DOI 10.1007/s10517-016-3596-7

METHODS

Short Peptides Regulate Gene Expression

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 162, No. 8, pp. 259-264, August, 2016
Original article submitted June 10, 2015

Short peptides constitute the system of signal molecules regulating the functions of the organism at the molecular, genetic, subcellular, cellular, and tissue levels. One short peptide can regulate dozens of genes, but the molecular mechanism of this process remains unclear. We suppose that short peptides penetrate through the cytoplasmic and nuclear membrane and bind to DNA. Spatial models of DNA—peptide complexes are constructed for 19 short peptides by the docking method. Some peptides have the same binding sites. Peptides KE and EDP bind agat sequence, peptides KEDW and AED to acct sequence, and peptides AEDL and EDL to ctcc sequence.

Key Words: *short peptides; DNA—peptide interactions; molecular simulation*

Short peptides created at St. Petersburg Institute of Bioregulation and Gerontology regulate gene expression and protein synthesis, stimulate cell proliferation and differentiation, and inhibit apoptosis, thus restoring organ functions in various pathologies and during aging. Peptide treatment reduced the incidence of cancer and prolonged the median and maximum lifespan in experimental animals. In most experiments, the peptides were shown to increase the physiological resource of cells, tissues, and the body by 20-42% [6].

Peptides KE and AEDG injected to transgenic mice 2.0-3.6-fold suppress the expression of *HER-2/neu* (human breast cancer) gene in comparison with the control, which is associated with a significant decrease of tumor size. Addition of AEDG peptide to human lung fibroblast culture induces the expression of telomerase gene, telomerase activity, and promotes telomere elongation by 2.4 times [6]. Activation of the gene expression is accompanied by an increase (by 42.5%) in the number of cell divisions, which demonstrated the overcoming of the Hayflick limit [6,7].

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The effects of peptides KE, EW, AEDG, and AEDP on the expression of 15,247 genes of the heart and brain of mice are studied by the DNA-microarray technology. Each peptide specifically regulates the expression of a certain group of genes. These results attest to the existence of a mechanism of peptide regulation of genetic activity [6]. Peptide KE characterized by immunomodulating activity regulates the expression of *IL2* gene in blood lymphocytes [5]. Peptide AEDL activates the expression of bronchial epithelium differentiation genes *Nkx2.1*, *SCGB1A1*, *SCGB3A2*, *FoxA1*, and *FoxA2* in human bronchial epithelium cell cultures and increases the expression of *MUC4*, *MUC5AC*, and *SftpA1* genes, reduction of their activities correlating with the development of chronic bronchitis [12]. Peptide KEDW stimulates the expression of differentiation genes *PDX1*, *NGN3*, *PAX6*, *FOXA2*, *NKX2*, *NKX6.1*, and *PAX4* and reduced the expression of *MNX1* and *HOXA3* genes in human pancreatic cell cultures. Peptide EDG regulates mRNA expression of various genes in induced peptic ulcer in rats, and reduces the synthesis of superoxide dismutase (SOD), TNF-α, and Cox-2 mRNA [9].

Neuroprotective peptide EDR improved energy support of the muscle tissue, which correlated with

higher expression of *PPARA* and *PPARG* genes encoding proteins stimulating the oxidative capacity of the skeletal muscles. The peptide regulation of adaptive potential in this case was paralleled by higher expression of heat shock protein *HSPA1A* gene [3].

Studies by physicochemical methods (UV spectroscopy, circular dichroism, and viscosimetry) have demonstrated that short peptides bind to DNA in solution *in vitro* [12]. This process takes several hours and virtually does not involve electrostatic forces. Complex formation realized in the DNA groove with participation of nitrous bases and the peptide leads to destabilization of the secondary structure of the macromolecule. UV spectrophotometry detected a concentration-dependent hyperchromatism (increase in optical density at λ=260 nm) in a mixture of AEDG and AEDL peptides and double-stranded DNA. Hyperchromatism indicates partial destruction of hydrogen bonds between the nucleotide pairs in the double strand and local separation of DNA strands (allosteric conformational modification). Separation of strands (metling) in free synthetic DNA separate occurs at 69.5°C. In the DNA-AEDG peptide system, the strand melts at 28°C and the process is characterized by 2-fold drop of entropy and enthalpy. It has been previously shown that by their steric and geometrical characteristics, short peptides can incorporate into the DNA major groove and interact with nitrous bases [11]. Polar interactions of the peptides with the base pairs in the major groove are determined by electrostatic interactions of the peptide carboxyl groups with adenine and cytosine amino groups, by hydrogen bonds of protonated amino groups and carboxyl groups of the peptide with adenine and guanine nitrogen atoms N7 [7]. Hydrophobic interactions in the system are determined by peptide side groups and thymine methyl group. It is obvious that each sequence of nucleotide pairs in DNA exposes to the major groove a unique ornament of functional groups recognizing the peptide [11]. Based on this complementarity, binding sites for peptides KEDA, AEDG, KEDW, KED, EDR, and KE were determined and 2D and 3D models of the DNA—peptide complexes were constructed [10,11,15]. With development of computer technologies, the set of the analyzed parameters can be considerably extended.

TABLE 1. Presumable DNA Binding Sequences for Short Peptides Created at St. Petersburg Institute of Bioregulation and Gerontology

No.	Peptide name	Peptide structure	DNA binding sequence	Force of peptide binding to DNA
1	Vilon	KE	agat*	+
2	Epithalone	AEDG	aatg	++
3	Prostamax	KEDP	attc	++
4	Livagen	KEDA	tcct	+++
5	Cortagen	AEDP	aacc	++
6	Cartalax	AED	acct*	+
7	Pinealon	EDR	ttcc	+++
8	Chonluten	EDG	tttt	+
9	Ovagen	EDL	ctcc*	++
10	Cristagen	EDP	agat*	++
11	Vesugen	KED	gccg	+
12	Vesilute	ED	attt	+
13	Pancragen	KEDW	acct*	+++
14	Cardiogen	AEDR	agtc	+++
15	Testagen	KEDG	caac	++
16	Bronchogen	AEDL	ctcc*	++
17	Normophthal	K(gamma)E	act*	+
18	Thymogen	EW	aacg	+
19	AD7	DS (mod)	aata	+

Note. +: weak interactions, enthalpy change (dH=-3 kcal/mol); ++: strong interactions (dH=-4-4.5 kcal/mol); +++: very strong interactions (dH=-5-6 kcal/mol). *Common peptide binding sites.

Our objective was to create the models of DNA—peptide interactions for 19 short peptides using the docking method.

MATERIALS AND METHODS

Molecular simulation of DNA—peptide complexes was carried out with the use of Molecular Operating Environment 2012 (MOE 2012) software. The peptides were constructed in the left-handed conformation. The spatial structure of double-stranded DNA was constructed for B-form with standard major (2.1 nm) and minor (1.1 nm) grooves. For calculation of the interaction energy and search for specific peptide binding sites, MOE 2012 standard docking package, Amber99 field of force, and Affinity dG genetic searching algorithm were applied. Semiflexible docking was used assuming peptide conformation flexible and DNA nitrous bases conformation rigid. Docking solutions were ranked by the estimator values. Docking of peptides with all combinations of 4 b.p. DNA duplexes was carried out. The solvent was taken into consideration as a continuous medium around the molecule, which simplified estimation of the contribution of solvation.

RESULTS

Peptides KEDA, KEDW, AEDR, and EDR — virtually all having polar positively charged side chains — demonstrate strong interactions with DNA. Dipeptides form weak interactions with DNA, presumably because of small area of contact (Table 1). Peptides KE and EDP bind to agat sequence of DNA (Fig. 1, *a*). Both peptides are immunomodulators. The peptides form hydrogen bonds with N7 and N6 in adenine and phosphate backbone. However, EDP forms one more hydrogen bond with N4 of cytosine, due to which the energy of EDP—agat complex formation is higher than of KE—agat complex.

Peptides KEDW and AED bind to acct sequence of DNA (Fig. 1, *b*). The area of KEDW contact is larger than of AED due to the presence of bulky lysine and tryptophan side chains. Hence, the peptide can completely occupy the DNA major groove, thus preventing binding of other molecules. KEDW binds to acct sequence 3-fold stronger than AED (Table 1). Both peptides interact with cytosine N4 and adenine N7, but KEDW also binds to adenine N7 and forms a network of hydrogen and ion-ion interactions with the phosphate backbone.

Peptides AEDL and EDL bind to ctcc sequence of DNA (Fig. 1, *c*) with the same complex formation energy (Table 1). Both peptides bind cytosine N4 and adenine N6 acting as proton acceptors and do not interact with phosphate backbone, because of their strong negative charge. The data of molecular simulation seem to indicate similar mechanisms of action of AEDL and AED peptides, but this hypothesis should be experimentally validated.

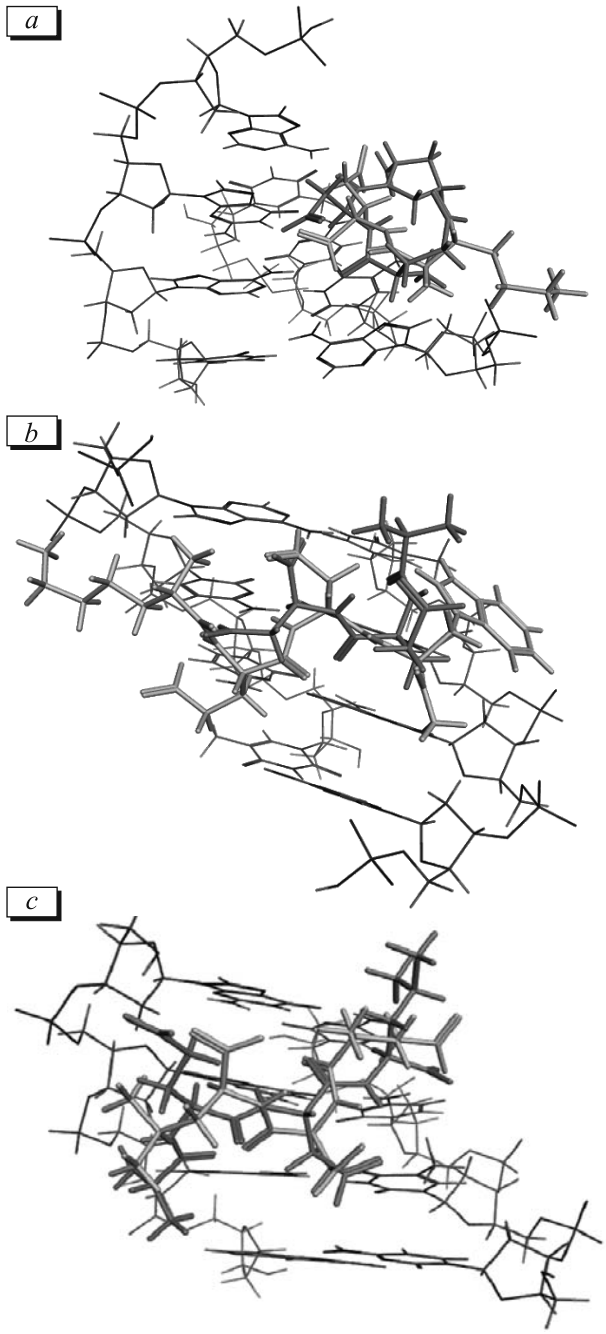

Fig. 1. Interactions between peptides KE (dark-gray) and EDP (light-gray) and the common binding sequence agat (*a*), between KEDW (dark-gray) and AED (light-gray) and common binding sequence acct (*b*), and between AEDL (dark-gray) and EDL (light-gray) and common binding sequence ctcc (*c*).

Our data on the effects of short peptides on gene expression are in good agreement with the results of other scientists. Peptide Selank (PGP) characterized by

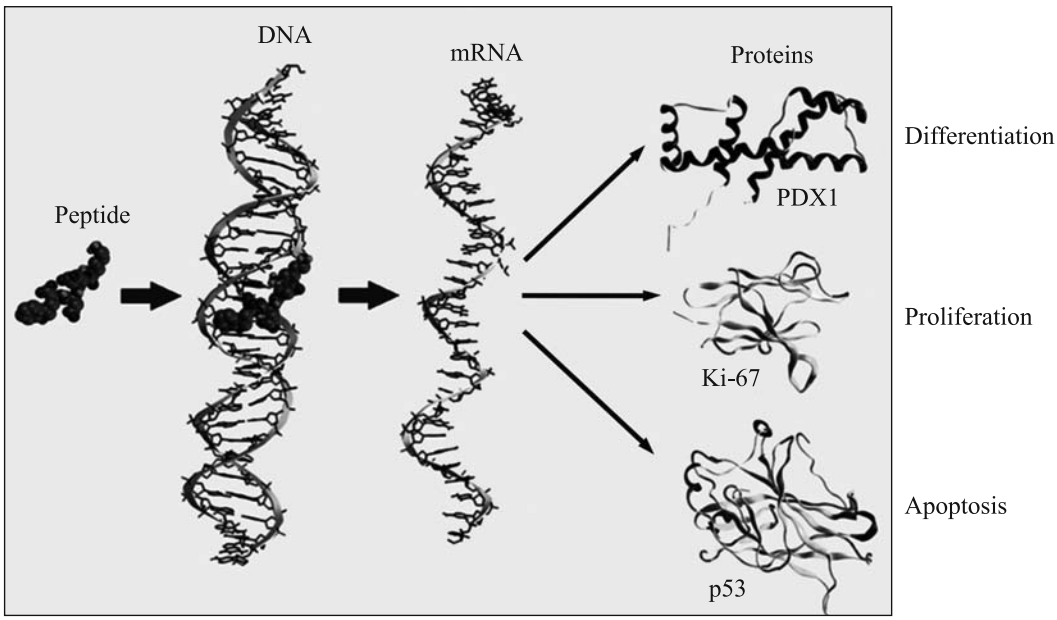

Fig. 2. Presumable mechanism of peptide regulation of gene expression and protein synthesis in the cell.

nootropic and antiviral activities regulates the expression of *Bdnf* mRNA in the rat brain. In addition, PGP peptide regulates the expression of mRNA for 15 of the studied 84 genes in rat spleen involved in inflammation processes and encoding chemokines and cytokines [13,14]. Administration of delta-sleep peptide (WAGGDASGE) to rats of different age stimulates the expression of SOD gene (*Sod1*) and glutathione peroxidase 1 gene (*Gpx1*) in the brain and in the blood nuclear cells, which is liable to reduce with physiological aging [2]. Semax (MEHFPGP), a modified fragment of the adrenocorticotropic hormone, attenuates stress-induced expression of *c-Fos* gene in the hypothalamic paraventricular nucleus in rats prone to mental stress [4]. In addition, peptide MEHFPGP activates the expression of NGN and BDNF neurotrophic factor genes in experimental animals [14]. Receptor binding of Semax to cytoplasmic membranes of the rat brain neurons and glial cells and to whole neurons was not detected so far. Presumably, semax can bind to neuronal membranes specifically and reversibly; the receptors capable of semax binding are really scanty and present in just few parts of the brain [1].

Hence, modern reports confirm that short peptides can regulate the gene expression. It remains unclear how this regulation is realized: whether short peptides penetrate through the cytoplasmic and nuclear membrane and then bind to DNA or they bind to receptors (intracellular or on cell membranes) and activate the intracellular signal cascades, which results in modification of the gene expression.

We have previously shown that FITC-labeled di- and tripeptides penetrate into the cytoplasm, nuclei, and nucleoli of HeLa cells [8]. Eukaryotic cells nucleus has a system of nuclear pores formed by protein complexes nucleoporins. The inner diameter of nuclear pores is about 50 nm. Hence, they are permeable for free diffusing low-molecular-weight substances with a molecular weight ≤3500 Da. Therefore, short peptides by their physicochemical characteristics (charge, size, and hydrophoby) can penetrate through the cytoplasmic and nuclear membranes of the cell and interact with DNA.

Based on the data on peptide effects on gene expression and protein synthesis and the results of in silico abalysis (Table 1), we created a scheme of peptide regulation of gene expression (Fig. 2). Short peptides, penetrating into the cell, bind to complementary sequences in the gene promoters, which results in the synthesis of the relevant mRNA and triggering of the translation process. This is the way the peptides regulate the expression of genes and synthesis of proteins determining the most important stages of vital activity of the cell: proliferation, differentiation, and apoptosis.

Hence, specific (complementary) peptide—DNA interactions can epigenetically regulate the genetic functions of the cell; presumably, this mechanism played an important role at the earliest stages of life formation and subsequent evolution.

REFERENCES

1. Grivennikov IA, Dolotov OV, Gol'dina YuI. Factors of peptide nature in proliferation, differentiation, and maintenance of cell viability. Mol. Biol. 1999;33;(1):120-126. Russian.
2. Kutilin DS, Bondarenko TI, Kornienko IV, Mikhaleva II. Effect of delta sleep-inducing peptide on the expression of an-

tioxidant enzyme genes in the brain and blood of rats during physiological aging. Bull. Exp. Biol. Med. 2014;157(5):616-619.
3. Umnov RS, Linkova NS, Khavinson VKh. Neuroprotective effects of peptides bioregulators in people of various age. Uspekhi Gerontol. 2013;26(4):671-678. Russian.
4. Umriukhin PE, Koplik EV, Grivennikov IA, Myasoedov NF, Sudakov KV. Immediate early gene c-Fos expression in rats resistant and predisposed to emotional stress after intraperitoneal injection of the ACTH(4-10) analog — Semax. Zh. Vyssh. Nervn. Deyat. 2001;51(2):220-227. Russian.
5. Anisimov SV, Bokheler KR, Khavinson VKh, Anisimov VN. Studies of the effects of Vilon and Epithalon on gene expression in mouse heart using DNA-microarray technology. Bull. Exp. Biol. Med. 2002;133(3):293-299.
6. Anisimov VN, Khavinson VKh. Peptide bioregulation of aging: results and prospects. Biogerontology. 2010;11(2):139-149.
7. Chen XW, Jeong JC. Sequence-based prediction of protein interaction sites with an integrative method. Bioinformatics. 2009;25(5):585-591.
8. Fedoreyeva LI, Kireev II, Khavinson VKh, Vanyushin BF. Penetration of short fluorescence-labeled peptides into the nucleus in HeLa cells and in vitro specific interaction of the peptides with deoxyribooligonucleotides and DNA. Biochemistry (Mosc). 2011;76(11):1210-1219.
9. Khavinson VKh, Lin'kova NS, Dudkov AV, Polyakova VO, Kvetnoi IM. Peptidergic regulation of expression of genes encoding antioxidant and anti-inflammatory proteins. Bull. Exp. Biol. Med. 2012;152(5):615-618.
11. Khavinson VKh, Tarnovskaya SI, Linkova NS, Pronyaeva VE, Shataeva LK, Yakutseni PP. Short cell-penetrating peptides: a model of interactions with gene promoter sites. Bull. Exp. Biol. Med. 2013;154(3):403-410.
10. Khavinson VKh, Lin'kova NS, Tarnovskaya SI, Umnov RS, Elashkina EV, Durnova AO. Short peptides stimulate serotonin expression in cells of brain cortex. Bull. Exp. Biol. Med. 2014;157(1):77-80.
12. Khavinson VKh, Tendler SM, Vanyushin BF, Kasyanenko NA, Kvetnoy IM, Linkova NS, Ashapkin VV, Polyakova VO, Basharina VS, Bernadotte A. Peptide regulation of gene expression and protein synthesis in bronchial epithelium. Lung. 2014;192(5):781-791.
13. Kolomin T, Shadrina M, Andreeva L, Slominsky P, Limborska S, Myasoedov N. Expression of inflammation-related genes in mouse spleen under tuftsin analog Selank. Regul. Pept. 2011;170(1-3):18-23.
14. Shadrina M, Kolomin T, Agapova T, Agniullin Y, Shram S, Slominsky P, Lymborska S, Myasoedov N. Comparison of the temporary dynamics of NGF and BDNF gene expression in rat hippocampus, frontal cortex, and retina under Semax action. J. Mol. Neurosci. 2010;41(1):30-35.
15. Tarnovskaya SI, Yakutseni PP, Khavinson VKh. Study of interactions between DNA and tetrapeptides using methods of molecular mechanics. Bull. Exp. Biol. Med 2014;156(5):689-693.