Role of Peptide Bond in the Realization of Biological Activity of Short Peptides

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We performed a comparative analysis of biological activity of Lys-Glu peptide and its amino acid constituents. It was established that Lys-Glu stimulated proliferation of splenic cells in organotypic culture, while the mixture of glutamic acid and lysine inhibited culture growth. Using the method of molecular docking, we showed that glutamic acid, lysine, and Lys-Glu peptide can interact with different DNA sequences. The energy of interaction and the most beneficial localization of glutamic acid, lysine, and Lys-Glu peptide in DNA molecule was calculated. We demonstrated the interaction of the peptide and amino acids with DNA along the minor groove. The energy of DNA interaction with the peptide is higher than with individual amino acids. The peptide bonds increase the interaction of Lys-Glu peptide with DNA, which potentiates the biological effect on cell proliferation in organotypic culture of splenic cells.

Key Words: *dipeptide; amino acids; molecular modeling; docking; organotypic culture*

Short peptides and amino acids belong to a group of signal molecules involved in homeostasis maintenance. Hydrophilic amino acids with charged side groups stimulate cell proliferation in cultures of different origin [11,12,14] presumably via regulation of specific genes at the transcriptional and translational levels and thereby modulation of the expression of regulatory proteins in the cell [2,3].

Peptide Lys-Glu synthesized in the St. Petersburg Institute of Bioregulation and Gerontology acts an immunomodulator [7,10], stimulates proliferation and differentiation and inhibits apoptosis of T and B cells, NK-cells, and macrophages of the thymus and spleen [9]. The peptide increases functional activity of immune cells by inducing interferon and interleukin synthesis. Experiments showed that administration of Lys-Glu peptide to laboratory animals stimulates repair of the thymus, spleen, and gastrointestinal tract during accelerated aging induced by γ-irradiation [4,6]. This peptide stimulates liver regeneration and restores functional activity of the immune system in animals with severe infectious posttraumatic complications [8,9]. Moreover, the peptide Lys-Glu prolongs average lifespan and reduced the incidence of tumors in animals [4]. However, the question of whether biological activity of the peptide Lys-Glu is exclusively determined by its constituent amino acids or peptide bond remains unanswered.

The aim of this comparative study was to analyze the influence of amino acids lysine and glutamic acid, their mixture, and a peptide Lys-Glu on the development of organotypic culture of the spleen and calculation of the energy of their interaction with DNA sequences using the molecular docking technique.

MATERIALS AND METHODS

Three-month-old male Wistar rats were guillotined, the spleen was placed in sterile Petri dish and cut into explants (1-mm^3 fragments); 850 explants were examined. The explants were transferred into a Petri dish

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with collagen coating (3.5×2.5 mm, Jet Biofil; 10-12 explants per dish) and cultured in 3 ml nutrient medium consisting of Hanks saline (45%), Eagle medium (45%), fetal calf serum (10%), glucose (10 mg/ml), and gentamicin (0.5 mg/ml). The cultures were divided into 5 groups: without additives (control; group 1) and with addition of glutamic acid (group 2), lysine (group 3), mixture glutamic acid and lysine in a concentration of 0.05 ng/ml (group 4), and peptide Lys-Glu (group 5). Each bioactive substance was added in concentrations of 0.01, 0.05, 1.0, 10, 20, and 100 ng/ml. The explants were cultured for 3 days in a CO_2-incubator (5% CO_2) at 36.7°C. This duration of culturing corresponds to the formation of the growth zone consisting of proliferating and migrating lymphoblasts, lymphocytes, and fibroblasts [5,11].

The growth of spleen explants in culture was analyzed by vital light microscopy. In 3 days of culturing, two zones, central and peripheral, were clearly seen. The central zone was presented by cells initially placed on the collagen substrate. The peripheral zone (growth zone) consisted of outgrowing regenerating cells. For quantitative evaluation of the explant growth, the area index was calculated as the ratio (%) of explant to the initial area (central zone). The explants were photographed using a video camera for a MTH-13 microscope (Alpha-Telekom, series 10).

The data were processed statistically using Statistica 7.0 software. Intergroup differences were analyzed using nonparametric Mann–Whitney *U* test. The differences were significant at $p<0.05$.

Molecular model of the complex of DNA with amino acids lysine (Lys), glutamic acid (Glu), and peptide Lys-Glu was calculated using Molecular Operating Environment 2012 (MOE 2012.10) [13] for left-handed amino acids and peptides. B-form DNA duplexes 5'AAAAA3', 5'CCCCC3', 5'ATATA3', 5'CGCGC3', 5'ATGCA3', 'GCAGC', 'TGTGT' served as double-strand DNA.

The constructed molecules were protonated at pH 7.0, T=310 K, and 0.15 M NaCl, and then optimization of their geometry in water in Amber12EHT field was performed [1]. The influence of aqueous medium was estimated in generalized Born approximation using internal and external dielectric constants equal to 1 and 80, respectively. For calculation of DNA complexes with the amino acids and peptide we used the method of molecular docking, *i.e.*, computer modeling of the interaction between the ligand (peptide or amino acid) and the active site of the receptor (DNA). The method consisted in ligand substitution in different conformations into the binding site and calculation of the optimum geometry of the ligand and energy of its interaction with the presumptive site (kcal/mol). The contribution of the solvent and entropy into the system was estimated.

The ligand was considered as labile and DNA and rigid structures. When calculating the optimum pose of the ligand in the double-stranded DNA, the contact area, number of hydrogen bonds, and parameters of hydrophobic and electrostatic interactions were taken into account. Docking solutions were ranked by the values of estimator function ΔG calculated by the formula:

$$\Delta G \approx c+\alpha[2/3(\Delta E_{coul}+\Delta E_{sol})+\Delta E_{vdw}+\beta\Delta SA_{weighted}],$$

where c is the loss of rotational and translational entropy of the complex; α and β are experimentally determined constants that depend on force field; E_{coul} is Coulomb energy calculated for system charge at dielectric constant 1; E_{sol} is electrostatic energy of the solvent; E_{vdw} is van der Waals contribution into interaction energy; $SA_{weighted}$ is the contribution of molecular sheaths into the energy. The lowest ΔG characterizes

TABLE 1. Effects of Lysine (Lys), Glutamic Acid (Glu), Mixture of Amino Acids (Lys+Glu) and Dipeptide Lys-Glu on the Area Index of Splenic Explant Growth Zone (*M*±*m*)

Concentration, ng/ml	Area index, %			
	Lys	Glu	Lys+Glu	Lys-Glu
0.01	5.4±0.2	9.4±0.1	0.7±0.1	9.4±0.3
0.05	28.0±1.4*	25.0±1.2*	-18.0±1.3*	32.0±1.6*+
1	20.0±2.6*	17.8±1.9*	-15.5±2.0*	31.0±1.1*+
10	12.1±1.3	14.0±1.6	-9.5±0.7	27.5±1.0*+
20	3.0±0.4	7.0±0.9	-11.00±0.47	11.00±0.48
100.00	2.3±0.3	4.6±0.1	5.3±0.2	5.3±0.3

Note. $p<0.05$ in comparison *with the control (100%) and +with Lys, Glu, and Lys+Glu.

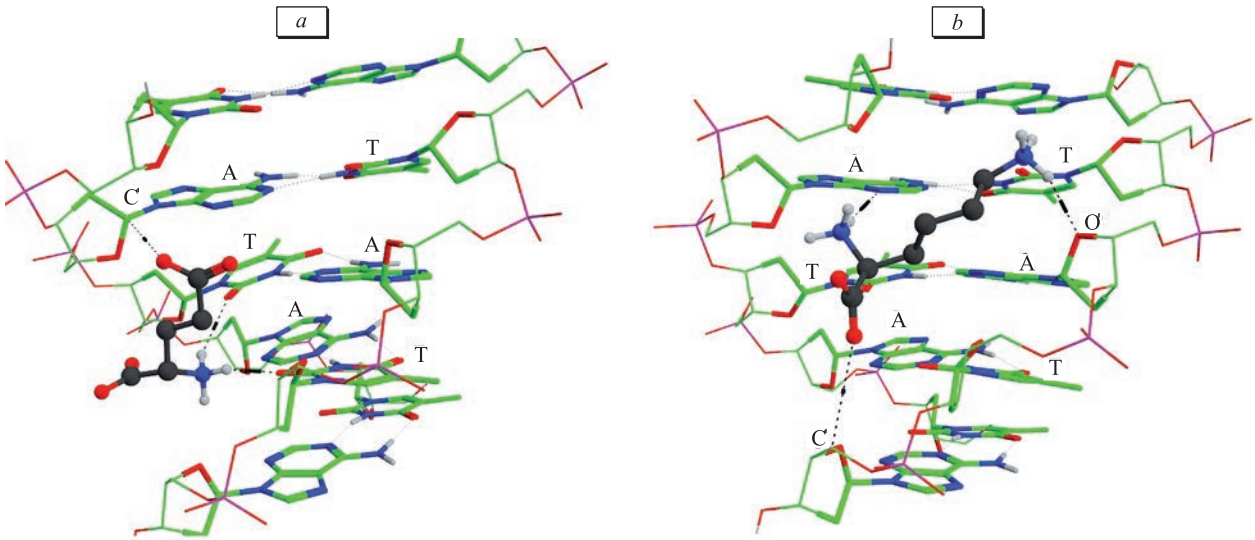

Fig. 1. Model of interaction of glutamic acid (*a*) and lysine (*b*) with DNA sequence 5'ATATA3'. Here and in Fig. 2: peptide molecules are shown with *ball and sticks* and DNA molecule with *tubes*. Hydrogen bonds are shown with dotted line.

the most effective conformation of the ligand in the binding site. The lower is ΔG, the higher is the energy of ligand–DNA interaction.

RESULTS

The growth zone in organotypic culture of the spleen considerably increased in the presence of lysine and glutamic acid in concentrations of 0.05 and 1.00 ng/ml and did not differ from the control in the presence of other concentrations (Table 1). However, addition of the mixture of lysine and glutamic acid in concentrations of 0.05 and 1.0 ng/ml to the culture produced an opposite effect: the amino acids significantly reduced the area of the growth zone in comparison with the control. After addition of the mixture of amino acids in concentrations of 10 and 20 ng/ml, the area index of the growth zone tended to decrease, but this effect did not attain statistical significance (Table 1). The peptide Lys-Glu in concentrations of 0.05, 1.00, and 10 ng/ml significantly increased the explant growth area index (Table 1). Thus, the minimum effective concentration of the peptide, amino acids, and their mixture was 0.05 ng/ml.

The results of docking analysis confirm these experimental data. We calculated the interaction energy for lysine and glutamic acid and peptide Lys-Glu with various short DNA sequences consisting of 5 nucleotides (Table 2). It was found that the energy of DNA interaction with Lys-Glu for all sequences except 5'CGCGC3' was higher than with individual amino acids. In all cases, the peptide and amino acids bind to DNA along the minor groove. The positions of lysine and glutamic acids in DNA minor groove of 5'ATATA3' calculated by docking analysis are presented in Figure 1. Hydrophobic, van der Waals, electrostatic, conformational, and donor-acceptor interactions contribute to the interaction energy ΔG (Table 2). Glutamic acid forms a network due to three hydrogen bonds between glutamic acid amino group and thymine oxygen atoms and between the oxygen in the glutamic acid side chain and adenine deoxyribose carbon atom (Fig. 1, *b*). Lysine forms hydrogen bonds with DNA nitrogenous bases: between lysine amino group in the main chain and adenine N3, between lysine amino group in the side chain and oxygen atom of adenine deoxyribose, and between glutamic acid car-

TABLE 2. Interaction of Amino Acids and Peptide with DNA Sequences

Ligand	DNA sequence, ΔG kcal/mol						
	AAAAA	CCCCC	ATATA	CGCGC	ATGCA	GCAGC	TGTGT
Glu	-3.85	-4.21	-4.57	-3.96	-4.27	-4.30	-4.50
Lys	-4.10	-4.55	-4.87	-5.10	-5.22	-5.03	-5.12
Lys-Glu	-4.86	-4.98	-7.03	-4.96	-6.14	-5.72	-6.01

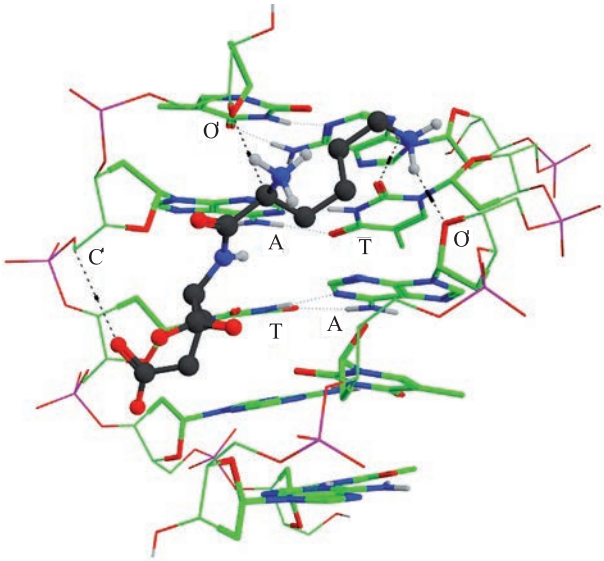

Fig. 2. Model of Lys-Glu interaction with 5'ATATA3' sequence.

boxyl group and carbon atom of the sugar-phosphate backbone of adenine.

Model of Lys-Glu interaction with 5'ATATA3' sequence is shown in Figure 2. The peptide interacts with the same atoms as individual amino acids. Its location also coincided with the location of individual amino acids in the minor groove. The energy of peptide interaction with 5'ATATA3' sequence 1.55- and 1.44-fold surpassed the energy of its interaction with individual amino acids, which explains the more pronounced stimulating effect of the peptide on spleen cell cultures (Table 1). It question remains unanswered why the mixture of lysine and glutamic acid produces a negative effect and inhibits cell proliferation. The total energy of interaction of these amino acids was on average -9.05 kcal/mol. Most likely, simultaneous addition of lysine and glutamic acid to the culture of splenic cells reduces gene expression due to high total energy of interaction. It is known that the concentration dependence in living systems is always described by a bell-shaped curve, *i.e.* after attaining an effective concentration (corresponding to the maximum effect), further increase in substance concentration leads to a decrease the system response to super stimulation [11,14]. Simultaneous addition of two amino acids to the culture medium was a super strong stimulus for cell proliferation and led to inhibition of expression of proliferation genes. Peptide Lys-Glu forms the most energetically effective complex with sequence 5'ATATA3' that repeats TATA box in the promoter region. Binding to this region can trigger expression of proteins involved in cell proliferation, which manifests in enlargement of explant growth zone.

Thus, DNA can be a target of lysine, glutamic acid, and Lys-Glu peptide. The interaction with DNA can lead to activation of the expression of genes responsible for cell proliferation, which explains the increase in splenic explant growth zone. The calculated models of interaction between the peptide and amino acid with various DNA sequences and the revealed correlation between the interaction energy and biological effect of the amino acids and peptide suggests that peptide bond potentiates binding of Lys-Glu peptide to DNA resulting in a greater biological effect on cell proliferation in organotypic culture of the spleen.

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