Histone Acetylation is not Affected by Chloroacetamides *in vitro*

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The effects of chloroacetamides on the acetylation of histone protein in maize *(Zea mays)* were studied in an *in vitro* assay. Neither alachlor nor metazachlor showed any influence on both of the investigated acetylating enzymes, the nuclear histone acetyltransferase A and the cytoplasmic histone acetyltransferase B. Furthermore, an effect of these herbicides on deacetylation of histones could be excluded.

Introduction

Chloroacetamides are widely used as preemergent herbicides for the control of grasses and some broadleaf weeds. Although the first active compound of this group of herbicides has been developed more than 40 years ago (Jaworski, 1965), and a dozen or more of these chemicals have become the active ingredient of commercially important herbicides, their primary mode of action remains unknown. Many different effects on biochemistry, physiology, and morphology of various treated plant species have been reported (for review see Fuerst, 1987; LeBaron *et al.,* 1987). Generally, disturbance of cell metabolism leads to a significant inhibition of germination of susceptible weeds. Apart from inhibition of cell division and synthesis of flavonoids, isoprenoids, epicuticular waxes, and protein, especially synthesis (Weishaar *et al.,* 1988) and desaturation (Couderchet and Böger, 1993) of fatty acids and lipids (Mann and Pu, 1977) seem to be affected.

Recently, evidence for inhibition of acetylation of DNA-associated protein was presented (Weisshaar and Böger, 1991). In eukaryotic cells the DNA is associated with histones to form nucleosomes, containing two molecules of each of four histone species. 26 to 28 possible acetylation sites within a nucleosome (Doenecke and Gallwitz, 1982) ensure a dynamic state of histone acetylation, controlled by acetylating and deacetylating enzyme activities (Loidl, 1988; López-Rodas *et al.,* 1993). Disturbance of this enzyme activities leading to impaired regulation of histone acetylation with its implications on replication and transcription processes in the affected cells would explain the variety of reported effects of the chloroacetamides.

The present investigation was performed to elucidate the influence of chloroacetamides on the histone-acetylating and -deacetylating enzymes in plants. We studied the effect of alachlor and metazachlor on histone acetylation using an *in vitro* system.

Materials and Methods

Seeds of maize (*Zea mays* M320) or barley (*Hordeum vulgare* var. Gimpel) were germinated in the dark for 3 to 4 days. The root tips were ground to powder in liquid nitrogen. Resuspension, homogenization and dialysis were performed as described (López-Rodas *et al.,* 1991). The dialysate was centrifuged at 27,000×*g* for 10 min and the supernatant loaded onto a column of DEAE-Sepharose CL-6B (1×11 cm), equilibrated with buffer (15 mM Tris-HCl, pH 7.9 (N-tris-(hydroxymethyl)-aminomethane), 10 mM NH_4Cl, 0.25 mM EDTA, 10 mM β-mercaptoethanol, 10% (v/v) glycerol). Elution was performed with a linear gradient of 10–350 mM NH_4Cl in the same buffer with a flow rate of 18 ml/h. Fractions of 2.8 ml were collected and assayed for enzyme activities. Alternatively, a Mono-Q column was used for HPLC separation of the acetyltransferases from an extract of maize seedlings after 12 h or 40 h of germination. The nuclear histone acetyltransferase (A) was always separated from the cytoplasmic

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acetyltransferase (B). The preparations were partially pure as reported previously (López-Rodas *et al.*, 1991).

Histone acetyltransferase activity was measured according to López-Rodas *et al.* (1991): 100 µl of the enzyme-containing chromatographic fraction were mixed with 120 µg of histones from chicken erythrocytes (López-Rodas *et al.*, 1991) or calf thymus (Sigma, Deisenhofen) and 50 nCi (1.85 kBq) [1-^{14}C]acetyl-CoA (Amersham, Braunschweig). After a 20 min incubation period at 37 °C with herbicide present in the concentration indicated or of an equal volume of solvent, respectively, aliquots of this reaction mixture were placed on glass fiber filters, which were subsequently air-dried for 5 min. The filters were then submersed in ice-cold 25% (w/v) trichloroacetic acid, after 20 min washed twice with fresh 25% (w/v) trichloroacetic acid and once with ethanol, ethanol:ethylether (1:1, v/v) and ethylether, respectively, to remove radioactive label not bound to the histones. Finally, the filters were dried at 70 °C for 10 min and after adding 5 ml of INSTA Gel (Packard, Groningen) the radioactivity remained was measured by scintillation counting.

Histone deacetylase activity was assayed according to Sendra *et al.* (1988). To 200 µl of enzyme-containing solution herbicide or an equal amount of solvent was added. The mixture was incubated at 37 °C for 2 h with 10 µl of histones (4 mg/ml) from chicken erythrocytes, prelabeled with [^{3}H]acetate (2500 cpm/µg, Amersham, Braunschweig) as described by Ferenc and Nelson (1985). The reaction was stopped by acidification of the solution with 70 µl of 1 M HCl/0.4 M H_2SO_4 to protonate the released acetate. After addition of 1 ml ethylacetate, the mixture was vortexed thoroughly and centrifuged for 5 min at 10,000×*g*. The supernatant (upper phase), containing the released acetate, was carefully removed from the lower aqueous phase, mixed with 5 ml of INSTA Gel (Packard, Groningen) and radioactivity detected by liquid scintillation counting.

Results and Discussion

Assays of the chromatographic fractionation of extracts of meristematic maize cells reveal at least two distinct peaks of enzyme activity of histone acetyltransferases A and B, representing a nuclear and a cytoplasmic form, with differences in histone specificity. Fig. 1 (upper part) shows the enzymatic activity of the A-form after incubation with and without alachlor and metazachlor, respectively. The good reproducibility of the enzyme activity is by no means affected by the two herbicides. Lack of any inhibiting effect of the tested herbicides on the enzymatic activity *in vitro* is obvious. Analogous results have been found for the histone acetyltransferase B (Fig. 1, lower part). Unfortunately, no naturally occurring *in vitro* inhibitor of these enzymes is available to be compared with the chloroacetamides.

Analogous experiments with extracts from meristematic barley cells *(Hordeum vulgare)*, fractionated by anion-exchange chromatography on DEAE-Sepharose, repeated the zero effect of chloroacetamides on the acetylating enzymes.

Similar results were found with the histone-deacetylating enzyme (Fig. 2). The activity of this enzyme is neither inhibited nor increased in the presence of any of the chloroacetamides tested in this assay. Furthermore after incubation of plant material with chloroacetamides up to 100 µM the isolated enzymes revealed no significant differences in HPLC elution profile and enzyme activity (data not shown).

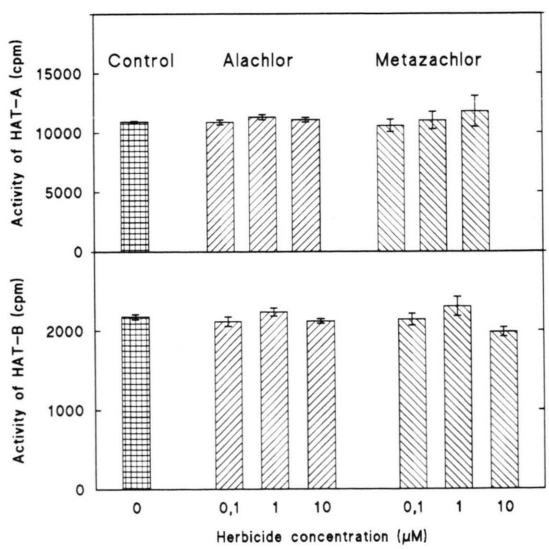

Fig. 1. *In vitro* activity of histone acetyltransferase A (HAT-A, upper part) and histone acetyltransferase B (HAT-B, lower part) from maize in presence or absence of the chloroacetamide herbicides indicated.

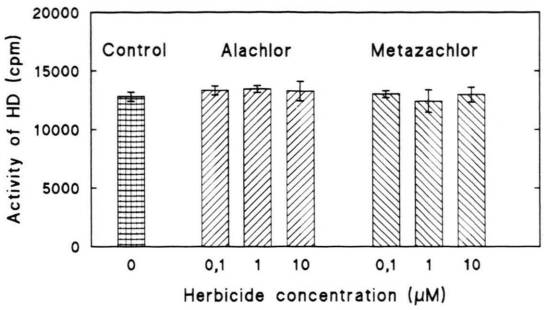

Fig. 2. *In vitro* activity of histone deacetylase (HD) from maize in presence or absence of the chloroacetamide herbicides indicated.

Our original hypothesis about chloroacetamide action affecting the acetylation level of histone protein (Weisshaar and Böger, 1991) has to be revised and some former results should be reinterpreted.

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