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BRIEF COMMUNICATION

Improved method of total histone isolation from *Arabidopsis thaliana*

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Abstract

Standard methods of isolation of chromatin histones from *Arabidopsis thaliana* yield strongly degraded proteins when applied to plants grown from seeds in axenic liquid media. For isolation of undegraded histones from *Arabidopsis* grown in liquid media we used extraction with guanidine hydrochloride followed by selective binding of histones on *BioRex 70* resin in the batch system. The quality of obtained proteins is confirmed by SDS polyacrylamide gel electrophoresis.

Additional key words: quanidine hydrochloride, SDS PAGE.

Growing *Arabidopsis* from seeds in axenic liquid medium is a convenient and fast method of producing large quantities of material for biochemical isolations (Karesch *et al.* 1991). However, compared to soil-grown plants the material obtained by liquid culturing may have considerably changed properties making it particularly difficult for isolation of certain types of proteins. We faced this problem when attempting to isolate the highly basic histone proteins from *Arabidopsis* grown in liquid media. The standard procedure of plant histone isolation published by Moehs *et al.* (1988) while proved satisfactory in our hands when used for soil-grown *Arabidopsis* (Fig. 1a), gave completely unreproducible results with plants obtained from seeds grown in liquid medium (Fig. 1b). In the latter case, the isolated core histones H2A, H2B, H3 and H4 were usually strongly degraded despite using protease inhibitors (phenylmethylsulphonyl fluoride, pepstatin A) and carrying all procedures at 4 °C

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and in a minimum time. *Arabidopsis* histone H1 stains very weakly on SDS gels (Moehs *et al.* 1988). We checked whether similar difficulties with isolation of histones also occur with tobacco grown in liquid media. Total histone preparation isolated from soil- and liquid-grown tobacco plants did not show considerable differences and were both not degraded (results not shown). The reason of the increased difficulties with histone isolation in the case of liquid-grown *Arabidopsis* could be partly due to increased production of proteolytic enzymes. The other reason could be elevated level in plants grown in liquid culture of the polycarbohydrates with the property of strongly bindidng to DNA (Prymakowska-Bosak, unpublished). Because of our previous experience with the material with similar properties, we adopted for histone isolation from liquid-grown *Arabidopsis* the method successfully used for histone isolation from a slime mold *Physarum polycephalum* (Meade *et al.* 1983, Jerzmanowski and Maleszewski 1985). This method after modification and shortening proved to be effective and reproducible.

Material was produced by sowing sterile seeds of *Arabidopsis thaliana* cv. Columbia in flasks containing MS medium (Murashige and Skoog 1962). Flasks were shaken gently under 16-h photoperiod at 20 - 22 °C (night) and 24 - 26 °C (day). Plants were harvested after 4 weeks. Approximately 40 g of plant material was homogenized in Waring blender in 200 cm^3 of solution containing 0.4 M saccharose, 10 mM Tris-HCl, pH 8, 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol and 1 mM PMSF. Homogenate was filtered through 4 layers of cheesecloth and 2 layers of *Miracloth* (Calbiochem, La Jolla, CA), then centrifuged at 12 000 *g* for 25 min in *GS3* rotor (*Sorvall*, DuPont) to obtain cell nuclei (Moehs *et al.* 1988). The nuclear pellet was resuspended in 15 cm^3 of 40 % GP buffer [40 % (m/v) guanidine hydrochloride, 50 mM KH_2PO_4, 50 mM K_2HPO_4, pH 6.8, adjusted with KOH] and sonicated 4 × 20 s in ice bath in *UP 200H* sonicator (*Dr. Hielscher GmbH*, Stahdorf, Germany) with 14 mm probe at amplitude set at 125 μm and power at 105 W cm^{-2}. The solution was centrifuged for 10 min at 38 000 *g* in *SS34* rotor (*Sorvall*, DuPont) and the pellet was discarded. Concentrated HCl was added to the supernatant to a final concentration of 0.25 M. Solution was gently mixed on ice for 7 h and centrifuged as before. 100 mM phosphate buffer pH 6.8 was added to the supernatant to make the refractive index the same as that of 6 % GP buffer. Roughly 70 cm^3 of *BioRex 70* (*Bio Rad Laboratories*, Richmond, USA) cation exchange resin (10 g *BioRex 70* and 60 cm^3 of 6% GP buffer, pH 6.8) was added to the supernatant and the mixture was gently aggitated at room temperature overnight. The resin was then allowed to settle and was decanted and washed twice with 6 % GP buffer. Equal volume of 40 % GP buffer was then added to the slurry and agitated on magnetic stirrer for 1 h at room temperature. The suspension was centrifuged in *SS34* rotor for 5 min at 500 *g* at 4 °C. Supernatant was poured off carefully and dialyzed for 6 h against 0.01 M sulfuric acid, 0.1 mM PMSF, with 3 changes. The solution was subsequently made 25 % with trichloroacetic acid by adding 100 % (m/v) trichloroacetic acid, stirred overnight in cold room and centrifuged for 45 min at 43 000 *g* in *SS34* rotor. The precipitate was washed with 20 cm^3 of cold acetone and centrifuged for 30 min at 38 000 *g* in *SS34* rotor. The pellet was dried under cold air, dissolved in 0.5 cm^3 of ultrapure water and concentrated in vacuum centrifuge.

Proteins were electrophoresed in SDS-PAGE system (Laemmli 1970). Separated proteins were electroblotted onto nitrocellulose membrane using semi-dry blotter (*Sigma*, St. Louis, USA) and incubated with mouse polyclonal anti-wheat H1 antiserum. Goat anti-mouse IgG coupled to alkaline phosphatase (*Dako*, Glostrup, Denmark) was used as secondary antibody according to manufacturer recommendations. All washings and detection were as described (Mazzoloni *et al.* 1989, Otto 1993).

The analysis of obtained histone preparation by SDS-PAGE shows a typical pattern of undegraded *Arabidopsis* core histones (Fig.1*c*) and a distinct band representing histone H1 (Fig. 1*c,d*).

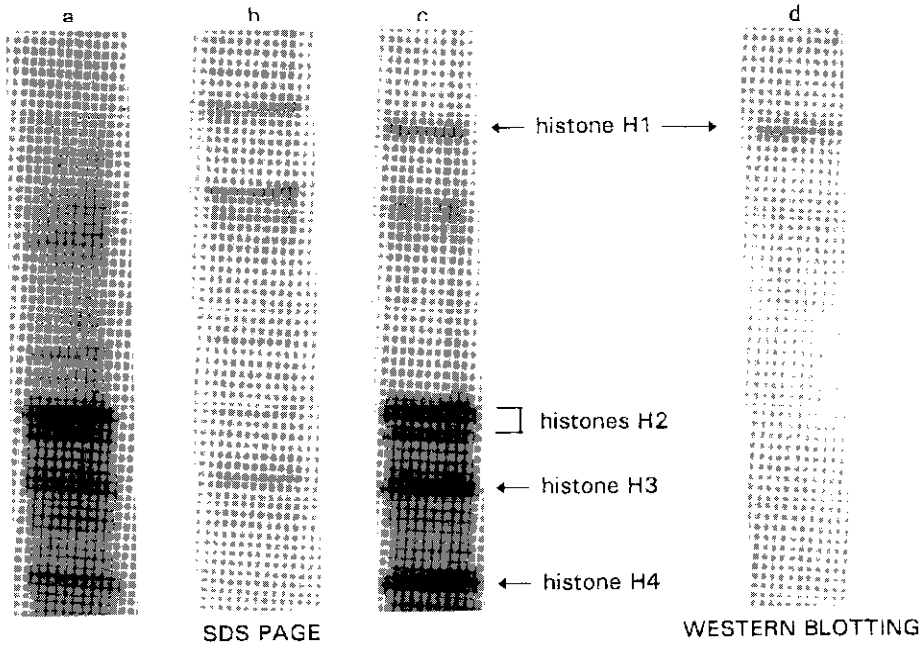

Fig. 1. Total histone proteins of *A. thaliana* separated by SDS polyacrylamide gel electrophoresis: *a* - soil-grown plants, *b,c* - liquid medium-grown plants; *a,b* - histones isolated by standard procedure (Moehs *et al.* 1988), *c* - histone isolated with improved method using guanidine hydrochloride; *d* - immunoblotting of lane *c*.

Chromatin structure is implicated in many regulatory mechanisms affecting transcription of genes. Due to availability of well characterized mutants *Arabidopsis* has become a material of choice also for the studies of chromatin-related transcription regulatory mechanisms. The reliable and reproducible procedure of histone preparation described in this work can be of use in both structural and functional analysis of chromatin form *Arabidopsis* grown under different natural and laboratory conditions.

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