techniques. NIH Image software was used to evaluate the blots. Actin levels in 20 day post anthesis cotton fibers appear to be higher than in 10 day post anthesis cotton fibers. Actin levels appear to be higher in tissue from fiber cells compared to tissues from leaves and cotyledons. Roots appear to have a higher level of actin than leaves or cotyledons.

Introduction

Cotton fiber develops from a differentiating epidermal cell of a fertilized ovule. The importance of cell wall organization in cotton fiber has been established (Delanage, 1986). Microtubules and microfilaments, and their interrelationships, are critical to cotton fiber development. Seagull (1992) identifies 3 important periods in fiber development: 1) the transition from fiber initiation to elongation, when microtubules undergo a reorientation and ordering, 2) the transition between primary and secondary wall synthesis, when microtubules change orientation from shallow-pitch helices to steeply-pitched helices, and 3) early in secondary wall synthesis when the number of microtubules increase 4-fold. The importance of tubulin, a cytoskeletal protein has been described (Kloth, 1989; Dixon et al., 1994).

Actin, the subunit component of microfilaments, is also an important part of the cytoskeleton. There are several functions of actin within the cell (Alberts et al., 1994). The purpose of this study was to estimate relative levels of actin protein in developing cotton fibers at two stages of development. The first being at 10 days post anthesis (DPA) when primary wall synthesis is occurring, and the second at 20 DPA when primary wall synthesis is complete, but secondary wall synthesis is continuing. Other plant tissues were sampled to compare to the actin levels within the developing fiber. A better understanding of the role of actin in fiber development will provide insight as to how cell wall organization affects fiber properties such as length, strength, and micronaire.

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Material and Methods

Cotton seeds, Gossypium hirsutum (DES 119), were planted in 8 inch pots and thinned to one plant per pot. Plants were grown under greenhouse conditions and watered daily and fertilized weekly, with Peters 20-10-20 liquid fertilizer. The first bloom was tagged on each of the first three fruiting branches of each plant. The bolls were randomally harvested at 10 and 20 days post anthesis (DPA). Cotyledon, leaf, and root tissues were also collected for comparison to fibers. Fibers were removed from harvested bolls and ground to a powder and stored in liquid nitrogen. Other plant tissues were also ground in liquid nitrogen and stored for analysis at a later time. Protein was extracted according to the procedure of Barent and Elthon (1992). The protein pellet was resuspended in three proteinase inhibitors, leupeptin, PMSF, and benzamidine-HCl. Protein samples from selected plants and from purified actin (rabbit muscle), were loaded onto a 10% Laemmli polyacrylamide gel. The gel was electrophoresed for 2 hours at 100 volts using a Hoefer Mighty-Small II electrophoresis unit. The proteins were then blotted to a 0.22 um nitrocellulose membrane, for 2 hours at 100 volts (20-35 mAmps), using a Bio-Rad Mini Trans-Blot apparatus. The membrane was blocked with Tris buffered saline with Tween (TBST) and 5% Carnation non-fat dry milk, and was probed with an antibody (mAb3H11) raised against pea root actin (Andersland et al., 1994). A second membrane, prepared as described above, was probed with a commercial antibody (JLA 20, Developmental Studies Hybridoma Bank). The membranes were probed with a second antibody conjugated to horseradish peroxidase (Amersham). A chemiluminescence substrate system (Pierce) for western blotting was used to expose X-ray film. Developed X-ray film was scanned into a Power Macintosh 8100 computer using an Apple Color OneScanner. Image analysis of the scanned film was performed using NIH 1.57 Image software. The blot areas were used to establish relative levels of proteins in the sampled tissues.

Results

There were two replications of plants in this study. Computer scans of the western blots of the proteins from these two replications are shown in Figures 1 and 2. The blot using the mAb3H11 antibody, Figure 1, indicates the presence of actin in roots, cotyledons, 10 DPA cotton fiber, and 20 DPA cotton fiber. No actin was detected in leaves of either replication. A small amount of actin was detected in the cotyledon tissue. Blots of the same tissue samples are shown in Figure 2, the only difference being the primary antibody and the presence of purified muscle actin in lane one. In Figure 2 only the purified actin and the 20

DPA cotton fiber produced a reaction when incubated with the JLA20 antibody.

The mAb3H11 antibody prepared against plant actin may detect degradation products of actin. These are indicated by the bands that have migrated slightly lower in the gel and appear below the major band on the western blot.

The relative amounts of actin for the blot using the mAb3H11 antibody (Figure 1.) are indicated in Table 1. Actin levels increased from 2 to 5 fold in the time period between 10 and 20 DPA.

1 2 3 4 5 7 8 9 10 11

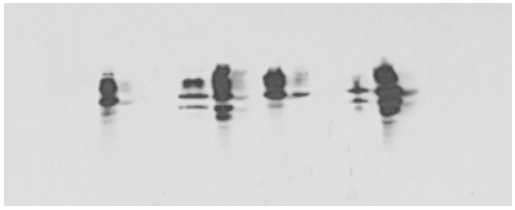

Figure 1. Western blot using mAb3H11 as the primary antibody (4 ug total protein per lane).

Lanes 1 and 7-Root tissue
Lanes 2 and 8-Cotyledon tissue
Lanes 3 and 9-Leaf tissue
Lanes 4 and 10- 10 DPA Fiber
Lanes 5 and 11- 20 DPA fiber

1 2 3 4 5 6 8 9 10 11 12

Figure 2. Western blot using JLA20 as the primary antibody (5 ug total protein per lane except where noted).

Lane 1 purified muscle actin (1 ug)
Lanes 2 and 8-Root tissue
Lanes 3 and 9-Cotyledon tissue
Lanes 4 and 10-Leaf tissue
Lanes 5 and 11- 10 DPA Fiber
Lanes 6 and 12- 20 DPA Fiber

Table 1. Relative levels of actin in blot probed with mAb3H11 antibody (Figure 1).

Replication 1

Lane	Tissue	Area	Relative % of 10 DPA
1	Root	2481	138
2	Cotyledon	242	14
3	Leaf		
4	10 DPA	1792	100
5	20 DPA	3829	214

Replication 2

Lane	Tissue	Area	Relative % of 10 DPA
7	Root	2829	284
8	Cotyledon	664	67
9	Leaf		
10	10 DPA	996	100
11	20 DPA	5200	522

Discussion

The blot probed with the mAb3H11 antibody indicates a difference in the actin protein levels of the different tissues. The root tissue collected was from young seedlings approximately five days after emergence. This tissue was from a highly meristimatic region and it is not surprising to find high amounts of actin in this tissue. The cotyledon tissue was also collected from young seedlings. Only a relatively small amount of actin was detected in this tissue. Actin is probably present in the leaf tissue but was not detected with this method. The 10 DPA cotton fiber is the developmental stage with no secondary wall synthesis and actin is present at this stage. In the 20 DPA cotton fiber primary cell wall synthesis is practically complete and secondary wall synthesis has begun. At this stage actin levels have increased two to five times relative to the 10 DPA cotton fiber. Tubulin protein levels increased four-fold during the 10 to 20 DPA cotton fiber in a similar study (Kloth, 1989). The second blot was probed with the JLA20 antibody (Figure 2) and only the purified actin and protein from the 20 DPA fiber were detected. This suggest that there is more actin protein in the 20 DPA fiber than in the 10 DPA cotton fiber, which is consistent with the results from Figure 1 using the mAb3H11 antibody.

Actin levels appear to have increased over the time course of 10 days. Blots probed with both antibodies indicated greater amounts of actin protein at 20 DPA compared to 10 DPA. The JLA20 blot indicates that there is more actin in the 20 DPA cotton fiber since no protein was detected in the 10 DPA cotton fiber. Andersland et al.,(1994) established the specific nature of the mAb3H11 antibody, its higher affinity for plant actin and its lower affinity for rabbit muscle actin. Both tubulin (Dixon et al., 1994; Kloth, 1989) and actin protein levels (Table 1) increase between 10 and 20 DPA. There are many complex interactions involving the cytoskeleton during this time period. More work is needed to make more conclusive statements regarding actin levels in developing cotton fibers.

Acknowledgment

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