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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

Short Peptides Modulate the Effect of Endonucleases of Wheat Seedling

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Short peptides (2,3,4-amino acids) inhibit or enhance the hydrolysis of phage λ DNA by site-specific wheat endonucleases WEN1 and WEN2 depending on the DNA methylation status. The effect of endonucleases is modulated by peptides presumably as a result of their binding to DNA. Peptides recognize not only specific DNA sequences but also their methylation status. Peptides bind to both DNA and single- and double-stranded deoxyribonucleotides containing CNG and CG sites that are methylated in eukaryotes. During the hydrolysis of double-stranded structures by endonucleases, peptides influence the set of hydrolyzed sites. The effect of peptides (bronchogen, epithalon, pinealon, etc.) on the hydrolysis of DNA by endonucleases is different and is modulated by histones (histone H1). Site-specific interactions of peptides with DNA can epigenetically control the genetic functions of the cell.

The physiological effect of the studied peptides is based on their tissue- or gene-specific interaction with DNA [1]. However, the patterns and mechanisms of selective binding of peptides to DNA and the resulting changes in transcription are still poorly understood. The binding of peptides to DNA should determine the effects of many proteins (enzymes, including restriction endonucleases) that operate with DNA and compete for the same binding sites.

We investigated the effect of short peptides on the hydrolysis of phage λ DNA and oligonucleotides by wheat endonucleases and studied how this process can be influenced by histone H1. We assayed changes in the fluorescence intensity of labeled oligonucleotides under the influence of peptides. The peptides used in this study were epithalon (Ala–Glu–Asp–Gly), pinealon (Glu–Asp–Arg), bronchogen (Ala–Asp–Glu–Leu), testagen (Lys–Glu–Asp–Gly), cardiogen (Ala–Glu–Asp–Arg), and pankragen (Lys–Glu–Asp–Trp) [2]. Endonucleases WEN1 and WEN2 were isolated from wheat coleoptiles [3] as described earlier [4, 5]. Endonuclease WEN1 hydrolyzes methylated phage λ DNA (dcm^+, dam^+) [4], which contains 5-methylcytosine residues in Cm^5CWGG sequences and N^6-methyladenine residues at Gm^6ATC sites; methylated DNA is hydrolyzed preferably at CNG sites. Endonuclease WEN2 cleaves unmethylated DNA (dcm^-, dam^-) [5]. DNA was hydrolyzed by WEN1 and WEN2 under standard identical conditions either without [4, 5] or with the addition of peptides (1.2 μg per 1 μg DNA) to the reaction mixture. The hydrolysis products of DNA and oligonucleotides were separated by electrophoresis in 1.5% agarose gel and 20% polyacrylamide gel, respectively. The fluorescence spectra of oligonucleotides were recorded with a Perkin-Elmer LS55 spectrofluorometer (Perkin-Elmer, United States).

Epithalon inhibited the hydrolysis of unmethylated DNA by WEN2 (Fig. 1a, lane *5*); in the presence of histone H1, this inhibitory effect of the peptide was less pronounced (lane *6*). Apparently, histone H1 suppresses the inhibitory effect of epithalon, making some sites in DNA more accessible to hydrolysis by this enzyme. In the presence of bronchogen, DNA was selectively hydrolyzed to fragments approximately 140 nt long. Apparently, bronchogen binds to DNA sites other than the sites for epithalon, protecting them from hydrolysis. Under the influence of histone H1, the protective effect of bronchogen was abolished (lane *8*) and DNA was completely hydrolyzed (similarly to the control). Pinealon had almost no effect on the hydrolysis of unmethylated DNA by WEN2 (lane *9*); however, in the presence of histone H1, it strongly inhibited the hydrolysis of this DNA (lane *10*). Histone H1 per se did not affect the hydrolysis of DNA (lane *4*). Therefore, various peptides modulated the effect of endonucleases in different ways and their effect may be mediated by histones. This is a very important fact because, in the cell, peptides should initially find the sites in chromatin that are accessible

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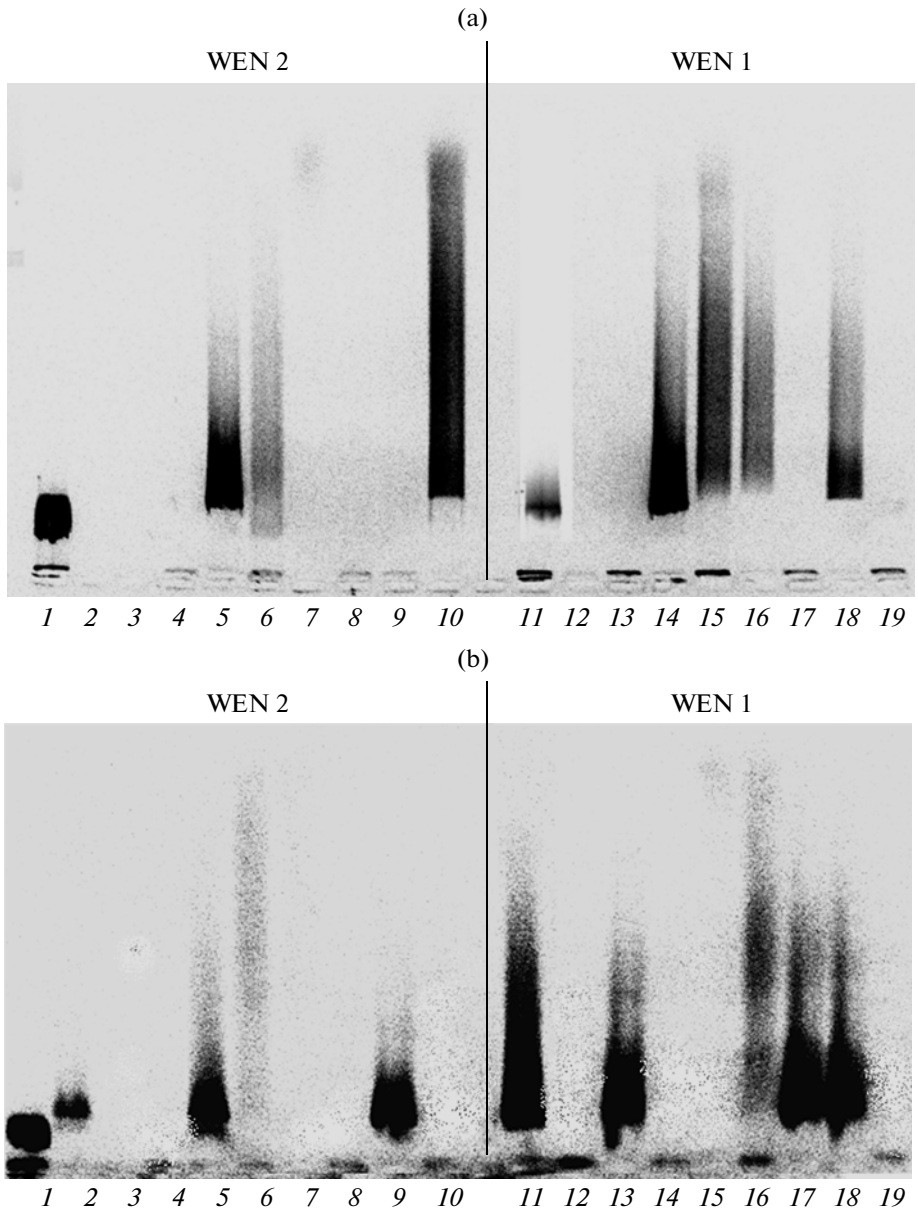

Fig. 1. Electrophoretic separation in 1.5% agarose of the products of hydrolysis of (a) unmethylated and (b) methylated phage λ DNA by endonucleases WEN1 and WEN2. Designations: *1*, unmethylated phage DNA; *2*, *1* + WEN2; *3*, *2* + Mg^{2+}; *4*, *2* + histone H1; *5*, *2* + epithalon; *6*, *4* + epithalon; *7*, *2* + bronchogen; *8*, *4* + bronchogen; *9*, *2* + pinealon; *10*, *4* + pinealon; *11*, *1* + WEN1; *12*, *11* + Mg^{2+}; *13*, *11* + histone H1; *14*, *11* + epithalon; *15*, *13* + epithalon; *16*, *11* + bronchogen; *17*, *13* + bronchogen; *18*, *11* + pinealon; *19*, *13* + pinealon.

for binding to DNA; this accessibility can be determined by histones.

Epithalon had almost no effect on the hydrolysis of methylated DNA by WEN2 (Fig. 1b, lane *5*) but completely inhibited the hydrolysis of unmethylated DNA (Fig. 1a, lane *5*). This means that the peptide is able to discriminate between the methylated and unmethylated DNA and, apparently, differentially interacts with them. Bronchogen strongly activated the hydrolysis of methylated DNA by WEN2 (Fig. 1b, lane *7*). Therefore, epithalon and bronchogen had opposite effects on the hydrolysis of methylated DNA. Pinealon did not affect the hydrolysis of DNA with different methylation status; however, in the presence of histone H1, this peptide inhibited the hydrolysis of unmethylated DNA (Fig. 1a, lane *10*) and, conversely, stimulated the hydrolysis of methylated DNA (Fig. 1b, lane *10*).

Epithalon, bronchogen, and pinealon inhibited the hydrolysis of unmethylated DNA by WEN1 (Fig. 1a, lanes *14*, *16*, and *18*). Unlike WEN2, endonuclease WEN1 in the presence of pinealon completely hydro-

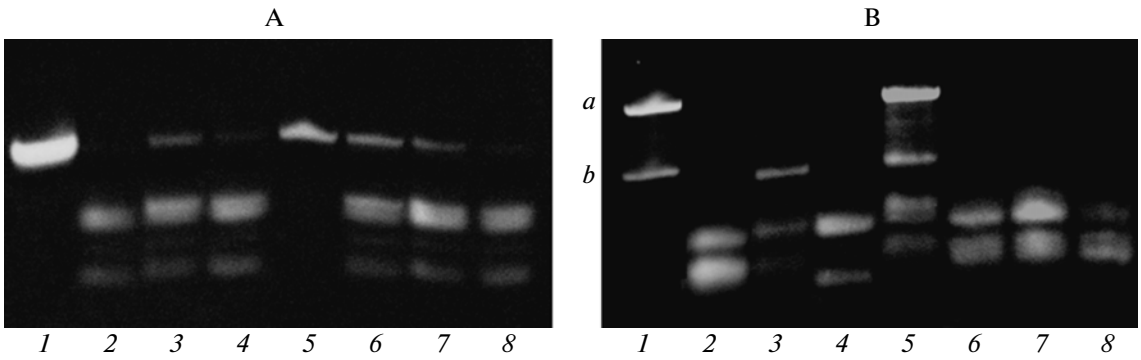

Fig. 2. Electrophoretic separation in 20% polyacrylamide gel of the products of hydrolysis of fluorescently labeled (A) single-stranded 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3' and (B) double-stranded 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3'/3'-GCG GCG GTC CGC GGC GGC GC-FAM-5' deoxyribooligonucleotides by the WEN1 endonuclease. Designations: *1*, deoxyribooligonucleotides; *2*, *1* + enzyme + Mg^{2+}; *3*, *2* + epithalon; *4*, *2* + bronchogen; *5*, *2* + cardiogen; *6*, *2* + pankragen; *7*, *2* + pinealon; *8*, *2* + testagen. Designations on panel B: (*a*) double-stranded and (*b*) mixture of single-stranded deoxyribooligonucleotides.

lyzed the unmethylated DNA in complex with histone (Fig. 1a, lane *19*). The degree of hydrolysis of the methylated DNA by WEN1 increased in the presence of epithalon (Fig. 1b, lane *14*).

Epithalon, bronchogen, pinealon, pankragen, and testagen partly and to different degrees inhibited WEN1-catalyzed hydrolysis of the deoxyribooligonucleotide 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3' containing CNG and CG sites, which are methylated in eukaryotes. Cardiogen blocked this hydrolysis (Fig. 2A, lane *5*). Pankragen, pinealon, and testagen did not affect the hydrolysis of the double-stranded oligonucleotide 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3'/3'-GCG GCG GTC CGC GGC GGC GC-FAM-5' (Fig. 2B, lanes *6*–*8*). Epithalon inhibited the hydrolysis of the single-stranded oligonucleotide (Fig. 2A, lane *3*) but stimulated the hydrolysis of the double-stranded oligonucleotide (Fig. 2B, lane *3*). Judging by the intensity of fluorescence bands (Fig. 2B, lane *3*), epithalon promoted unwinding of DNA strands. Bronchogen did not block the hydrolysis of single- and double-stranded structures but changed their site specificity. Cardiogen, in contrast to the blockade of hydrolysis of single-stranded structures (Fig. 2A), only partially inhibited the hydrolysis of the double-stranded oligonucleotide (Fig. 2B, lane *5*).

Epithalon strongly quenched the fluorescence of deoxyribo-5'-FAM-CGC CGC CAG GCG CCG CCG CG-3' (Fig. 3A), which is indicative of a high binding constant of the peptide to the oligonucleotide. Bronchogen quenched its fluorescence less markedly. Cardiogen did not alter the fluorescence of the oligonucleotide. At the same time, cardiogen completely inhibited the hydrolysis of this oligonucleotide by WEN1 (Fig. 2A, lane *5*). Apparently, the drastic suppression of DNA hydrolysis by cardiogen was due to its interaction with the enzyme rather than with DNA. None of the studied peptides quenched the fluorescence of labeled polydeoxyribo-C. Hence, peptides bind to DNA (oligonucleotides) in a site-selective manner. The peptides also quenched the fluorescence of the double-stranded oligonucleotide (Fig. 3D); i.e., they can intercalate into the DNA helix. Judging by the different degrees of fluorescence quenching (Figs. 3A, 3D), the peptides preferably bind to the single-stranded structures.

The specific binding of peptides to single-stranded oligonucleotides, revealed in this study, is of particular importance. Single-stranded regions (strands) either always exist or appear in DNA, especially during replication, recombination, and repair. The interaction of short peptides with such sites can control these genetic processes. Thus, this is the first to demonstrate that short peptides modulate the effect of endonucleases and that this modulation is realized primarily through site-specific peptide–DNA binding.

We discovered tissue, subcellular, and age specificity of DNA methylation [6] and were the first to show that the DNA methylation pattern in cancer cells differs from that in normal cells [7]. We postulate that the same biologically active peptide will bind to DNA in different manner depending on its methylation status and will differentially affect the genome in various tissues (cells), in the nucleus and mitochondria, in young and old cells, and normal and cancer cells. Almost all of these postulates have been confirmed experimentally [1, 2].

The discovered phenomenon of modulation of the effect of endonucleases by short peptides may be only part of the global biological law: peptides that site-specifically bind to DNA (especially to the regulatory elements of the genome) should modulate the functions of many proteins interacting with DNA (RNA and DNA polymerases, DNA methyltransferases, and many regulatory factors). For example, some hexapeptides are strongly selective ligands for protein-free Holliday junctions and block recombination [8].

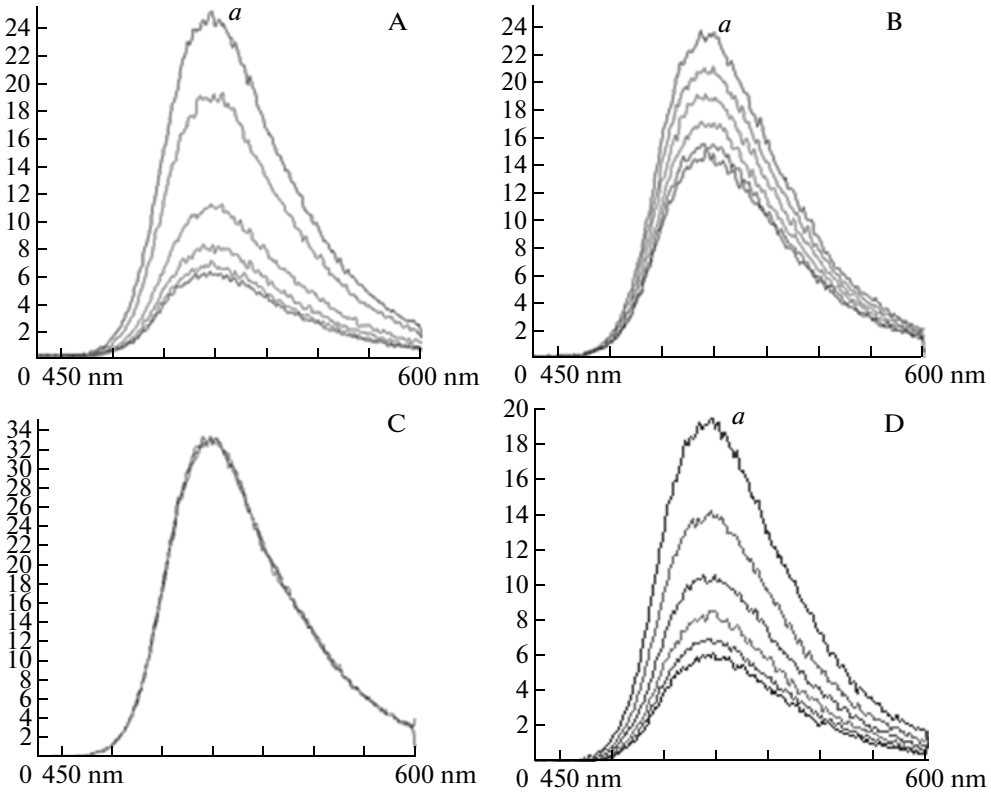

Fig. 3. Fluorescence spectra of the fluorescently labeled single-stranded deoxyribooligonucleotide 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3 ' before (curves *a*) and after (all other curves) titration with peptides (A, epithalon; B, bronchogen; C, cardiogen) and double-stranded oligonucleotide 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3'/3'-GCG GCG GTC CGC GGC GGC GC-FAM-5' (D, titration with epithalon).

We have proposed one of the most probable mechanisms of gene activation by short peptides: peptides selectively bind to promoter CNG or CG sites, making these sites inaccessible for DNA methyltransferases; as a result, the promoter remains unmethylated, which is the crucial element of gene activation.

Thus, the specific (complementary) peptide–DNA interactions can epigenetically control the genetic functions of cells. Probably, they played a very important role at the earliest stages of life origin and evolution.

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