



Research article

Regulation of DNA methyltransferase gene expression by short peptides in nicotiana tabacum regenerants

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Abstract: Methylation of cytosine DNA residues is the most studied and stable of all epigenetic modifications. Methylation of eukaryotic DNA is carried out by DNA methyltransferases. DNA methylation is an active mechanism for controlling gene transcription and is usually associated with prolonged silencing of DNA. The effect of peptides AEDG and AEDL on the growth of calluses of tobacco (*Nicotiana tabacum*) at low concentrations (10^{-7} M) is similar to the effect of phytohormones, has a regulatory character, and is possibly epigenetic in nature. Peptides increase the expression of DNA methyltransferase genes. One of the possible mechanisms of regulation of DNA methyltransferase genes by AEDG and AEDL is their ability to bind to free DNA regions at certain CNG sites, which are also methylation sites of plant cytosine methyltransferases. The AEDG peptide preferably binds to the CAG site and the AEDL peptide to the CTG site. By binding to the same sites as DNA methyltransferase, peptides block methylation sites, thereby reducing the level of DNA methylation. The specific binding of peptides to different sites that we discovered can be of great importance in gene regulation, since peptides with different structures can block different DNA regions for methylation of certain genes, thereby activating or silencing their expression.

Keywords: DNA methyltransferase; gene expression; *Nicotiana tabacum*; short peptides

1. Introduction

Fixed plants adapt to unfavorable environmental factors, often exhibiting striking phenotypic

plasticity. By its nature, this plasticity is epigenetic, since a plant with the same genotype under different conditions may have different phenotypes. The term epigenetics [1] is used to describe inherited differences not caused by changes in DNA sequence, any changes in chromatin modifications, or, simply, unusual patterns of inheritance [2].

DNA methylation and histone modifications are key mediators of epigenetic modifications. DNA methylation is usually associated with prolonged gene silencing, while histone modifications promote both activation and repression of gene transcription and can be removed after several cell cycles [3,4]. DNA methylation in plants controls development, participates in tissue-specific gene repression and parental imprinting, and serves as a mechanism to limit the expression of multicopy genes within acceptable limits. The most important role of DNA methylation is the inactivation of potentially dangerous elements in the genome, such as transposons and foreign DNA sequences. Methylation of gene promoters usually leads to their inactivation, while demethylation leads to reactivation. DNA methylation is a covalent modification of nucleotides in DNA.

The most well studied and most stable of all epigenetic modifications is the methylation of cytosine DNA residues [5–7]. Methylation of eukaryotic DNA is carried out by DNA enzymes methyltransferases (DMTs), which transfer the methyl group from *S*-adenosyl-*L*-methionine (SAM) to the cytosine carbon at the fifth or fourth position. Plant DNA is methylated by a vast arsenal of specific cytosine DMTs, some of which have no analogs in animals [5,6]. These enzymes in plants, as well as in animals, are homologous to bacterial DMT and are obviously a very ancient acquisition adapted for the particular needs of multicellular organisms. These enzymes are subdivided into supporting and *de novo* DMT, depending on whether the recognition site is already methylated or not. There are two types of DNA supporting methyltransferases in plants: DNA methyltransferase (MET) and chromomethyltransferase (CMT) [7]. Plant genomes have three types of methylation sites CG, CNG, and CNN (where N = C, T, or A), in contrast to the single methylation site type (CG) observed in animals, with the exception of embryonic stem cells and neurons [8,9]. Methylation of previously unmethylated DNA *de novo* is carried out by the DMT family, called domains rearranged methyltransferases (DRMs).

The main cytosine DMTs in plants are MET1, CMT3, and DRM2. These enzymes methylate different DNA sites (Table 1) and have different methylation mechanisms. The DMT enzyme MET1 methylates daughter DNA strands directly during replication. It is assisted in this by three related proteins (VIM1 to VIM3) containing the SRA domain, which recognizes semimethylated CG sites in DNA [5,6].

The mechanism of DNA methylation by the CMT3 enzyme is based on the presence of positive links between CMT3 and the H3K9 methylation enzymes, the main one of which is SUVH4 (KRYPTONITE, KYP). It was shown that CMT3 recognizes and methylates CNG sites in chromatin loci containing H3K9me2 molecules, while KYP, in turn, methylates histone H3 molecules in CNG-methylated loci [10].

It is known that DRMs mainly methylate asymmetrical CNN sites, but are capable of *de novo* methylating cytosines in any sequence context in a process called RNA-directed DNA methylation (RdDM). Interfering short RNAs (RNAi) (24 nucleotides) are formed as a result of the action of specific RNA polymerases that have been found only in plants [11].

Asymmetric methylation (which lacks an adjacent methylcytosine to provide epigenetic information after DNA replication) is largely controlled by DMT DRM2 [12]. For normal DNA methylation by the RdDM type, an SNF2-family nucleosome remodeler (DDM1) ATP-dependent

helicase is required [13,14]. The SNF2 ATPase domain hydrolyzes ATP by moving along the chromatin DNA, thereby changing the chromatin structure, allowing other proteins to access the DNA [15]. The DDM1 mutation causes a profound loss of methylation in some transposon elements and repetitions [13,14]. It is thought that DDM1 is involved in the methylation of CNN sites. The protein SUV2, which has an SUV domain, is also involved in DNA methylation by the RdDM mechanism; however, this protein does not have histone methyltransferase activity [16].

Chromomethyltransferase CMT2, along with DMT DRM2, is responsible for methylation of CNN sites in the *Arabidopsis* genome [7]. On the phylogenetic tree of chromomethylases, methyltransferase CMT2 forms a separate branch. In most organs, the *CMT2* gene is expressed much more weakly than *CMT3*.

In recent years, interest in studying the action of short peptides has increased dramatically [17]. The secreted peptides, like phytohormones, are important in the regulation of numerous intercellular connections and physiological activities, and respond to various influences [18]. Peptides interact with signaling phytohormones and are involved in regulation with the environment, modulating a wide range of biological processes. It was found that peptides are involved in the regulation of seed development, vascular formation, and lateral root formation, and participate in stem cell homeostasis in the apical meristem of seedlings and roots [19,20]. In plants, short peptides induce the expression of genes encoding factors of transcription, cell differentiation, growth, and development [21,22].

The action of exogenous peptides is gene-specific; it has a signal regulatory nature and, apparently, an epigenetic nature [18]. The molecular mechanisms of the effect of exogenous peptides on cellular processes are still not studied. One possible mechanism of action of exogenous peptides is the regulation of gene transcription.

The aim of this study is to consider possible mechanisms of regulation of the expression of DNA methyltransferase genes in *Nicotiana tabacum* regenerants by short peptides AlaGluAspLeu and AlaGluAspGly.

2. Materials and methods

2.1. Plant material

Tobacco seeds (*N. tabacum* L. cultivar Samsun) were germinated in flasks with agarose hormone-free Murashige–Skoog (MS) medium. Seeds of tobacco (*Nicotiana tabacum* L., Samsun serotype) were allowed to germinate in flasks with an agarized hormone-free Murashige and Skoog (MS) nutrient medium (Sigma, United States). The emerging cotyledons were detached with a scalpel and were placed on an agarized MS medium containing 10^{-7} M AEDL or AEDG. The control MS medium did not contain the peptides. The medium also contained phytohormones: 2 mg/L 6-benzylaminopurine, 0.2 mg/L naphthylacetic acid, and 0.2 mg/L indole-3-butyric acid. At the end of the experiment (after 21 days), the normally developed regenerated plants possessing shoots and roots were registered [23].

2.2. DNA and RNA isolation

DNA from tobacco regenerants was isolated using a standard method, utilizing a HigherPurity Plant DNA Purification Kit (Canvax, Spain). The purity of the preparations was determined by electrophoresis in 1% agarose. The mass of the obtained DNA was determined relative to DNA

markers. RNA was isolated from regenerants of tobacco according to a standard method, using reagent kits for the isolation of RNA (Extran RNA Synthol, Russia). The concentrations of isolated DNA and RNA preparations were determined spectrophotometrically using a NanoPhotometer IMPLEN.

2.3. cDNA

cDNA was obtained by a standard method, using a set of reagents (Synthol, Russia) for reverse transcription. The concentrations of cDNA preparations were determined spectrophotometrically using a NanoPhotometer IMPLEN.

2.4. Real-time PCR (PCR-RT)

Real-time polymerase chain reaction (PCR-RT) was carried out using a CFX 96 Real-Time System thermal cycler (BioRad, USA). Information on the primary structure of the DNA methyltransferase genes of *N. tabacum* was taken from the NCBI database. Primers for these genes were selected using the online service NCBI Primer-BLAST and synthesized by Synthol (Table 1). Samples were prepared by the standard method, using a set of reagents for PCR-RT in the presence of SYBR Green I (Synthol). The PCR-RT reaction was carried out under identical conditions for all samples: 95 °C for 5 min, then 45 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. The reaction was carried out in three repeats. The relative level of gene expression was calculated using a calibration curve constructed with PCR products obtained with primers for the GaPDh gene. The effectiveness of PCR-RT with primers for the studied genes reached 95–96%.

Table 1. Primers for PCR-RT.

Gene	5'-3'- sequence	Coding protein	Function of coding protein
<i>MET1B</i>	GACCACTTCTTCGCCAAAGC CGTCCTGACCGATAAGTTGCT	DNA (cytosine-5) methyltransferase 1B	Methylate CG sites in DNA
<i>CMT3</i>	TCAAGGAGAGATGGGTCTGTT AGGCAGGTCCCTGAAGTTTG	DNA (cytosine-5) methyltransferase CMT3	Methylate CNG sites in DNA
<i>CMT2</i>	GCTGGTCGAAAAACGAAGCG CCCACCCCTTGGTGCTTGAT	DNA (cytosine-5) methyltransferase CMT2	Methylate CNN (CNG) sites in DNA
<i>DRM2</i>	TGCTGGGTTTAGCCTGATGG GGAACCTGTTAGTTTCGCCCC	DNA (cytosine-5) methyltransferase DRM2	Methylate <i>de novo</i> CNN (CNG) sites in DNA

2.5. Statistical processing of results

The arithmetic mean values were calculated as $M_x = \Sigma X_i/n$. Standard deviations were calculated as:

$$\sigma_x = \sqrt{D_x} = \sqrt{\frac{\Sigma(X_i - M_x)^2}{(n - 1)}}$$

where D_x is the dispersion.

The calculation of the main statistical parameters was carried out according to standard methods, and Statistica 10.0 for statistical data processing were used. Standard deviations are shown according to Student's criterion, $p < 0.05$.

2.6. PCR amplification

PCR amplification was performed in a thermal cycler (DNAEngine, Biorad). The PCR reaction was carried out with DNA under the same conditions as for PCR-RT with cDNA. The obtained products were analyzed by electrophoresis in 1.5% agarose.

2.7. Digestion DNA by restriction endonuclease HpaII

Digestion of genomic plant DNA (1 μ g) by restriction endonuclease HpaII was performed according to a standard protocol using a Thermo Scientific Epi JET DNA Methylation Analysis Kit (Thermo Scientific).

2.8. Fluorescence

Fluorescence spectra were recorded using a PerkinElmer LS 55 spectrofluorometer (USA). Titration of FITC-labeled deoxyribooligonucleotides (oligos) with peptides AEDL and AEDG was carried out as described in the literature [23]. To determine the binding constants, we used the following equation:

$$-\log \left[\frac{F_0 - F}{F} \right] = \log K_b + n \log [Q] \quad (1)$$

where K_b is a constant and n is the number of binding sites. From equations (1) and (2), it is possible to derive $-\log [(F_0 - F)/F] = \log K_b + n \log [Q]$ (2), where K_b and n are the constant and the number of binding sites: $-K_{sf} = K_b[Q] n - 1$ (3). For $n = 1$, $K_{sf} = K_b$. This allows us to use the Stern–Volmer constant to evaluate the binding of peptides to various oligos [24,25].

2.9. Isothermal titration calorimetry

Isothermal titration calorimetry was carried out using a MicroCal VP ITC instrument (MicroCal, USA) at 25 °C. The obtained data were analyzed using the Origin 7 program, using the model of

ligand binding with one site type on the macromolecule (“one site” model). The obtained values of binding constant (K_b), number of binding sites (n), and excess molar enthalpy (ΔH) were used to determine the free energy of binding (ΔG) and standard entropy of binding (ΔS), as

$$RT \ln K_b = \Delta G = \Delta H - T\Delta S \quad (2)$$

The size of the binding site was determined as the number of nucleotides per ligand molecule ($1/n$).

Deoxyribooligonucleotides, FAM-deoxyribooligonucleotides, and peptides AEDG and AEDL were synthesized by Syntol (Russia). The equipment of the Center for Collective Use of VNIISB RAS was used in the work.

3. Results

3.1. Cultivation of tobacco callus in the presence of AEDL and AEDG peptides

When growing calluses of *N. tabacum* on a standard medium in the presence of 10^{-7} M peptides AEDG and AEDL, a significant increase in the mass of calluses is observed, and the total number of regenerants per explant increases. On media with tetrapeptides, the formation of large regenerants with a large leaf area was observed (Figure 1).

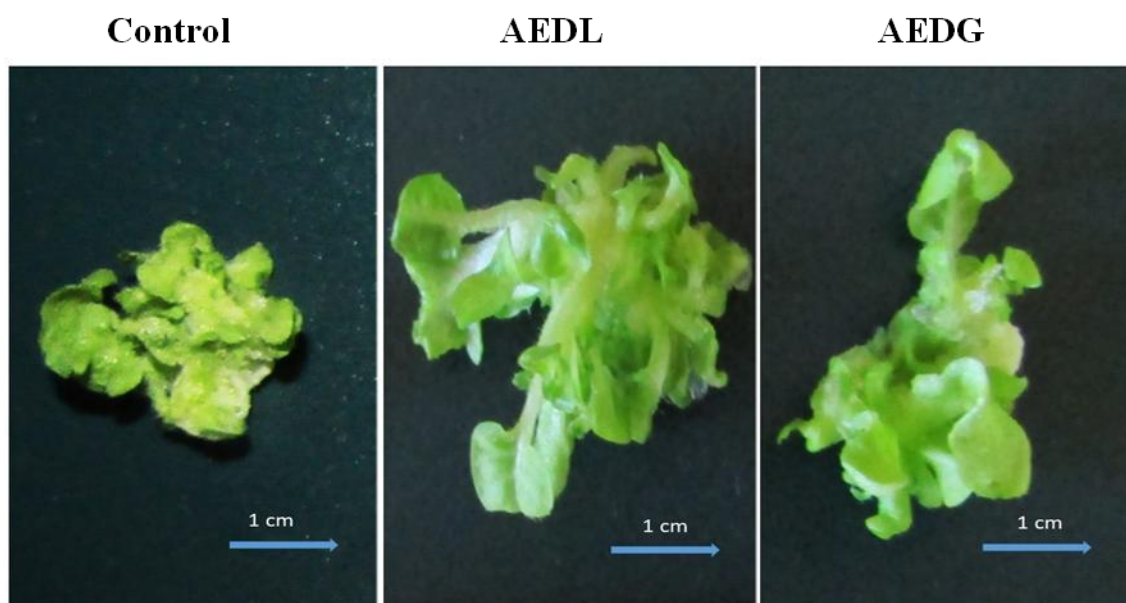


Figure 1. Regenerants of *Nicotiana tabacum* callus (28 days) grown without peptides (control) and in the presence of AEDL and AEDG at a concentration of 10^{-7} M.

Thus, short exogenous peptides have a pronounced physiological effect on plants. The action of peptides is likely to have a regulatory character and is similar to the action of phytohormones.

3.2. Gene expression of DNA methyltransferases

Expression of genes from *N. tabacum* encoding cytosine DNA methyltransferases is shown in Figure 2. In regenerants from tobacco calluses grown in the presence of AEDG and AEDL peptides, the expression level of the MET1B gene, encoding maintenance cytosine DNA methyltransferase, is activated by 30–60%. This DNA methyltransferase methylates the daughter DNA strands directly during replication.

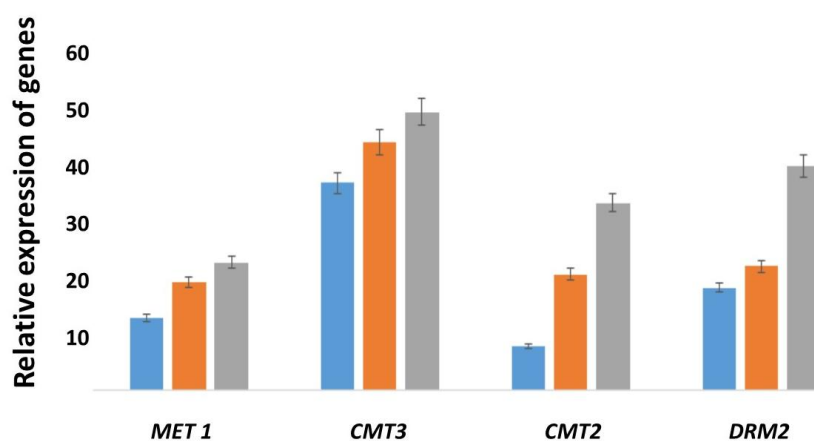


Figure 2. Relative gene expression of different DNA methyltransferase. Blue: cDNA from control tobacco regenerants; orange: from regenerants grown in the presence of peptide AEDL; grey: from regenerants grown in the presence of peptide AEDG. The mean values and their standard deviations.

DNA methyltransferase CMT3 provides downstream methylation of symmetric CNG sites. The relative level of expression of the *CMT3* gene in tobacco regenerants is significantly higher than in the *CMT2* and even *MET1* genes. In regenerants grown in the presence of peptides, the expression of *CMT3* genes increases in comparison with the control variant by approximately 25–35% (Figure 2).

De novo methylation of previously unmethylated DNA is performed by DRM2. It is known that DRM2 mainly methylates asymmetrical CNN sites during RdDM. In regenerants from tobacco calluses grown in the presence of the AEDL peptide, the expression level of the *DRM2* gene encoding the cytosine DNA methyltransferase involved in *de novo* DNA methylation increases insignificantly, by $\approx 20\%$. At the same time, the AEDG peptide almost doubles the expression of this *DRM2* gene.

3.3. Digestion DNA by restriction endonuclease HpaII

Bacterial restriction endonucleases cleave DNA highly specifically at certain sites. Restrictase HpaII cleaves DNA if the DNA is not methylated at the 5'-CCGG-3' site. We processed DNA isolated from tobacco regenerants grown both in the presence of peptides and without them with HpaII restriction enzyme (Figure 3). The enzyme weakly hydrolyzes plant DNA; to increase its capacity for restriction, we significantly increased the exposure time to 4 h. DNA restriction from tobacco regenerants grown in the presence of AEDL and AEDG peptides differs from the control in

terms of both quantity and mobility.

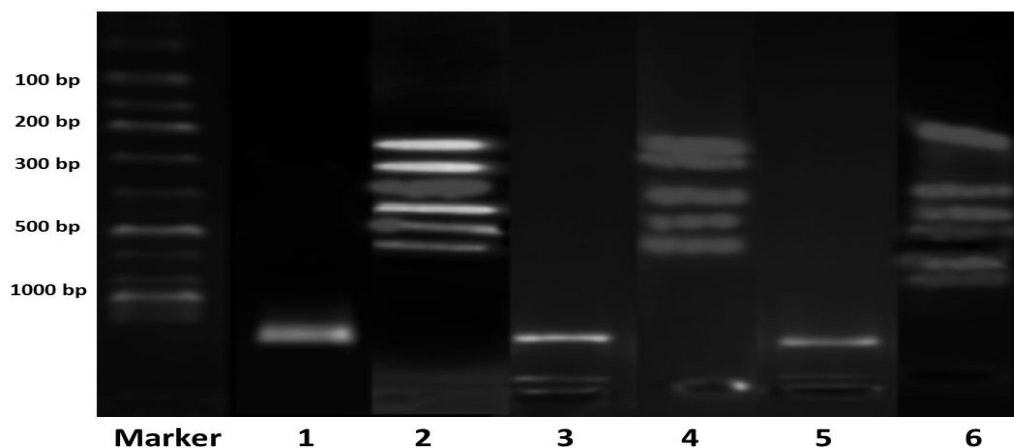


Figure 3. Digestion of DNA (1 μ g) with a restriction enzyme HpaII. 1: DNA without peptides; 2: DNA (1) + HpaII; 3: DNA with AEDL; 4: DNA (3) + HpaII; 5: DNA with AEDG; 6: DNA (5) + HpaII.

The decrease between the number of oligonucleotides after DNA hydrolysis from the regenerants grown in the presence of AEDL and that in the control regenerants is possibly due to the binding of the AEDL peptide to DNA at certain sites. The binding site of the AEDL peptide differs from the binding site of the AEDG peptide because the oligonucleotide sets are different, which supports the assumption that the peptides can directly bind to DNA. By binding to certain DNA sites, peptides can block their cleavage. Moreover, peptides can also block post-translational DNA methylation, thereby altering the sites of HpaII restriction enzyme cleavage.

3.4. PCR amplification

PCR amplification was carried out with primers for DMT genes with DNA from tobacco regenerants grown in the presence of peptides and without (Figure 4). As follows from Figure 4, the composition of the products on amplification of DNA from tobacco regenerants grown in the presence of peptides differs from that for control DNA, with all primers, except for primers for the *CMT2* gene. This fact possibly indicates the binding of peptides to DNA, which leads to the appearance of new amplification products. The composition of DNA amplification products from tobacco regenerants grown in the presence of AEDG and AEDL peptides is the same; however, there are some differences in the intensity of high molecular weight oligonucleotides during PCR amplification with *MET1B* and *DRM2* genes (Figure 4). Possibly, peptides bind to free regions of DNA and, during amplification, primers can bind to peptide-DNA motifs, and, as a result of sandwich formation during PCR amplification, nonspecific products are synthesized.

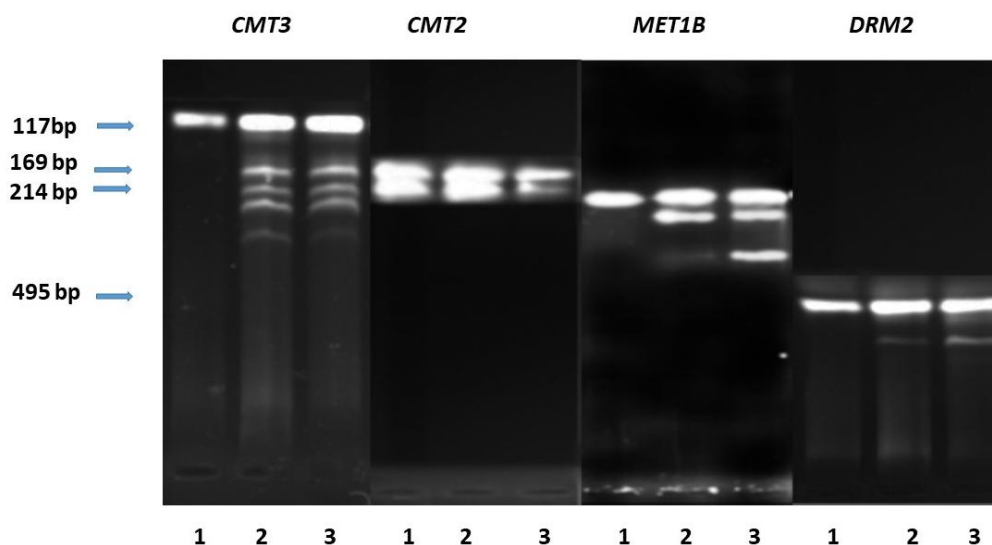


Figure 4. PCR amplification of DNA from tobacco regenerants with primers for the *DMT* genes. 1: DNA from tobacco regenerants without peptides; 2: DNA from tobacco regenerants with peptide AEDL; 3: DNA from tobacco regenerants with peptide AEDG.

3.5. Interaction of peptides AEDL and AEDG with deoxyribooligoribonucleotides

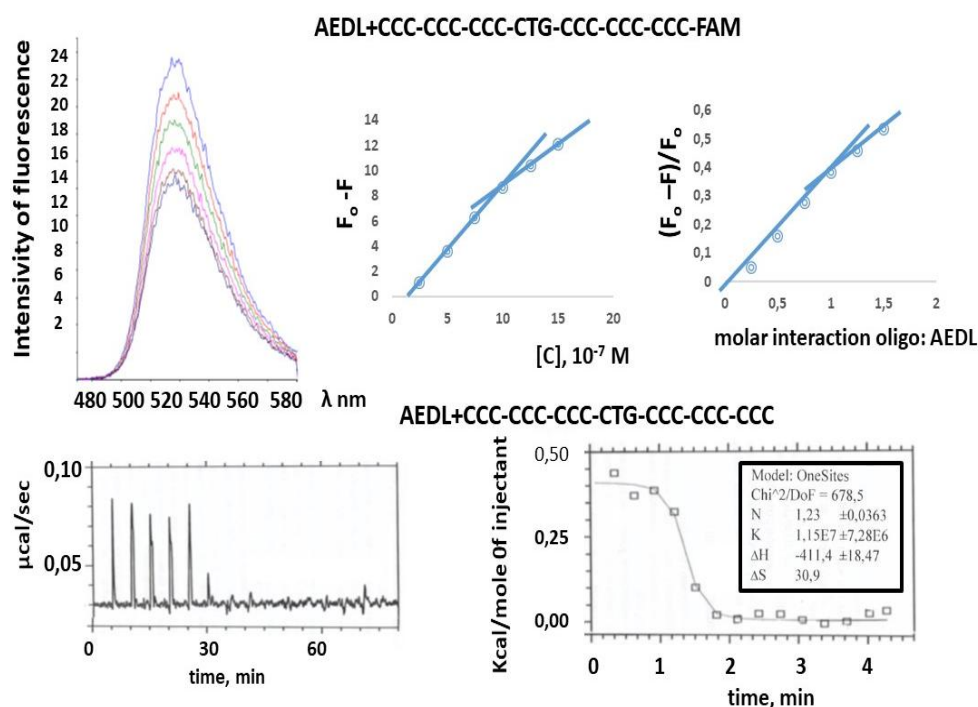


Figure 5. Fluorescent titration of FAM-oligo with CTG site by AEDL peptide and isothermal titration oligo by AEDL peptide.

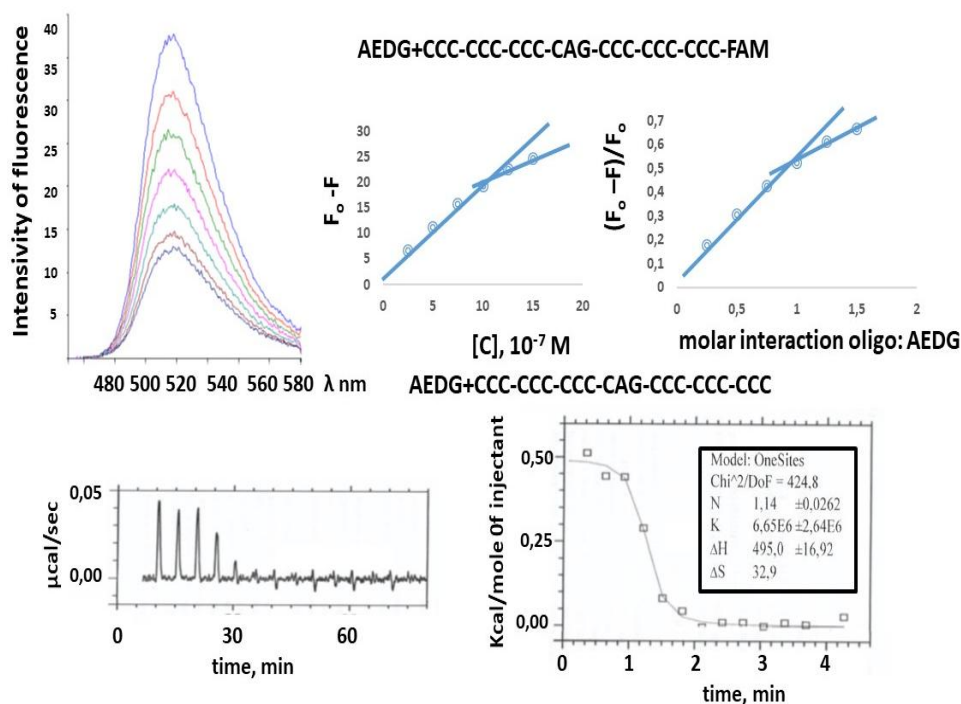


Figure 6. Fluorescent titration FAM-oligo with site CAG by AEDG peptide and isothermal titration oligo by AEDG peptide.

Static quenching of FAM-oligo during oligo–peptide complex formation can be described by the Stern–Volmer equation [24,25]. It was found that although the peptides are close in amino acid sequence and differ only in one amino acid, the AEDG peptide predominantly binds to the oligo, which includes the CAG site, and the AEDL peptide predominantly binds to the CTG site.

As an example, Figure 5 shows graphs of the fluorescence quenching of olig FAM-CCCCCCCCCTGCCCCCCCCC with the AEDL peptide and ITC titration of its analog without a fluorescent probe, and Figure 6 shows graphs of the fluorescence quenching of FAM-CCCCCCCCCAGCCCCCCCCC with the AEDG peptide and ITC titration of its analog without a fluorescent probe. On the basis of these graphs, the interaction constants were calculated, which characterize the interaction of the studied peptides with oligos, as well as the molar ratio of peptide: oligo. Peptides AEDG and AEDL bind to oligos in a 1 M: 1 M ratio. However, their binding constants differ significantly from each other. This is probably because their hydrophobicity indexes differ significantly. The AEDG peptide on the Kyte–Doolittle scale is more hydrophilic (−5.6) than the AEDL (−1.4) peptide [26]. In addition, oligo binding is probably sterically hindered by the long hydrophobic end of leucine in the AEDL peptide.

Methylation of cytosine at the CNG site leads to a decrease in the binding constant for both peptides, especially in the case of the AEDG peptide (Table 2). This is probably because cytosine methylation results in inaccessibility of the CNG site for peptide binding. Fusion of complementary oligos containing and not containing 5-methylcytosine leads to a decrease in the binding constant in the case of AEDG. Therefore, the AEDG peptide prefers to bind to single-stranded unmethylated oligos at the CAG site. Unlike the AEDG peptide, the AEDL peptide prefers to bind to double-stranded unmethylated oligos with the CTG site.

Table 2. Binding constants of peptides AEDG and AEDL with oligos.(revise)

	Oligos	K, M
AEDG	FAM- CCC CCC CCC CCC CCC CCC CCC CCC	1.1×10^{-4}
	FAM-CCC CCC CCC CAG CCC CCC CCC CCC	2.0×10^{-7}
	FAM-CGC GCG CGC GCG CGC GCG CGC GCG	0.9×10^{-4}
	FAM-CGC GCG CGC CAG CGC GCG CGC GCG	2.1×10^{-7}
	FAM-CGC GCG CGC CAG CGC GCG CGC GCG- CGC GCG CGC GCG CTG GCG CGC GCG	8.9×10^{-5}
	FAM-CGC GCG CGC meCAG CGC GCG CGC GCG	5.7×10^{-5}
	FAM-CGC GCG CGC meCAG CGC GCG CGC GCG- CGC GCG CGC GCG CTG GCG CGC GCG	1.3×10^{-5}
	AEDL	FAM- CCC CCC CCC CCC CCC CCC CCC CCC
FAM-CCC CCC CCC CTG CCC CCC CCC CCC	0.8×10^{-7}	
FAM-CGC GCG CGC GCG CGC GCG CGC GCG	1.2×10^{-4}	
FAM-CGC GCG CGC CTG CGC GCG CGC GCG	1.2×10^{-7}	
FAM-CGC GCG CGC CTG CGC GCG CGC GCG- CGC GCG CGC GCG CAG GCG CGC GCG	1.7×10^{-7}	
FAM-CGC GCG CGC meCTG CGC GCG CGC GCG	5.3×10^{-5}	
FAM-CGC GCG CGC meCTG CGC GCG CGC GCG- CGC GCG CGC GCG CAG GCG CGC GCG	5.4×10^{-5}	

4. Discussion

The effect of peptides AEDG and AEDL on the growth of tobacco calluses at low concentrations (10^{-7} M) is similar to the effect of phytohormones, has a regulatory character, and is possibly epigenetic in nature. The most studied of all epigenetic modifications is the methylation of cytosine DNA residues [5,6].

The mechanisms of gene expression regulation by DNA methylation are poorly understood. It was assumed that the selection of methylation targets is a passive process. However, the discovery of the effect of methylated individual CG sites in the promoter region of rRNA genes in plants on the specific binding of nuclear proteins to the promoter region shows that the methylation process is an active mechanism for regulating the transcription of these genes, and not a passive result of the absence of their transcription [27].

Comparison of the relative expression levels of genes encoding cytosine DNA methyltransferases from *N. tabacum* regenerants grown in the presence and absence of AEDG and AEDL peptides revealed the activation of these genes in the presence of peptides, as compared with the control (Figure 2).

In tobacco regenerants grown in the presence of AEDG and AEDL peptides, the level of gene expression of all cytosine DMTs is activated. While the expression of the methyltransferase genes *MET1* and *CMT3* increases insignificantly, the expression of the *CMT2* gene increases 2–3 times, and the expression of the *DRM2* gene doubles in regenerants grown in the presence of AEDG. It should be noted that in regenerants grown in the presence of the AEDG peptide, the level of expression of all DMTs is higher than in regenerants grown in the presence of the AEDL peptide.

Although the peptides differ from each other by only one amino acid, they significantly differ in binding constants and have different binding sites: the AEDG peptide predominantly binds to the

CAG site, and the AEDL peptide predominantly binds to the CTG site. The CAG and CTG sites are methylation sites for the cytosine methyltransferase CMT3, and in some cases also CMT2 and DRM2.

The composition of the products of the amplification of DNA from tobacco regenerants grown in the presence of peptides differs from those of the control DNA, with all primers, except for the primers for the *CMT2* gene, which may indicate the binding of peptides to DNA, which leads to the appearance of new amplification products. The composition of DNA amplification products from tobacco regenerants grown in the presence of AEDG and AEDL peptides is the same; however, there are some differences in the intensity of high molecular weight oligonucleotides during PCR amplification with *MET1B* and *DRM2* genes (Figure 4).

The set of oligonucleotides obtained after DNA restriction from regenerants grown in the presence of AEDL differs from DNA restriction from control tobacco regenerants, as well as regenerants grown in the presence of AEDG, in both quantity and mobility.

The decrease in the number of oligonucleotides after DNA hydrolysis from the regenerants grown in the presence of AEDL, compared with the control, is possibly due to the binding of the AEDL peptide to DNA at certain sites. The binding site of the AEDL peptide differs from the binding site of the AEDG peptide, since the sets of oligonucleotides differ from each other. These data support the assumption that peptides can directly bind to DNA. By binding to sites, peptides can block their cleavage. Moreover, peptides can also block post-translational DNA methylation, thereby altering the sites of HpaII restriction enzyme cleavage.

The DMTs are encoded in the genome and it is logical to assume that their activity is also regulated by DNA methylation. In this case, a drop in the level of DNA methylation, which is caused by peptide binding to sites involved in the methylation process, should lead to increased expression of *DMT* genes and, as a consequence, to a compensatory increase in the activity of methylation systems. Thus, an increase in the activity of the expression of *DMT* genes in tobacco regenerants is because short peptides bind to CNG sites, blocking methylation sites, thereby reducing the level of DNA methylation.

Titration of synthetic oligos with peptides revealed that although the peptides are close in amino acid sequence and differ only in one amino acid, they bind to different sites and have different binding constants. The AEDG peptide preferentially binds to the oligos, which includes the CAG site, and the AEDL peptide binds to the CTG site. This selective binding of structurally related peptides is explained by their different hydrophobicity and steric effects.

The specific binding of peptides to single-stranded oligos that we have discovered may be of particular importance. Single-stranded regions always exist or appear in DNA; for example, they arise during replication, recombination, and repair of the genome. The interaction of short peptides with such regions can control these genetic processes in a targeted manner. In addition, intercalation of short peptides into DNA is accompanied by local untwisting of DNA strands [28], which leads to the emergence of single-stranded targets for binding of peptides to DNA. This takes on a special meaning in the event of the possible combined action of different peptides in the cell, when some serve as inducers of the emergence of single-stranded structures in the genome, while others, as a result, are actually initiator regulatory agents of the biological effect.

5. Conclusion

The regulation of DNA methylation can occur by different mechanisms, since many players are

involved in this process, but, first of all, regulation is carried out through the expression of genes of DNA methyltransferases. Controlling the regulation of *DMT* genes is of particular importance, since the process of DNA methylation in plants controls most of the processes occurring in plants. Methylation of gene promoters usually leads to their inactivation, while demethylation leads to reactivation.

It has been shown that short peptides are able to regulate the expression of *DMT* genes. One possible mechanism for the regulation of *DMT* genes by short peptides is their ability to bind to free DNA regions at certain CNG sites, which are also methylation sites of plant cytosine methyltransferases. By binding to the same sites as DNA methyltransferases, peptides block methylation sites, thereby reducing the level of DNA methylation, and causing an increase in the expression of *DMT* genes, especially *CMT2* and *DRM2*. The AEDG peptide has higher oligo binding constants than the AEDL peptide. This fact probably explains the higher expression of *DMT* genes by AEDG.

The specific binding of peptides to different sites that we have discovered can be of great importance in the regulation of *DMT* genes, and not only these genes, since the use of peptides with different structures can block different DNA regions for methylation of certain genes, thereby activating or silencing their expression.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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