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H1 Histone Modulates DNA Hydrolysis with WEN1 and WEN2 Endonucleases from Wheat Coleoptiles

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Abstract—We show that total H1 histone from wheat seedlings or rat liver enhances hydrolysis of λ phage DNA with plant endonucleases WEN1 and WEN2 isolated from wheat coleoptiles. Optimal DNA/protein weight ratio in the hydrolysis reaction is 1 : 1. The action of fractions I and IV (obtained from total wheat H1 histone by electrophoresis) on DNA hydrolysis with WEN1 and WEN2 enzymes depends on the DNA methylation status. Fraction IV of wheat histone H1 stimulates hydrolysis of unmethylated λ phage DNA with WEN1 and WEN2 enzymes. Hydrolysis of methylated λ phage DNA (it contains 5-methylcytosine in Cm^5CWGG sequences and N^6-methyladenine in Gm^6ATC sites) with WEN1 is inhibited with fractions I and IV of wheat H1 histone. Fractions II and III of wheat H1 histone do not influence DNA hydrolysis with WEN1 and WEN2. S-Adenosyl-*L*-methionine (SAM) stimulates activity of these plant enzymes. But in the presence of H1 histone, SAM does not add to the ability of the enzyme to hydrolyze more DNA compared with that induced with H1 histone itself. Therefore, the stimulating effects of SAM and H1 histone on DNA hydrolysis with plant endonucleases may be similar. It could be suggested that SAM and H1 histone can induce more or less analogous allosteric transformations in the structure of the investigated plant endonucleases. Thus, DNA hydrolysis with plant endonucleases is modulated with total H1 histone. H1 histone fractions affect DNA hydrolysis in a different fashion; they enhance or inhibit hydrolysis depending on the DNA methylation status. We suggest that H1 histone changes site specificity of endonucleases or it might be responsible for formation of new or masking of old sites available for these enzymes due to changes in DNA structure induced in a DNA–histone complex.

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H1 histone is one of the functionally significant nuclear proteins in eukaryotes. It belongs to the lysine-rich proteins, and like other histones it is characterized with high evolutionary conservativeness of amino acid sequence. H1 histone has a central hydrophobic globular domain and highly basic N- and C-terminal domains. Chromatin of eukaryotes contains about one H1 histone molecule per nucleosome. The globular domain of H1 histone is localized in where the DNA goes in and out of a nucleosome and stabilizes the nucleosome, but the terminal domains take part in internucleosomal interactions and chromatin condensation. It is commonly known that H1 histone is responsible for formation of higher levels of chromatin organization and inhibits transcription [1].

H1 histone has many isoforms (subfractions) [2]. They are different in amino acid composition, expression period, phosphorylation degree, and turnover velocity [3, 4]. Individual H1 histone fractions possess unique functions and play different roles in chromatin structural organization [5].

New data that appeared recently show that H1 histone might take part in apoptosis. Apoptosis (programmed cell death, PCD) is an obligatory element of ontogenesis in eukaryotes. Internucleosomal DNA fragmentation is one of the specific biochemical features of apoptosis. Unfortunately, the mechanisms of this DNA fragmentation are practically unknown. In animals, the

Abbreviations: bp, base pair; BSA, bovine serum albumin; endo G, endonuclease G; SAM, S-adenosyl-*L*-methionine; TBE, Tris-borate buffer containing EDTA.

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apoptotic DNA fragmentation is catalyzed by various endonucleases. Among them the mitochondrial caspase-independent endonuclease (endo G) and nuclear endonuclease DFF40/CAD (after its activation with caspase 3) are the most thoroughly investigated ones [6, 7]. Two stages are usually observed in the apoptotic DNA fragmentation: i) initial DNA macrofragmentation with formation of chromatin domains having about 50,000 bp, and ii) subsequent internucleosomal DNA fragmentation, the products of which are seen on electrophoresis in agarose gel as a typical "ladder" of DNA fragments [6, 7].

Activity of animal nuclear nucleases DFF40/CAD and DNase γ on the hydrolysis of the isolated DNA is significantly increased due to the presence of some chromatin proteins (H1 histone, HMG1/2, or topoisomerase II) [8, 9]. The mechanism of the H1 histone-dependent activation of endonucleases is unknown. It has been suggested that endonucleases can directly bind to H1 histone, and the complex formed acts on the substrate DNA in a different manner compared with the free enzyme. H1 histone is considered to be a sort of "gate" to nucleosomal DNA, and endonuclease interacting with H1 histone may substitute for it [9]. It is probable that H1 histone interacting with DNA also induces formation of some specific DNA structure that can serve directly as a more favorite target for endonuclease action.

The data on plant endonucleases participating in fragmentation of nuclear DNA during apoptosis are rather scarce. It is still unclear what kind of plant endonuclease takes part in this DNA fragmentation and, in particular, at the terminal stages of apoptosis. There are no data on the action of H1 histone on the activity of plant endonucleases.

We detected, isolated from aging wheat coleoptiles, and characterized earlier the endonucleases WEN1 [10] and WEN2 [11] – neutral Ca^{2+}-, Mg^{2+}-, and S-adenosyl methionine-dependent enzymes that recognize the methylation status of substrate DNAs.

In the present work we have investigated the influence of total plant and animal H1 histones and of separate fractions of wheat H1 histone on hydrolysis of methylated and unmethylated λ phage DNA with wheat endonucleases WEN1 and WEN2.

MATERIALS AND METHODS

Isolation of vesicular fraction. Seeds of winter wheat (Mironovskaya 808 variety) were germinated and seedlings were grown in the darkness at 26°C. Eight-day-old seedlings were collected, washed thoroughly with tap water, and coleoptiles were separated and homogenized at 4°C in homogenization buffer (50 mM Tris-HCl, pH 7.5, 0.4 M sucrose, 1 mg/ml bovine serum albumin (BSA)) (about 5 ml buffer/g tissue). The homogenate was filtered sequentially through four-layers of gauze and one layer of Miracloth. Nuclei were sedimented by 15 min centrifugation at 600*g*. The supernatant was centrifuged for 15 min at 3000*g* to obtain a pellet (vesicular fraction). The vesicles were washed with homogenization buffer that did not contain BSA.

Isolation of nuclei. Three-day-old wheat seedlings collected and washed with tap water were homogenized at 4°C in buffer containing 25 mM Tris-HCl, pH 7.5, 0.4 M sucrose, 5 mM EDTA, 1 mM dithiothreitol, and 1 mg/ml BSA (5 ml buffer/g tissue). The homogenate was filtered through four layers of gauze and one layer of Miracloth. Nuclei were isolated from the filtrate by 15 min centrifugation at 600*g* and washed with the same buffer but without BSA.

Isolation and purification of endonucleases WEN1 and WEN2 from vesicular fraction. Endonuclease activities were extracted from the vesicular fraction with buffer containing 50 mM Tris-HCl, pH 7.5, 0.8 M sucrose, and 0.35 M NaCl. The endonucleases were purified by sequential ion-exchange chromatography on DEAE-cellulose, gel filtration through Superdex-200 and Superdex-75, and chromatography on Toyopearl-HW50. Enzymes WEN1 and WEN2 were isolated and purified according to a procedure that was described in detail earlier [11, 12].

Treatment (hydrolysis) of isolated wheat nuclei with endonucleases. Endonucleases (1 μg) were added to isolated nuclei (equivalent to 6 μg DNA) suspended in buffer (25 mM Tris-HCl, pH 7.5, 3 mM $MgCl_2$, 1 mM $CaCl_2$, and 1 mM dithiothreitol; total volume 20 μl), and the suspension was incubated for 30 min at 37°C. The reaction was stopped by addition of one-half volume of solution containing 0.6% SDS, 50 mM EDTA, and proteinase K (6 mg/ml), and the mixture was incubated (deproteinized) for 1 h at 42°C. DNA was isolated from the nuclear hydrolyzate by adding three volumes of cold ethanol, and the precipitate was then dissolved in 50 mM Tris-HCl buffer, pH 7.5, containing RNase A. The mixture was then incubated for 30 min at 37°C and the reaction stopped by addition of cold ethanol (three volumes). The DNA hydrolyzed in the nuclei due to their treatment with added endonucleases was analyzed by electrophoresis in 1.5% agarose.

Isolation of H1 histone. H1 histone was isolated from the fraction of partially purified nuclei obtained from coleoptiles of 4-5-day-old wheat seedlings [12]. Coleoptiles were cut with scissors and ground in a porcelain mortar with a pestle in cold with three volumes of cold solution containing 0.4 M sucrose, 20 mM TEA (triethanolamine)-buffer, pH 7.5, 30 mM KC1, 6 mM $MgC1_2$, 1 mM EDTA, and protease inhibitors (1 mM phenylmethylsulfonyl fluoride and 1 mM iodine acetate) (buffer A). The homogenate was filtered through four layers of gauze, plant debris was ground again in two volumes of buffer A, and the homogenate was filtered. The combined filtrate was centrifuged for 15 min at 600*g*. The sed-

iment obtained (fraction of partially purified nuclei) was washed twice with buffer A. To obtain H1 histone, the partially purified nuclei isolated from coleoptiles were extracted with a small volumes 0.74 M $HClO_4$ with ultrasonic treatment (10 sec at amplitude 16-20 μm on an MSE (England) disintegrator). H1 histone was precipitated from the combined extracts with addition 3.5 volumes of acetone + 0.03 volume concentrated HCl and incubated at −20°C overnight. Precipitates collected by centrifugation were washed twice with acetone, dissolved in water, and analyzed by means of electrophoresis.

Determination of endonuclease activity. Unmethylated λ phage (dcm^-, dam^-) DNA and methylated λ phage (dcm^+, dam^+) DNA were used as substrates for determination of endonuclease activities. Reaction mixture (10 μl) contained 1 μg substrate (phage DNA) and 0.25-0.50 μg enzyme in 50 mM Tris-HCl buffer, pH 7.5. The mixture was incubated for 2 h at 37°C, and then 2 μl buffer (10% glycerol, 0.01% bromophenol blue, and xylene cyanol in TBE buffer (Tris-borate buffer containing EDTA)) was added and the solution applied onto a 0.7% agarose gel in TBE buffer with 0.0005% ethidium bromide added. After electrophoresis, the products of DNA hydrolysis were visualized under UV light.

To investigate the influence of H1 histone on the endonuclease action, H1 histone (0.5-2 μg) or its fractions (0.3 μg) were added to substrate DNA (1 μg) and the mixture was preincubated for 15 min at 37°C, and then endonucleases (0.25 μg) were added and the mixture incubated for 2 h at 37°C. The reaction (DNA hydrolysis) products were electrophoresed and visualized as described above.

The molecular masses of proteins were determined by electrophoresis in 12.5 or 15% polyacrylamide gels according to Laemmli's SDS-PAGE procedure [13]. Phosphorylase (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carboanhydrase (30 kDa), soybean trypsin inhibitor (20 kDa), and lactalbumin (14.4 kDa) were used as molecular mass markers. Densitometry and analysis of electrophoregrams were performed using the Scione computer program. H1 histone fractions were eluted from gels in an LKB 2014-001 Extraphor Electrophoretic Concentrator apparatus (Sweden) in solution containing 50 mM Tris-buffer, pH 8.9, 50 mM glycine, and 3 M NH_4HCO_3. Proteins were precipitated with acidified acetone and prepared for analysis similarly as performed with total H1 histone isolation.

Protein concentration was determined spectrophotometrically by measuring the absorbance value at λ = 280 nm and by Bradford's method [14].

RESULTS AND DISCUSSION

We have isolated endonucleases WEN1 and WEN2 from the cytoplasmic vesicle fraction that was obtained from aging wheat coleoptiles. These vesicles appear in cellular vacuoles due to apoptotic fragmentation of cytoplasm and contain mitochondria with intense replication of mtDNA [15]. According to SDS-PAGE data, the molecular mass of WEN1 is about 27 kDa, and that of WEN2 is near 22 kDa [10, 11].

Unmethylated (dam^-, dcm^-) λ phage DNA and methylated (dam^+, dcm^+) λ phage DNA were used as the substrates for the isolated wheat endonucleases. Unlike unmethylated DNA (dam^-, dcm^-), the methylated DNA (dam^+, dcm^+) does contain 5-methylcytosine residues in Cm^5CWGG sequences and N^6-methyladenine in Gm^6ATC sites. Endonucleases WEN1 and WEN2 recognize the methylation status of these DNAs [10, 11]. Under standard conditions, WEN1 preferentially hydrolyzes methylated and WEN2 hydrolyzes unmethylated λ phage DNA (Fig. 1). Unlike WEN1, the endonuclease WEN2 hydrolyzes only double-stranded DNA.

A specific feature of these endonucleases is their dependence on S-adenosyl methionine (SAM) that is a source of methyl groups for transmethylation reactions including DNA methylation [10, 11] and an allosteric

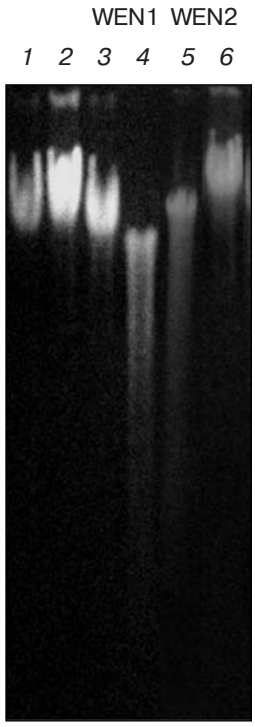

Fig. 1. Electrophoregram of λ phage DNA and products of its hydrolysis with endonucleases WEN1 and WEN2: *1*) unmethylated λ phage DNA (dam^-, dcm^-); *2*) methylated λ phage DNA (dam^+, dcm^+); *3*) unmethylated λ phage DNA (dam^-, dcm^-) treated with endonuclease WEN1; *4*) hydrolyzate of methylated λ phage DNA (dam^+, dcm^+) treated with endonuclease WEN1; *5*) hydrolyzate of unmethylated λ phage DNA (dam^-, dcm^-) treated with endonuclease WEN2; *6*) methylated λ phage DNA (dam^+, dcm^+) treated with endonuclease WEN2.

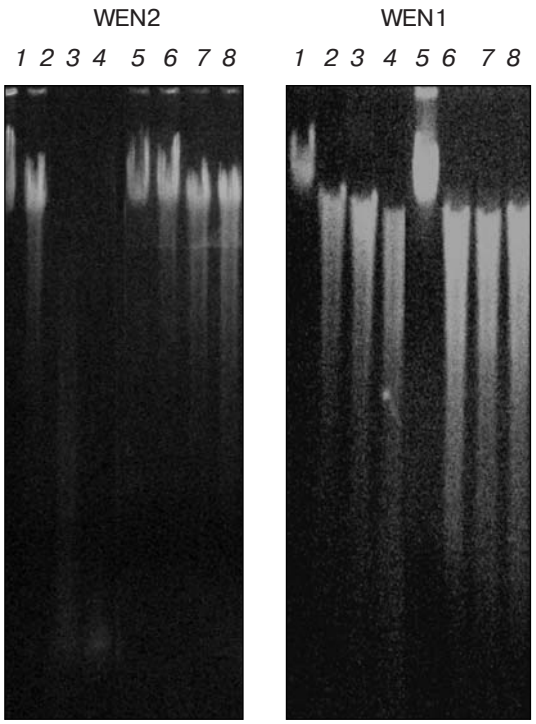

Fig. 2. Influence of S-adenosyl methionine on activity of endonucleases WEN1 and WEN2: *1*) unmethylated λ phage DNA (dam$^-$, dcm$^-$); *2*) lane *1* + enzyme; *3*) lane *2* + SAM (0.1 mM); *4*) lane *2* + SAM (1 mM); *5*) methylated λ phage DNA (dam$^+$, dcm$^+$); *6*) lane *5* + enzyme; *7*) lane *6* + SAM (0.1 mM); *8*) lane *6* + SAM (1 mM).

effector of many bacterial restriction endonucleases. SAM strongly affects the WEN2 endonuclease activity: in the presence of SAM this endonuclease hydrolyzes unmethylated λ phage completely to oligonucleotides with length of about 140-160 bp (Fig. 2), which is not the case in the absence of an effector. SAM also strongly stimulates the hydrolysis of methylated DNA with WEN2 enzyme. In contrast to WEN2, the WEN1 activity is stimulated by SAM insignificantly; in the presence of SAM, the endonuclease WEN1 practically does not recognize methylation status of substrate DNAs.

To establish whether WEN1 and WEN2 can in principle take part in the internucleosomal fragmentation of nuclear DNA, the nuclei isolated from wheat seedlings were incubated with these endonucleases. Pancreatic DNase I hydrolyzing DNA in chromatin at the internucleosomal sites was used as a comparative control [16] (Fig. 3, lane *2*).

Endonuclease WEN2 splits DNA in isolated nuclei to large fragments (Fig. 3, lane *3*); this is similar to the action of animal endonuclease DFF40/CAD that, as known, after activation with caspase-3 at the early stages of apoptosis hydrolyzes DNA in chromatin of animal cells to fragments with length of more than 50 kb. Double-stranded DNA is a target for DFF40/CAD endonuclease.

Endonuclease WEN1 splits DNA in isolated wheat nuclei to fragments of various length; on electrophoregams these fragments look like a characteristic "ladder" observed usually on apoptotic internucleosomal nuclear DNA fragmentation (Fig. 3, lane *4*). This is similar to the action of endonuclease G (endo G) on nuclei from animal cells. Endo G is localized along with cytochrome *c* in the intermembrane space of mitochondria and is a caspase-independent nuclease. The enzyme takes part in the second stage of apoptotic DNA degradation, hydrolyzing it to 140-180 bp fragments. Unlike DFF40/CAD enzyme, endo G preferentially hydrolyzes single-stranded DNA [8].

A clear picture of nuclear DNA degradation with appearance of fragments with size divisible per about 140 bp (Fig. 3, lanes *5* and *6*) was observed after simultaneous action of endonucleases WEN1 and WEN2 on the isolated wheat seedling nuclei; a similar picture is seen on internucleosomal DNA hydrolysis in nuclei treated with pancreatic DNase I (Fig. 3, lane *2*). Thus, similar to animal endonucleases DFF40/CAD and endo G participating in apoptosis, the wheat endonucleases found by us split DNA in the chromatin at the internucleosomal sites.

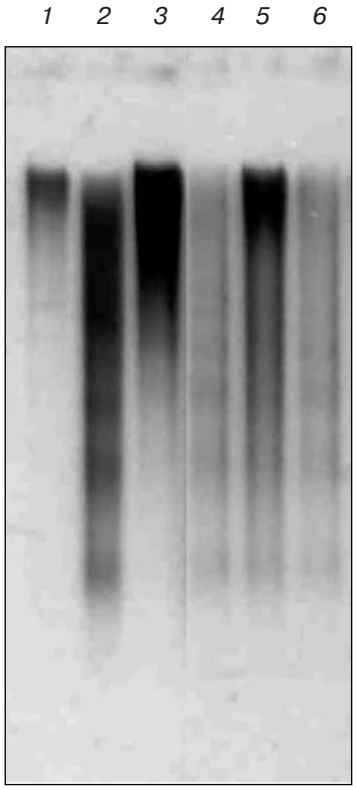

Fig. 3. DNA hydrolyzates from isolated wheat nuclei treated with endonucleases: *1*) DNA from isolated nuclei; *2*) nuclear digest with DNase I; *3*) nuclear digest with endonuclease WEN2; *4*) nuclear digest with endonuclease WEN1; *5*) nuclear digest with endonucleases WEN1 + WEN2 combined (2 : 1); *6*) nuclear digest with endonucleases WEN1 + WEN2 combined (1 : 2).

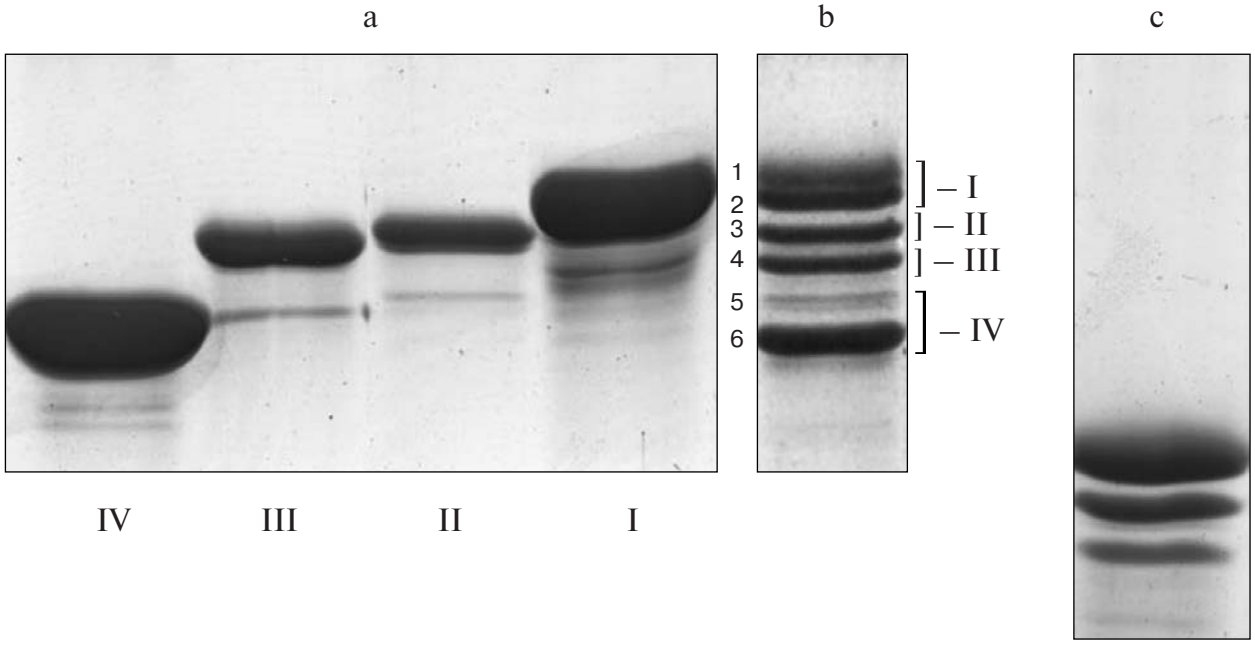

Fig. 4. SDS-PAGE of H1 histone and its fractions: a) wheat H1 histone fractions; b) total wheat H1 histone; c) total H1 histone from rat liver.

H1 histone isolated from 4-5-day-old wheat seedlings (Fig. 4b) has higher putative molecular mass (35-45 kDa) compared with that of animal H1 histone (isolated from rat liver, Fig. 4c). This is explained mainly by the longer molecular length of plant H1 histone [17]. Total plant H1 histone was separated by SDS-electrophoresis in more (six) components (subfractions) than animal H1 histone (Fig. 4).

Despite that total H1 content in wheat seedlings diminishes significantly with age, the ratio between different subfractions in it does not change [12].

H1 histone subfractions (1 + 2, 3, 4, 5 + 6) were cut from gel as it is shown in Fig. 4 and eluted. After subsequent electrophoresis (Fig. 4a), the proteins isolated from the gel represent in fact the H1 histone fractions required without significant admixtures. The quantitative ratio between corresponding fractions (I, II, III, IV) isolated from total wheat H1 histone is about 2 : 1 : 1 : 2.

We investigated the influence of total wheat H1 histone and its fractions on hydrolysis of DNA with endonucleases WEN1 and WEN2 at various ratios of DNA to H1 histone. Total H1 histone isolated from wheat seedlings or rat liver weakly increased hydrolysis of unmethylated phage DNA with endonuclease WEN2 (Fig. 5). Optimal weight DNA to H1 histone ratio is 1 : 1. Low-molecular-weight fraction IV from wheat H1 histone slightly stimulates hydrolysis of DNA with WEN2 at the ratio DNA to histone 1 : 0.3 (Fig. 6). On the contrary, the high-molecular-weight fraction I of wheat H1 histone at the same DNA to histone ratio (1 : 0.3) increases DNA hydrolysis with WEN2 enzyme quite significantly (Fig. 6, lane *6*); as a result, the enzyme is able to hydrolyze unmethylated DNA to 120-140 bp oligonucleotides. Fractions II and III of wheat H1 histone did not effect DNA hydrolysis with enzymes WEN1 and WEN2 (not shown).

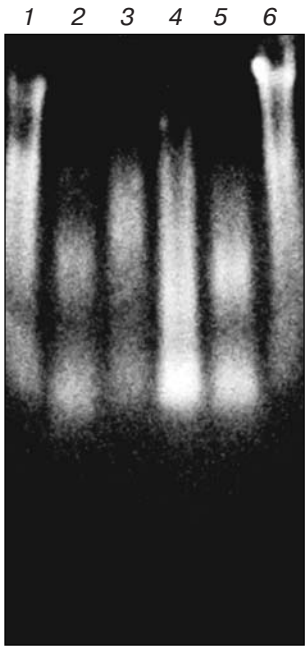

Fig. 5. Effect of total H1 histone on hydrolysis of unmethylated λ phage DNA (dam$^-$, dcm$^-$) with endonuclease WEN2. Total H1 histone from rat liver (*1-3*) or wheat coleoptiles (*4-6*) was added to the reaction mixture. The protein/DNA ratio was 0.5 : 1 (*1*, *4*), 1 : 1 (*2*, *5*), and 2 : 1 (*3*, *6*).

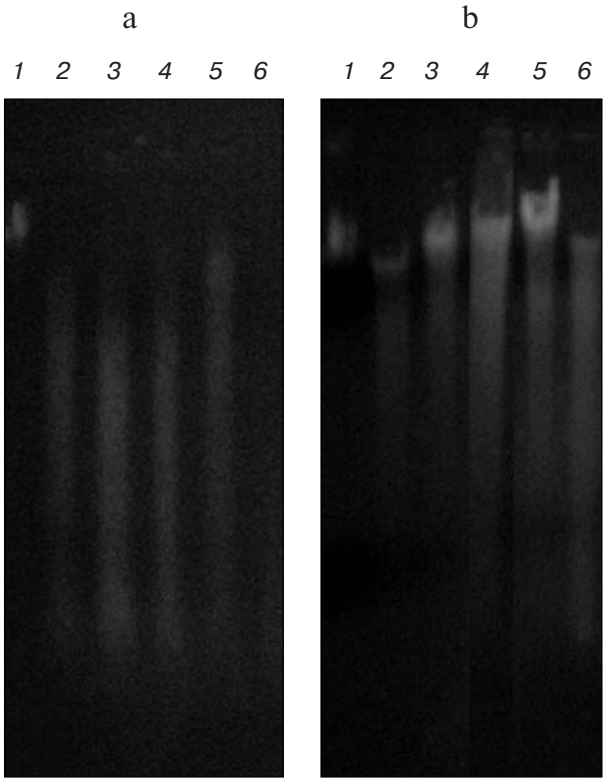

Fig. 6. Effect of wheat H1 histone and its fractions on hydrolysis of λ phage DNA with wheat endonuclease WEN2: a) substrate is unmethylated λ phage DNA (dam$^-$, dcm$^-$); b) substrate is methylated λ phage DNA (dam$^+$, dcm$^+$). *1*) λ phage DNA; *2*) DNA + WEN2; *3*) DNA + WEN2 + total wheat H1 histone; *4*) DNA + WEN2 + total H1 histone from rat liver; *5*) DNA + WEN2 + fraction IV of wheat H1 histone; *6*) DNA + WEN2 + fraction I of wheat H1 histone.

Total H1 histone stimulates hydrolysis of methylated λ phage DNA, but this hydrolysis is not complete as in agarose gel the 120-140 bp oligonucleotides were not observed among products of DNA hydrolysis (Fig. 6b). Low-molecular-weight fraction IV of wheat H1 histone has practically no influence on hydrolysis of methylated λ phage DNA with enzyme WEN2 (Fig. 6b, lane *5*).

There are no data on the influence of H1 histone on the activity of animal endo G. Here we can clearly see that total wheat H1 histone significantly affects the cleavage of DNA to relatively short internucleosomal fragments with plant enzyme WEN1. So, high-molecular-weight fraction I of wheat H1 histone increases the hydrolysis of unmethylated DNA with WEN1 enzyme (Fig. 7a, lane *6*). Low-molecular-weight fraction IV of H1 histone stimulates WEN1 enzyme action even more strongly.

Fractions I and IV of wheat H1 histone markedly inhibited hydrolysis of methylated λ phage DNA with WEN1 enzyme (Fig. 7b).

As we showed earlier, unlike in animal cells, relative increase in H1 histone/DNA ratio occurred in aging wheat coleoptile [12]. Therefore, especially in plants the significant influence of H1 histone on the apoptotic DNA fragmentation with endonuclease WEN1 was clearly observed.

The *in vivo* DNA methylation is one of the mechanisms of regulation of gene expression [18]. It effects the DNA–protein interactions and induces conformational transformations in chromatin [19]. It seems that isolated plant endonucleases are sensitive to similar conformational changes in substrate DNA complexes. The respective H1 histone–DNA complex can be formed after addition of H1 histone to our reaction mixture. Binding of H1 histone with a linear DNA under definite conditions and weight ratio H1/DNA up to 0.47 is cooperative, and nucleoprotein complexes formed can have a different structure [20]. It is known that H1 histone preferentially binds to AT-rich DNA sites [21]. As far as methylated DNA also preferentially binds with H1 histone [22], the complexes formed seem to be enriched with methylated DNA, but free DNA is relatively unmethylated. It should be noted that questions concerned the strength of H1 histone binding and its selectivity to bind one or another DNA sequence are still to be answered. Increase in hydrolysis of methylated or

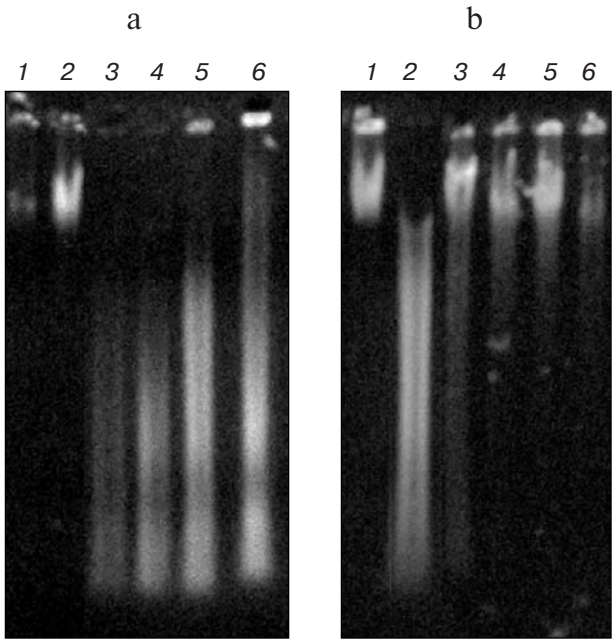

Fig. 7. Influence of wheat H1 histone and its fractions on hydrolysis of λ phage DNA with wheat endonuclease WEN1: a) substrate is unmethylated λ phage DNA (dam$^-$, dcm$^-$); b) substrate is methylated λ phage DNA (dam$^+$, dcm$^+$). *1*) DNA; *2*) DNA + WEN1; *3*) DNA + WEN1 + total wheat H1 histone; *4*) DNA + WEN1 + total H1 histone from rat liver; *5*) DNA + WEN1 + fraction IV of wheat H1 histone; *6*) DNA + WEN1 + fraction I of wheat H1 histone.

unmethylated DNA with endonucleases WEN1 and WEN2, respectively, induced by H1 histone can be explained by formation of the nucleoprotein structures [20] predominantly hydrolyzed with these endonucleases or by shifts induced in the balance between nucleoprotein structural transformations and appearance of such DNA conformation that is more sensitive to nucleases. On the other hand, we cannot rule out that H1 histone can modulate protein–protein interactions and, therefore, change the site specificity of action of endonucleases. Inhibition of the methylated DNA hydrolysis with endonuclease WEN1 under the influence of H1 histone fractions I and IV indicates that possible targets for the enzyme attack might be the DNA sites with methylated adenine residues; after binding with H1 histone, these sites seem to become sterically unavailable for an enzyme.

There are many data on direct H1 histone interactions with various functionally important proteins and, in particular, with animal apoptotic nuclease DFF40/CAD [8]. Formation of complex resulted in an increase of the constant of the enzyme binding to free DNA. Perhaps a similar situation takes place and with isolated plant endonucleases WEN1 and WEN2. One can suggest that the ability of an enzyme to hydrolyze DNA increases or diminishes due to specific features of the complex formed and, besides, the site specificity action of an enzyme can be changed.

We have established that SAM modulates the activity of plant endonucleases (Fig. 2). But in the presence of H1 histone, the SAM effector does not demonstrate an additional to H1 ability to modulate more DNA hydrolysis (data not shown). Therefore, the modulating actions of H1 histone and SAM on the DNA hydrolysis are similar to some extent. It is probable that SAM and H1 histone induce analogous allosteric transformations in the structure of investigated plant endonucleases. Anyway, the protein–protein interactions H1 histone–endonuclease play a significant role in regulation of DNA hydrolysis with the enzyme.

Interestingly, unlike animal H1 histone, the modulating action of various fractions of plant H1 histone on endonucleases is different. Taking into account the special dynamic properties and variability of H1 histone and of its fractions in the nucleus, one can suggest that the analogous diverse interactions of H1 histone with endonucleases might be realized *in vivo*.

Thus, based on the data obtained, we propose that H1 histone preferentially binds to methylated sites of phage DNA and after that they seem to become unavailable for endonucleases and hydrolysis of methylated DNA, particularly with endonuclease WEN1, appears to be practically impossible. But under these conditions the unmethylated DNA is hydrolyzed more actively. Inhibition of DNA hydrolysis seems also to show that H1 histone bound to endonuclease does not exchange with endonucleases and does not shift along the DNA molecule.

Significant differences in the influence of H1 histone fractions on the action of endonucleases seem to be due to both various length of their C-ends and different modifications of their amino acid residues. Therefore, different H1 histone fractions can bind to various DNA sites. In our opinion the most probable explanation of an increased endonuclease action on DNA hydrolysis induced with H1 histone is formation of a specific DNA–histone H1 structure that provides the most available DNA target sites for the enzyme.

We did not mention here possible changes in specific endonuclease activity due to induction with H1 histone. We do not rule out that it could occur. It is most probable that activity of the enzyme itself does not change under the H1 histone action, but availability of substrate DNA target sites to enzyme changes significantly.

Thus, undoubtedly, H1 histone modulates the *in vitro* action of plant endonucleases, and the individual fractions of H1 can either stimulate or inhibit DNA hydrolysis. As far as the nuclear H1 histone is located in the internucleosomal sites of chromatin, it is logical to suppose that H1 histone fractions can selectively stimulate or inhibit DNA hydrolysis in cell nuclei in these particular sites and *in vivo*. This may have an important significance for selective regulation of consecutive step-wise DNA degradation in the nuclear chromatin with endonucleases and, particularly, during apoptosis.

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