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HISTONE DEACETYLASE ACTIVITY AND REACTIVE OXYGEN SPECIES CONTENT IN THE TISSUE CULTURE OF *Arabidopsis thaliana* UNDER NORMAL CONDITIONS AND DEVELOPMENT OF ACUTE OSMOTIC STRESS

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The possible involvement of histone deacetylase (HDAC) in regulation of ROS content in the tissue culture of Arabidopsis thaliana under normal conditions and under development of acute osmotic stress was studied by using inhibition assay with application of trichostatin A (TSA). It was found that in the tissue culture grown under normal conditions a decrease in HDAC activity by means of TSA led to increase of the ROS content. Similar but more pronounced alterations occurred under stress. At the same time an increase in histone acetyltransferase (HAT) activity was also observed. The possible mechanisms of HDAC and HAT participation in regulation of ROS content by changes in expression of genes that are responsible for ROS production and antioxidant activity are discussed.

K e y w o r d s: histone deacetylase and acetyltransferase, reactive oxygen species, tissue culture, osmotic stress.

Acetylation and deacetylation of nuclear histones by histone acetyltransferases (HATs) and histone deacetylases (HDACs) play an important role in the regulation of gene expression in plants under stress [1-3].

HDACs (Histone deacetylases, HDAC, 3.5.1.98) are enzymes that remove acetyl groups from nuclear histones. This leads to a reduced ability of DNA transcription factors to access DNA that results in transcriptional repression and a decrease in gene expression. On the contrary, HATs (Histone acetyltransferases, HAT, 2.3.1.48) acetylate histones that results in transcriptional activation of DNA and an increase in gene expression [1, 4]. There is a dynamic balance between acetylation and deacetylation of histones in plants that regulates gene expression and cell metabolism as a whole [1, 5-7].

Reactive oxygen species (ROS), including H_2O_2, are important signaling components during the development of plant resistance to various stresses [8, 9], including osmotic stress [10]. Acetylation and deacetylation processes are also involved in regulation of development of oxidative stress in plants. It is known that the formation and utilization of reactive oxygen species (ROS) are regulated by a large network of genes [11, 12]. It was shown that HDAC inhibition by trichostatin A (TSA) in animal cells led to an increase of ROS level [13]. It was established that during an acute hyperosmotic stress in tissue culture of *Arabidopsis thaliana*, early H_2O_2-dependent increase in HAT and HDAC activities occurs. We have assumed that these changes are primarily aimed at increasing the antioxidant activity to prevent the development of oxidative stress [14]. However direct studies of HDAC role in the regulation of ROS content in plants have not been carried out.

The aim of our work was to study the interrelationship between HDAC activity and ROS content in tissue culture of *A. thaliana* under normal conditions and the early stages of acute osmotic stress induced by polyethylene glycol (PEG) by using inhibition assay with TSA.

Materials and Methods

Callus tissue culture of *A. thaliana* (12-14-days old), ecotype Columbia, at the stationary growth stage obtained by T. V. Vorobieva from plant leaves in our laboratory was used in experiments. The culture was grown on a solid agar medium Murashige and Skoog in the dark at 24 ºC.

Acute osmotic stress was caused by placing 1.0-1.5 g of tissue culture in 25% PEG-6000. The activity levels of HDAC and HAT, as well as inten-

sity of spontaneous chemiluminescence (SCHL) as an indicator of ROS level in the living native cells were determined after 3 h.

To obtain the supernatant, the tissue culture was homogenized in cooled mortars with cooled solution containing 50 mM Na_2HPO_4/KH_2PO_4 (pH 7.0), 0.8% Triton X-100 and 1% polyvinylpyrrolidone. The homogenate was then centrifuged at 17000 rpm/min for 17 min and HDAC and HAT activities were immediately determined in the obtained supernatant. All steps were carried out at 4 °C.

HDAC activity was assessed using "Colorimetric HDAC Activity Assay Kit" (BioVision, USA) with some modifications, in particular: 250 μg of cell homogenate protein was used; the reaction mixture was incubated for 3 h, then 140 μl of water was added to 110 μl of stained sample to a total volume 250 μl. Absorbance was measured at 405 nm using spectrophotometer (SF-2000, Russia). HDAC activity was calculated in absorbance relative units per mg of protein [15].

HAT activity was assessed using "HAT Activity Colorimetric Assay Kit" (BioVision, USA) also with some modifications, in particular: 70 μg of cell homogenate protein was used, the reaction mixture was incubated for 5-6 h, then 142 μl of water was added to 108 μl of stained sample to a total volume 250 μl. Absorbance was measured at 440 nm using spectrophotometer (SF-2000, Russia). HAT activity was expressed in absorbance relative units per mg of protein [16].

HDAC role in the regulating ROS content was studied using inhibition analysis with trichostatin A (TSA) based on the method described in [13] with some modifications such as: water insoluble TSA was dissolved in dimethylsulfoxide (DMSO) and then 0.01% DMSO+TSA solution was used. To exclude DMSO side effect, DMSO without TSA was used in corresponding control samples. The TSA inhibition effect on tissue culture cells was evaluated on reducing of HDAC activity. The tissue culture (1.0 g) was placed in 5 μM of TSA solution for 1 h. After that, HDAC and HAT activities as well as SCHL intensity were immediately determined in a sample (0.5 g) of the tissue culture. Another part of the tissue culture (0.5 g) was immediately placed in 25% PEG-6000 (TSA+PEG) and HDAC and HAT activities as well as SCHL intensity were determined after 3 h.

Determination of SCHL intensity. The tissue culture (1.0 g) was placed in a cuvette and in a special chemiluminometer chamber (ChLMTS-01, Ukraine). The luminescence intensity was measured after 10 min after "flashing effect" in the dark chamber. SCHL intensity was calculated in impulse/sec/g of wet weight of tissue culture [10].

Protein concentration was determined by Bradford assay [17]. All experiments were performed independently 3-5 times. Statistical processing of obtained data was performed using software "Microsoft™ Excel" and Student *t*-test. The data are presented as average values and standard errors and are considered significant at $P \leq 0.05$.

Results and Discussion

For control samples of tissue culture of *A. thaliana* growing under normal conditions the following parameters were characteristic: HDAC activity was 130-150 abs.rel.u per mg of protein; HAT activity was 30-40 abs.rel.u per mg of protein; SCHL intensity was 24-29 impulse/sec/g of wet weight. Changes of HAT and HDAC activities are represented in the figures as percentage with respect to corresponding control.

At first, the concentration of TSA inhibitor was selected for the most potent effect on HDAC activity. The optimal concentration was found to be 5.0 μM TSA (tested alternatives being 1.0 and 9.0 μM TSA). At this TSA concentration, HDAC activity decreased by 39-47% after 1 h of incubation with TSA and retained almost the same level after 3 h (Fig. 1, *A*).

TSA decreased HDAC activity in tissue culture growing under normal conditions and increased HAT activity. At that SCHL intensity was also increased significantly with respect to control (Fig. 1-3, *A*).

Significant increases in HDAC and HAT activities, as well as SCHL intensity were observed in the presence of PEG. While the effect of TSA+PEG resulted in a significant decrease of HDAC activity and a certain increase of HAT activity and SCHL intensity (Fig. 1-3, *B*).

The obtained data demonstrated that HDAC involved in the regulating of ROS level in tissue culture of *A. thaliana* under normal conditions and during the development of acute osmotic stress (Fig. 1-3).

The same effect was found in human culture cells [13], in particular, an increase of ROS level was also observed during HDAC inhibition by TSA however the authors did not investigate the mechanism behind the correlation.

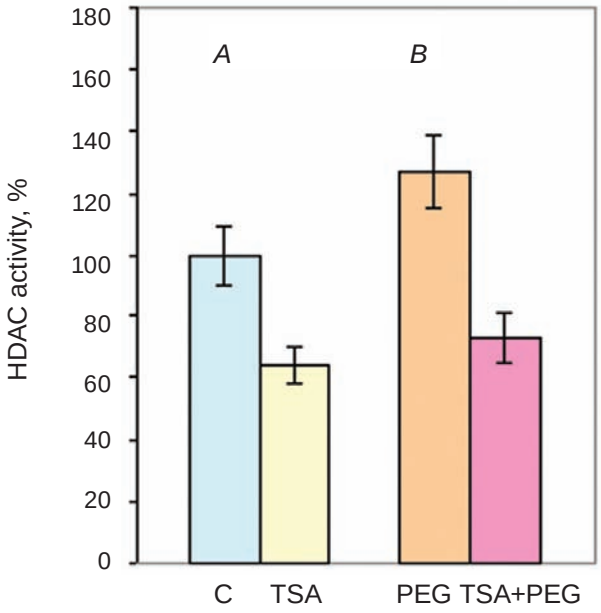

Fig. 1. Changes of histone deacetylase (HDAC) activity (% to control) in tissue culture of A. thaliana in the presence of trichostatin A (TSA) under normal conditions (A) and under PEG or TSA+PEG (B). C – control ($P \leq 0.05$)

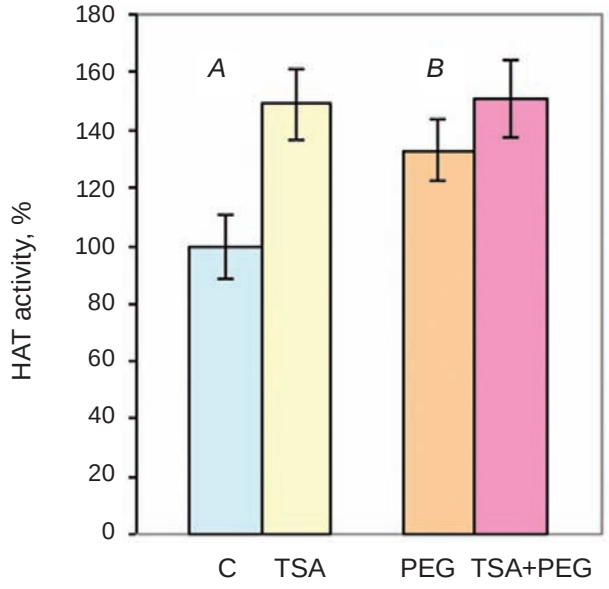

Fig. 2. Changes of histone deacetylase (HAT) activity (% to control) in tissue culture of A. thaliana in the presence of trichostatin A (TSA) under normal conditions (A) and under PEG or TSA+PEG (B). C – control (for A, $P \leq 0.05$, for B, $P > 0.05$)

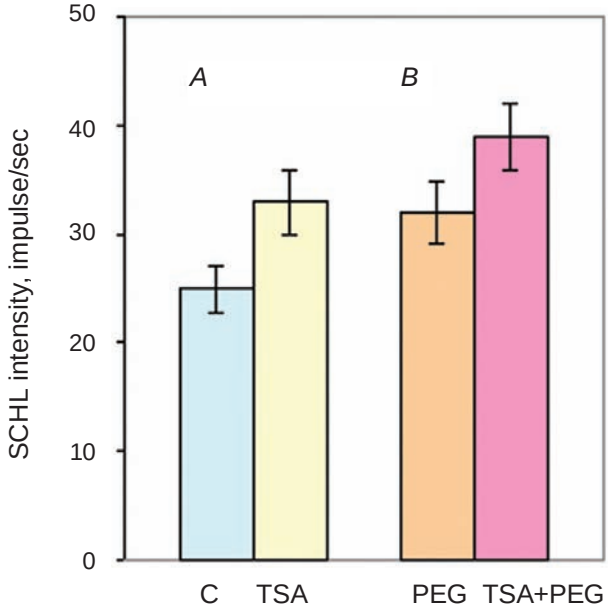

Fig. 3. Spontaneous chemiluminescence (SCHL) intensity changes in tissue culture of A. thaliana in the presence of trichostatin A (TSA) under normal conditions (A) and under the presence of PEG or TSA+PEG (B). C – control ($P \leq 0.05$)

HDAC in the studied tissue culture (Fig. 1-3) can actively be involved in the regulation of antioxidant activity of cells and in regulation of ROS level indirectly via alterations in chromatin structure and function as well as alterations in gene expression of the proteins which are responsible for ROS production and antioxidant activity.

It should be considered that under TSA inhibition of HDAC, an increase in histone acetylation may follow too, therefore HAT are also involved in stress-response reactions (Fig. 2).

Retaining a certain dynamic equilibrium between histones acetylation and deacetylation is important for cell metabolism [1, 3]. These processes may also be involved in maintaining a certain pro-antioxidant level with appropriate ROS level and antioxidant activity, particularly under plant stress [14]. Therefore a significant increase in ROS level occurred at inhibition of HDAC (Fig. 3, *B*).

A. thaliana plants contain many various HDAC and HAT isoforms. Therefore it should be considered which isoforms may be involved in stress-response reactions since each of the isoforms is involved in

the acetylation and deacetylation of certain lysine residues of histone tails that determines either expression or silencing of the respective genes [1, 7].

Alterations in HDAC and HAT activities via changes in gene expression may regulate activity of antioxidant enzymes such as ascorbate peroxidase, catalase, peroxiredoxin and thioredoxin since their activities are increased in the early stages of acute osmotic stress [10, 18].

Histones acetylation and deacetylation by HAT and HDAC may affect chromatin structure by altering the charge of the histone tails and assist in genes expression/silencing through mechanism of the so-called effect of "open/closed DNA" for transcription [1, 3]. Furthermore acetylated histone tails may serve as binding sites for proteins that may directly or indirectly regulate transcription. Bromodomain-extraterminal (BET) containing proteins BET9, BET10 and others in plants can specifically bind with acetylated lysine residues on the histones and activate transcription factors and the following respective regulation of gene expression [4].

All histone deacetylases, except for class III, contain zinc and are known as zinc-dependent deacetylases. TSA is an antifungal antibiotic that is able to inhibit HDAC effectively by displacing zinc ion from the active site of enzymes classes I and II of HDAC family including plant HDAC. Therefore, from the known HDAC inhibitors, TSA is the most effective one [13].

Thus, it was shown by inhibition assay with TSA that HDACs are involved in the regulation of ROS content in tissue culture of *A. thaliana* under normal conditions and under the development of acute osmotic stress. An increase of histone acetyltransferase activity (HAT) was also observed. This regulation was carried out possibly by alterations in histone acetylation and deacetylation that led to

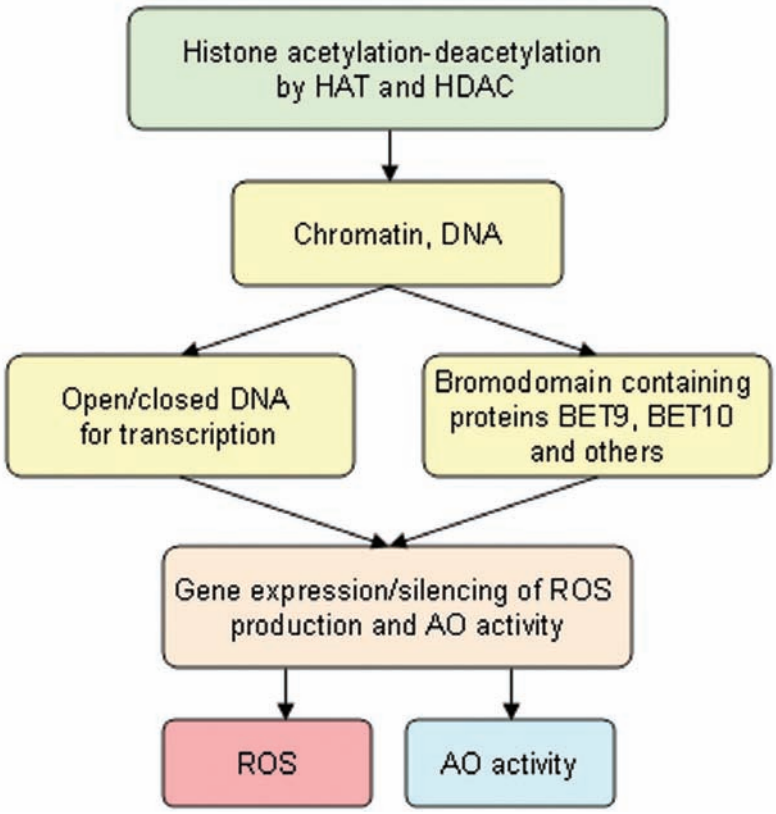

Fig. 4. Hypothetic scheme for involvement of histone acetyltransferase (HAT) and histone deacetylase (HDAC) in regulating of content of reactive oxygen species (ROS) and antioxidant (AO) activity in plants during the development of acute osmotic stress. BET9, BET10 – bromodomain-containing proteins

the alterations in chromatin structure and function, as well as expression of the genes that are responsible for ROS production and cell antioxidant activity (Fig. 4). However, further experimental verifications are required.

АКТИВНІСТЬ ГІСТОН ДЕАЦЕТИЛАЗИ І ВМІСТ АКТИВНИХ ФОРМ КИСНЮ В КУЛЬТУРІ ТКАНИНИ *Arabidopsis thaliana* В НОРМІ ТА ЗА РОЗВИТКУ ГОСТРОГО ОСМОТИЧНОГО СТРЕСУ

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Методом інгібіторного аналізу із застосуванням трихостатину А (ТСА) досліджували можливу участь гістон деацетилази (ГДА) в регуляції вмісту активних форм кисню (АФК) в культурі тканини *Arabidopsis thaliana* в нормі і за розвитку гострого осмотичного стресу. Встановлено, що в культурі тканини, що росла в нормальних умовах, у разі зниження активності ГДА за допомогою ТСА, відбувалося збільшення вмісту АФК. Аналогічні, але вираженіші зміни, відбувалися і під час розвитку стресу. При цьому також відбувалося збільшення активності гістон ацетилтрансферази (ГАТ). Обговорюються можливі механізми участі ГДА і ГАТ в регуляції вмісту АФК за допомогою змін в експресії генів, відповідальних за продукцію АФК і антиоксидантну активність.

Ключові слова: гістон деацетилаза і ацетилтрансфераза, активні форми кисню, культура тканини, осмотичний стрес.

АКТИВНОСТЬ ГИСТОН ДЕАЦЕТИЛАЗЫ И СОДЕРЖАНИЕ АКТИВНЫХ ФОРМ КИСЛОРОДА В КУЛЬТУРЕ ТКАНИ *Arabidopsis thaliana* В НОРМЕ И ПРИ РАЗВИТИИ ОСТРОГО ОСМОТИЧЕСКОГО СТРЕССА

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Методом ингибиторного анализа с применением трихостатина А (ТСА) исследовали возможное участие гистон деацетилазы в регуляции содержания активных форм кислорода (АФК) в культуре ткани *Arabidopsis thaliana* в норме и при развитии острого осмотического стресса. Установлено, что в культуре ткани, растущей в нормальных условиях, при снижении активности гистон деацетилазы посредством ТСА происходило увеличение содержания АФК. Аналогичные, но более выраженные изменения происходили и при развитии стресса. Установлено также увеличение активности гистон ацетилтрансферазы. Обсуждаются возможные механизмы участия этих энзимов в регуляции содержания АФК посредством изменений в экспрессии генов, ответственных за продукцию АФК и антиоксидантную активность.

Ключевые слова: гистон деацетилаза и ацетилтрансфераза, активные формы кислорода, культура ткани, осмотический стресс.

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