BIOGERONTOLOGY

Site-Specific Binding of Short Peptides with DNA Modulated Eukaryotic Endonuclease Activity

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Short peptides (2-4 amino acid residues) inhibit or stimulate hydrolysis of λ phage DNA by eukaryotic endonucleases WEN1 and WEN2 depending on DNA methylation status. Peptide modulation of endonucleases activity most likely appears as a result of their binding to DNA. Peptides discriminate (recognize) not only certain DNA sequences, but also their methylation status. Apart from intact DNA, the test peptides bind to single-stranded DNA structures (oligonucleotides) containing CNG- and CG-sites methylated in eukaryotes. Peptides affect the set of hydrolyzed sites during endonuclease hydrolysis of double-stranded structures. The effects of peptides with different primary structure on DNA hydrolysis by endonucleases are different and are modulated by histones (histone H1). Site-specific peptide interactions with DNA may epigenetically control genetic functions of the cell. These interactions probably played an important role at the very early stages of evolution.

Key Words: *peptides; site-specific binding; DNA endonucleases*

Peptides form a wide and diverse signal regulatory system controlling physiology, growth, and development of plants and animals [2,6,7].

Physiological effects of the studied short peptides are based on tissue- and gene-specific interaction with DNA [10] via site-specific or complementary peptide binding with DNA. This peptide binding in major DNA groove is associated with substantial changes in the organization of double-stranded DNA and gene expression [9]. However, detailed mechanisms of such specific binding of short peptides with DNA and transcription induction and suppression caused by this binding are still poorly studied. Unique abilities and regularities (rules) of effective in biological terms binding between primary DNA chain structures and peptides are virtually unknown.

Activity of numerous proteins competing for the same binding sites operating with DNA (enzymes, transcriptional factors, and others), *e.g.* endonucleases, apparently depends on this site-specific peptide binding with DNA. Endonucleases are essential enzymes hydrolyzing DNA involved in DNA recombination, replication, reparation, and apoptotic degradation.

Here we studied the effects of short peptides on hydrolysis of methylated and non-methylated λ phage DNA and fluorescein-labeled single and double stranded deoxyribonucleotides by CNG-site-specific endonucleases from wheat sprouts and investigated how this process can be affected by histone H1. We also studied changes in fluorescence spectrum of fluorescein-labeled deoxyribonucleotides under the influence of various peptides.

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MATERIALS AND METHODS

The following peptides were used in the study: epithalon (Ala-Glu-Asp-Gly), pinealon (Glu-Asp-Arg), bronchogen (Ala-Glu-Asp-Leu), testagen (Lys-Glu-Asp-Gly), cardiogen (Ala-Glu-Asp-Arg), and pancragen (Lys-Glu-Asp-Trp); the peptides were synthetized in St. Petersburg Institute of Bioregulation and Gerontology, Northwestern Division of the Russian Academy of Medical Sciences [3,7].

Fluorescence-labeled deoxyribonucleotides with specified primary structure were synthesized and kindly provided by Sintol Company.

Endonucleases WEN1 and WEN2 were separated and purified from the fraction of cytosolic vesicles of ageing wheat coleoptiles [4] by chromatography and electrophoresis [1,5]. These endonucleases site-specifically hydrolyse DNA. WEN1 preferentially hydrolyzes at CNG-sites of methylated λ phage DNA (dcm^+, dam^+) containing 5-methylcytosine residues in Cm^5CWGG sequences and N^6-methyladenine residues in Gm^6ATC sites [5]. WEN2 primarily hydrolyzes non-methylated DNA (dcm^-, dam^- [1]; phage DNA obtained from Fermentas).

DNA was hydrolyzed by WEN1 and WEN2 enzymes under identical conditions developed by us [4, 5] with or without peptide addition to the reaction mixture (1-2 μg peptide per 1μg DNA). DNA hydrolysis products were separated by electrophoresis in 1.5% agarose gel and then deoxyribonucleotide fragments were separated by electrophoresis in 20% polyacrylamide gel. Fluorescence spectra of fluorescein-labeled oligonucleotides were recorded on a Perkin-Elmer LS55 spectrofluorometer.

RESULTS

In the control, WEN 2 completely hydrolyzed unmethylated phage DNA (Fig. 1, *a*), therefore the corresponding tracks (*2-4*) looked empty on electrophoregrams. Epithalon suppressed hydrolysis of unmethylated DNA (Fig. 1, *a*, track *5*); in the presence of histone H1 this inhibitory peptide action on DNA hydrolysis by WEN2 was still well observed (Fig. 1, *a*, track *6*), however it was less marked, than without histone H1. Apparently, histone H1somewhat reduced the inhibitory effect of epithalon by making some DNA sites more susceptible for hydrolysis by the enzyme. The effect of bronchogen on DNA hydrolysis was different. In the presence of this peptide, DNA was selectively hydrolyzed to fragments of 140 nucleotides (Fig. 1, *a*, track *7*), which were not further hydrolyzed. It can be hypothesized that bronchogen interacts with another (than epithalon) and sufficiently specific DNA sites, protecting them from complete enzymatic hydrolysis. Histone H1 eliminated this protective effect of bronchogen (Fig. 1, *a*, track *8*), and DNA was hydrolyzed completely, like in the control. Pinealon had virtually no effect on WEN2 hydrolysis of unmethylated DNA (Fig. 1, *a*, track *9*), but markedly inhibited DNA hydrolysis in the presence of H1 histone (Fig. 1, *a*, track *10*). Histone H1 by itself did not affect DNA hydrolysis in the control (Fig. 1, *a*, track *4*). All this suggests that the test peptides modulate endonuclease DNA hydrolyzing activity in different manner, and their effects on the process to a certain extent can be mediated by histones. It is a very important observation, because peptides in the cell should first find chromatin sites accessible for interaction with DNA, and this accessibility is largely determined by histones, *e.g.* histone H1.

Epithalon had virtually no effect on WEN2 hydrolysis of methylated λ phage DNA (Fig. 1, *b*, track *5*), but completely suppressed hydrolysis of methylated phage DNA (Fig. 1, *a*, track *5*). It means that the peptide "recognizes" methylated and unmethylated DNA and, apparently, differently interacts with them. Bronchogen strongly activated hydrolysis of methylated DNA by WEN2 (Fig. 1, *b*, track *7*). Thus, epithalon and bronchogen have opposite effects on hydrolysis of methylated DNA. Pinealon by itself had no appreciable effect on hydrolysis of DNA with different methylation status, but peptide sensitivity to DNA methylation status is clearly seen in the presence of histone H1: under the influence of histone H1 pinealon inhibits hydrolysis of unmethylated DNA (Fig. 1, *a*, track *10*) and stimulates hydrolysis of methylated DNA (Fig. 1, *b*, track *10*).

Epithalon, bronchogen, and pinealon inhibited WEN1 hydrolysis of unmethylated DNA (Fig. 1, *a*, track *14*, *16*, *18*). Unlike WEN2, WEN1 completely hydrolyzed unmethylated DNA in complex with histone in the presence of pinealon (Fig. 1, *a*, track *10* and *19*). Epithalon increased the degree of WEN1 hydrolysis of methylated DNA (Fig. 1, *b*, track *14*). Different effects of the test peptides on endonuclease activity can be determined by different site specificity of peptide binding with DNA and with different site specificity of enzyme activity.

Since peptides discriminate not only individual DNA sequences, but also their methylation status, we intentionally constructed fluorescence-labeled deoxyribooligonucleotides containing CNG-sites methylated in eukaryotes and studied hydrolysis of these oligonucleotides by WEN1 in the presence of different peptides. Epithalon, bronchogen, pinealon, pancragen, and testagen partially and to different extent inhibited hydrolysis of single-stranded oligonucleotide 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3', and cardiogen completely blocked hydrolysis of this oligonucleotide (Fig. 2, *a*, track *5*). Hydrolysis of

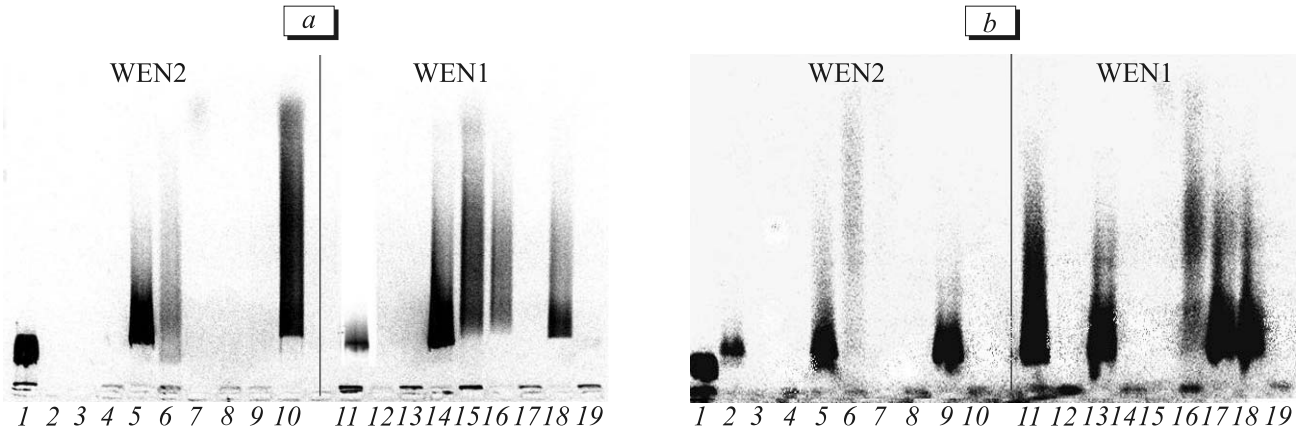

Fig. 1. Electrophoretic separation (in 1.5% agarose gel) of hydrolysis products of unmethylated (*a*) and methylated (*b*) λ phage DNA by endonucleases WEN1 and WEN2. *a*: *1*) unmethylated phage DNA; *b*: *1*) methylated phage DNA. *a*, *b: 2*) 1+enzyme WEN2; *3*) 2+Mg^{2+}; *4*) 2+histone H1; *5*)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

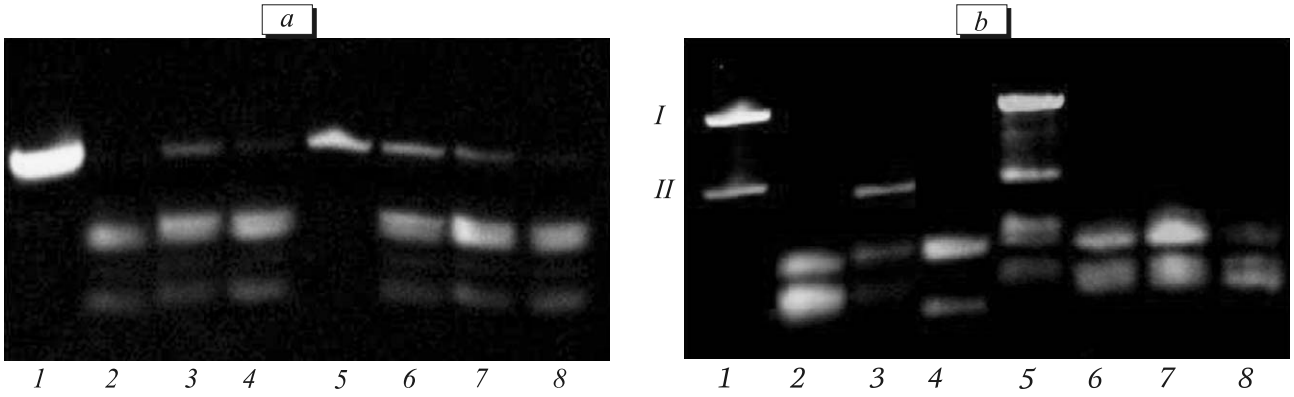

Fig. 2. Electrophoretic separation (in 20% polyacrylamide gel) of the hydrolysis products of fluorescence-labeled deoxyribooligonucleotide 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3' (*a*) and double-stranded fluorescence-labeled deoxyribooligonucleotide 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3'/3'-GCG GCG GTC CGC GGC GGC GC-FAM-5' (*б*) by endonuclease WEN1. *a*: *1*) oligonucleotide; *b*: *1*) oligonucleotides (*I* – double-stranded, *II* – mixture of single-stranded). *a*, *b*: *2*) 1+enzyme+Mg^{2+}; *3*) 2+epithalon; *4*) 2+bronchogen; *5*) 2+cardiogen; *6*) 2+pancragen; *7*) 2+pinealon; *8*) 2+testagen.

double-stranded oligonucleotides obtained by fusion of 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3' and complementary 3'-GCG GCG GTC CGC GGC GGC GC-FAM-5' in the presence of peptides proceeded in a different way. Pancragen, pinealon, and testagen had no effect on hydrolysis of the double-stranded oligonucleotide (Fig. 2, *b*, tracks *6-8*). Epithalon inhibited hydrolysis of single-stranded oligonucleotide (Fig. 2, *a*, track *3*), but stimulated hydrolysis of double-stranded one, however, some sites within the oligonucleotide remained unavailable for hydrolysis (the absence of the smallest fragment in comparison with the control; Fig. 2, *b*, track *3*). This agrees with the assumption on intercalation of epithalon into double stranded DNA upon binding to certain sites [10], which can determine the observed specific blockage of enzyme hydrolysis of certain sites in double-stranded oligonucleotide. It can be hypothesized that epithalon promotes DNA untwisting. It comes from the ratio of track fluorescence intensities for obtained fragments (Fig. 2, *b*, track *3*). In the presence of bronchogen, both single- and double-stranded structures are hydrolyzed; it does not block hydrolysis, but notably affects site-specificity of the hydrolysis. Similarly to epithalon, it seems to intercalate into DNA double helix with the same site-specificity. Cardiogen, in contrast to complete hydrolysis of single-stranded structures (Fig. 2, *a*), only partially inhibited hydrolysis of double-stranded oligonucleotide (Fig. 2, *b*, track *5*).

To confirm peptide binding to DNA or at least to relatively long deoxyribooligonucleotides, the fluorescence spectra for 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3' deoxyribooligonucleotide were analyzed in the presence of each investigated peptide. Fluorescence spectra for this oligonucleotide after addition of epithalon, bronchogen, or cardiogen to the medium are presented (Fig. 3). Epithalon markedly quenches fluorescence of labeled oligonucleotide, which indicated

high affinity constant for this peptide and oligonucleotide. Bronchogen also binds this oligonucleotide, but less markedly quenches its fluorescence. Unlike these peptides, cardiogen does not affect oligonucleotide fluorescence. It indicates that cardiogen does not bind to this oligonucleotide. Cardiogen completely inhibited hydrolysis of this oligonucleotide by WEN1 endonuclease (Fig. 2, *a*, track *5*). It cannot be excluded that such drastic inhibition of DNA hydrolysis with cardiogen is mediated by interaction with the enzyme, rather than with DNA. None of the test peptides quenched fluorescence of fluorescein-labeled monotonic oligonucleotide polydeoxy-C. Hence, peptides exhibit certain site-specificity of binding to DNA (oligonucleotides). We also found that the peptides also quenched fluorescence of the double-stranded oligonucleotide (Fig. 3, *d*), *i.e.* short peptides may intercalate into DNA helix [9]. Judging from different fluorescence quenching intensities (Fig. 3, *a*, *d*), peptides preferentially bind to single-stranded structures.

Specific peptide binding to single-stranded oligonucleotides revealed in our study is of particular importance. Single-stranded loci are always present or appear in DNA, particularly during genome replication, recombination, and reparation. Peptide interaction with these sites may be focused on the control of these genetic processes. In addition, intercalation of short peptide (epithalon) into DNA is associated with local DNA untwisting [9], which can lead to the appearance of single-stranded targets for peptide binding to DNA. This is most important during possible combined action of different peptides within the cell, when ones induce the appearance of single-stranded genomic structures, and others act initiatory regulating executors of the biological effect.

Thus, short peptides modulate endonuclease activity. We believe that peptide modulation of endonuclease activity appears due to site-specific peptide–DNA binding, which protects DNA against enzymatic hydrolysis. We found that modulation of endonuclease activity with the peptides in turn is controlled by histones (histone H1), therefore in cell (nucleus) chromatin histones may affect binding of short peptides to DNA. In addition, some peptides (cardiogen), can control endonuclease DNA hydrolysis at the level of interaction with the enzyme.

Tissue, subcellular, and age specificity of DNA methylation was previously reported [13] and different DNA methylation status in cancer and normal cells was demonstrated [12]. Considering these facts we hypothesized that the same bioactive peptide can differently bind to DNA depending on its methylation status and differently affect gene functions in different tissues (cells), in nucleus and mitochondria, in young and old cells, in normal and malignant cells. Almost all these hypothesizes were confirmed in experiments [3,7-9].

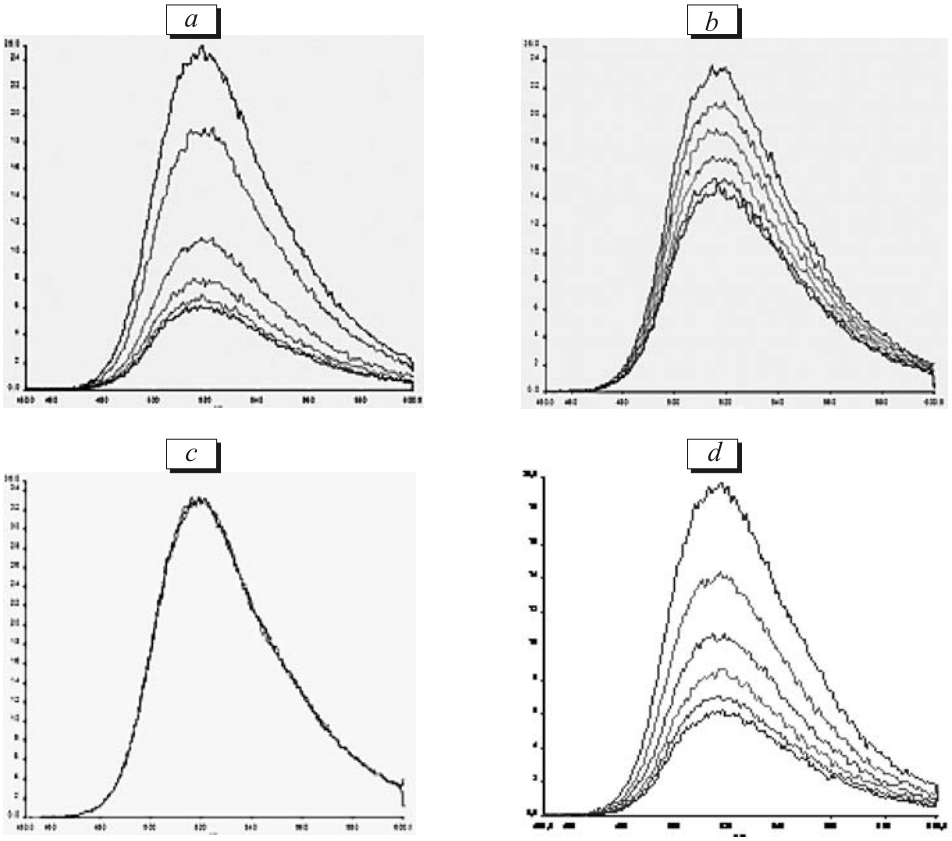

Fig. 3. Florescence spectra of fluorescence-labeled deoxyribooligonucleotide 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3' without (upper curves) and after titration by peptides (titration: *a*) by epithalon, *b*) by bronchogen, *c*) by cardiogen) and of double-stranded oligonucleotide 5'-FAM-CGC CGC CAG GCG CCG CCG CG-3'/3'-GCG GCG GTC CGC GGC GGC GC-FAM-5' (*d*; titration by epithalon). Abscissa: λ=450-600 nm, ordinate: fluorescence intensity 0-30 units.

The phenomenon of endonuclease activity modulation by short peptides revealed in our study may be just a part of global biological principle: peptides site-specifically and complementary binding with DNA (especially with regulatory sequences) should modulate the function of numerous specific proteins that interact with DNA (RNA- and DNA-polymerases, DNA-methyltransferases, reparation DNA enzymes, and numerous regulatory proteins). For example, certain hexapeptides are strictly selective structural ligands for protein-free *Holliday junctions* and block recombination [11]. We proposed one of the most probable mechanisms for gene activation by short peptides: peptides selectively bind to promoter CNG- or CG-sites, what make them inaccessible for DNA-methyltransferases and promoter remains unmethylated, what is crucial for activation for the majority of genes. Thus, specific (complementary) peptide-DNA interaction may epigenetically control genetic cell functions and probably this mechanism played an important role at the very early stage of life development and evolution.

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