DNase I affinity column chromatography

DNase I binds tightly to a globular actin (G-actin) monomer and forms a complex, although the physiological function of this complex has not yet been elucidated. A DNase I affinity column has been widely used to identify and isolate not only G-actin but also several kinds of plant G-actin-binding-proteins, including a fragmin-like protein (Yamashiro et al. 2001), an actin-filament-severing protein (Fan et al. 2004), a gelsolin-like protein (Huang et al. 2004), and villins (Yokota et al. 2005), which are all adsorbed onto the column through a G-actin-DNase I interaction.

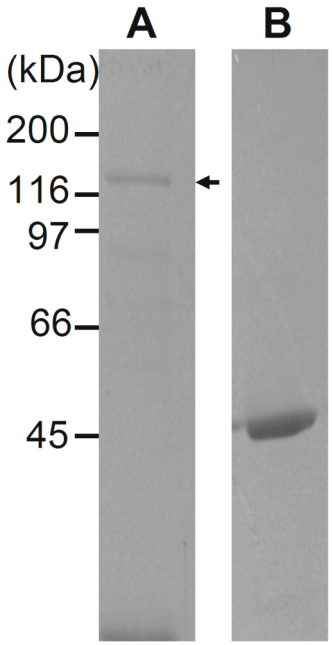
DNase I (grade II; Roche Diagnostics, Mannheim, Germany) was coupled to Affi-gel 10 resin (Bio-Rad, Richmond, VA, USA) according to the manufacture's instructions. Ten to 15 ml Affi-gel 10 resin was rinsed with 250 ml cold deionized water, then equilibrated with 200 ml Buffer A (10 mM $CaCl_2$, 1 mM PMSF, and 100 mM HEPES-KOH at pH 7.5) on a glass filter. Fifty mg DNase I was dissolved in 20 to 30 ml Buffer A and dialyzed in 500 ml Buffer A at 4^{o}C. A dialysate was mixed with the resin gently on a rotator at 4^{o}C overnight, following which 2-amonoethanol (pH 8.0) was added to the final concentration of 100 mM and further mixed gently on a rotator at 4^{o}C for 1 hour. After washing thoroughly with Buffer A, the resin was packed into a column, such as a disposal syringe.

Plant materials, such as seedling of Arabidopsis (Fig. 1) and germinating pollen (Yokota et al. 2005), were homogenized in Buffer B (60 mM KCl, 1 mM $MgCl_2$, 0.2 mM ATP, 2 mM DTT, 1 mM PMSF, 0.2 mg/ml leupeptin, and 30 mM HEPES-KOH at pH 7.5) supplemented with 0.2 M sucrose, 0.2 mM $CaCl_2$, and 1% casein, which potentially protects cytoskeletal proteins from degradation caused by endogenous proteases. After centrifugation at 15,000 *g* for 10 minutes, the supernatant was further centrifuged at 100,000 *g* for 30 minutes. After adjusting pH of a resulting supernatant to 7.5 with KOH, the extract was applied to the DNase I affinity column pre-equilibrated with Buffer B supplemented with 1 mM $CaCl_2$. After the column was thoroughly washed with the pre-equilibrated solution, the adsorbed materials were eluted successively with Buffer B supplemented with 5 mM EGTA, and then with 50% formamide or 3 M guanine hydrochloride.

Figure 1 shows an elution pattern from the DNase I affinity column of the extract

prepared from Arabidopsis seedling. The molecular masses of standard proteins are indicated on the left in kDa. A polypeptide with a molecular mass of about 123 kDa, which is most probably Arabidopsis villin, was eluted with 5 mM EGTA (indicated by an arrow in lane A). G-actin was dissociated from DNase I by the elution with 50% formamide (lane B). Generally, since the actin molecules are substantially denatured, they are no longer able to polymerize into actin filaments. Nevertheless, there is only one report that showed that G-actin prepared from animal tissues by a DNase I affinity column exhibited significant polymerization activity (Schafer et al. 1998).

Fig. 1



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