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IN VITRO INTERACTION OF THE AEDL PEPTIDE WITH DNA

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In the cell culture experiments, the AEDL peptide proved to be an efficient agent stimulating the cell renewal processes and the enhancement of the functional activity of bronchial epithelial cells. A presumed target of the peptide action is a DNA molecule. The work studies the peptide binding with high-molecular DNA in solutions with different ionic strengths. The spectral (UV spectophotometery and circular dichroism) and hydrodynamic (viscosimetry) methods show that, under the experimental conditions, the AEDL peptide forms a complex with DNA and that nitrogen bases are involved in the binding. The character of spectral changes in DNA suggests a possible interaction of the AEDL peptide with DNA in the major furrow at the guanine N7 site without a visible distortion of the double helix structure.

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INTRODUCTION

Short peptides consisting of no more than 20 amino acid residues with a molecular weight of up to 3.5 kDa play an important role in various physiological processes. Specifically, they are signal molecules involved in the regulation of homeostasis at various levels of living matter organization. The main physiological mechanism for the formation of short peptides is the hydrolysis of high-molecular proteins.

At the St. Petersburg Institute of Bioregulation and Gerontology (SPIBG), natural polypeptide extracts derived from cattle organs were obtained. Their short analogues possessing a pronounced tissue-specific activity were also synthesized [1]. The regulatory activity of some natural and synthetic peptides has been studied on cell cultures and in the experimental models on animals of different age and in clinical trials on humans. It was found that peptides stimulated the gene expression and protein synthesis in the cells of those organs from which they were derived [2].

The AEDL peptide (Fig. 1) regulating the bronchopulmonary system functions was synthesized at SPIBG by the classical peptide synthesis in solution [3].

The biological activity of AEDL was established in the analysis of the course of acute bacterial pneumonia, in the chronic fibrotic inflammatory process, and the sublethal hyperoxic lung injury in animals [3]. All these pathologies are

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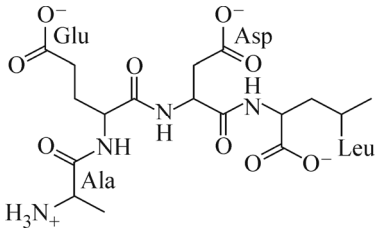

Fig. 1. Structural formula of the AEDL peptide.

characterized by substantial changes in the lung morphology and cellular structure of the bronchoalveolar fluid. It was found that the AEDL peptide promoted the normalization of the cell structure of bronchial tissues in various pathological processes [4]. A peroral intake of a dietary supplement, whose active ingredient is the AEDL peptide, proved to be efficient and safe in complex treatment of patients with chronic bronchitis; thus, it can be recommended for patients with lung and bronchial dysfunctions in chronic respiratory diseases of different origins, as well as to maintain the respiratory functions of the older-age group persons.

The molecular mechanism of the biological peptide activity is related to its ability to epigenetically regulate the synthesis of a wide spectrum of proteins in human bronchial epithelium [5, 6]. The AEDL peptide activates the cell renewal processes in bronchial epithelium by stimulating the synthesis of Ki67, Mcl-1, p53 proteins and enhances the functional state of the cells by increasing the expression of CD79 and NOS-3 molecules. The tetrapeptide activates the expression of the Nkx2.1, SCGB1A1, SCGB3A2, FoxA1, FoxA2 genes of human bronchial epithelial cell differentiation and the MUC4, MUC5AC, SftpA1 genes, whose decreased expression correlates with the development of chronic bronchitis [6]. It was found that, on ageing and under the action of the AEDL peptide, the methylation profile of the promoter regions of the NKX2-1 and SCGB1A1 genes in the bronchial epithelial cells changes, which correlates with changes in the expression level of these genes.

The study of the molecular mechanisms of the AEDL peptide action and its efficiency with respect to treatment of bronchopulmonary pathology allows us to conclude that it epigenetically regulates the expression of genes and the synthesis of proteins involved in the differentiation and maintenance of the functional activity of bronchial epithelial cells. A presumed target of the peptide action *in vivo* is a DNA molecule [7]. This work was aimed to examine the possibilities of the AEDL peptide binding with a DNA molecule in solution using the methods allowing to monitor the state of the secondary and tertiary structures of the macromolecule.

EXPERIMENTAL

In the work, we used a commercial DNA sample of calf thymus (Sigma) with a molecular weight of $8.8 \cdot 10^6$ Da determined from the intrinsic viscosity in 0.15 M NaCl. The AEDL peptide was synthesized at SPIBG. The DNA sample was dissolved in distilled water for 5 days at a temperature of 4°C; then we mixed it with a NaCl solution of a certain concentration until the specified ionic strength was reached, filtered, and determined the DNA concentration from the difference in the absorption of its hydrolyzed solutions (15 min at a temperature of 100°C in 6% $HClO_4$). The peptide was dissolved in a 5 mM NaCl solution. The initial concentration was about 10 mM. The salt concentration in the DNA solutions with the peptide was varied (0.005 M, 0.15 M, and 1 M NaCl). In the research, the DNA solutions with the peptide prepared by pouring equal volumes of the solutions of the interaction components were used. The DNA (or peptide) concentration was maintained constant when the concentration of the second component changed. To characterize the solutions, we used the ratio between the molar peptide concentration and that of the DNA base pair *r*, showing the number of peptide molecules per base pair in the solution.

The absorption spectra of the solutions were recorded on a SF-56 spectophotometer (Russia). The circular dichroism spectra of DNA and its complexes were measured on a Mark IV autodichrograph (France).

Melting of DNA and its complexes with peptides was examined on a Shimadzu UV-1700 spectophotometer (Japan) with thermostated cuvettes; the solutions were heated with a rate of 2°/min. In all systems the DNA concentration was 0.001% (0.015 mM (base pair)) and the AEDL peptide amount corresponded to the ratio of two peptide molecules to one DNA base pair. In the result, we got the melting curves of the reference solutions of DNA and DNA in the presence of the AEDL peptide, from which the melting point and the hypochromic effect were calculated.

To examine the effect of the AEDL peptide on the DNA macromolecule tertiary structure, viscosimetry was applied. To determine the viscosity of the solutions, a modified low-gradient Zimm–Crozers type viscometer was used. We determined the relative viscosity of the solutions η_r, which was used to calculate the reduced viscosity $(\eta_r - 1)/C$, where C is the DNA concentration.

RESULTS AND DISCUSSION

The absorption spectra of DNA, the AEDL peptide, and their complex at the 5 mM NaCl concentration are presented in Fig. 2. The spectra were measured on the day when the systems were prepared.

The analysis of the data evidenced that the AEDL peptide interaction with DNA in a 5 mM NaCl solution manifested itself immediately after the solutions were prepared and retained seven days later on keeping the solutions at 4°C. Since the peptide does not absorb in the wavelength region above 240 nm, we can follow a change in the absorption spectrum of DNA in the complex in this frequency range. The experiment showed that the peptide binding with DNA led to a hypochromic effect in the wavelength region below 272 nm and at greater wavelengths a shoulder appeared. These spectral changes can be due to the attachment of ligands to the guanine N7 group in the DNA major furrow [8]. The spectral analyses also showed that in 0.15 M and 1 M NaCl solutions the interaction is weak.

Fig. 3 depicts the spectra of the complexes at different peptide concentrations (C_{DNA} = 0.005%). With an increase in r, the hypochromic effect gradually increases and at $r > 10$ the studied type of binding reaches saturation, i.e., all possible binding sites of this type on the DNA are occupied. The circular dichroism spectra also show (Fig. 4) that the AEDL peptide has a specific effect on the DNA secondary structure in 5 mM NaCl.

Therewith, the shape of the circular dichroism spectra suggests a considerable influence of the peptide concentration in the DNA solution on the binding process. It should be noted that in 0.15 M and 1 M NaCl no visible changes in the circular dichroism spectrum of DNA were observed.

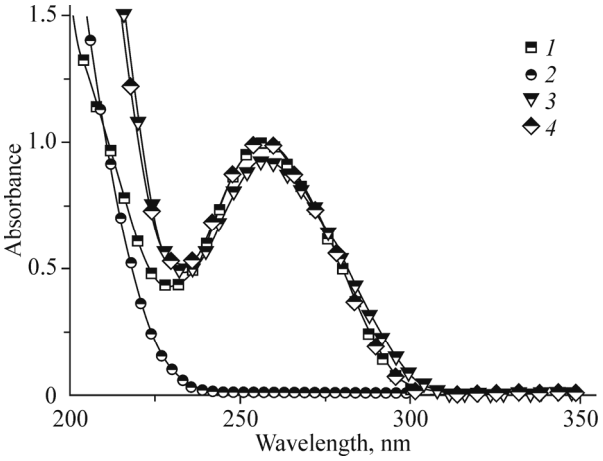

Fig. 2. UV absorption spectra of DNA (*1*), the peptide (*2*), their complex at $r = 2$ (*3*) in the 5 mM NaCl solution and the calculated total absorption spectrum of the components (*4*).

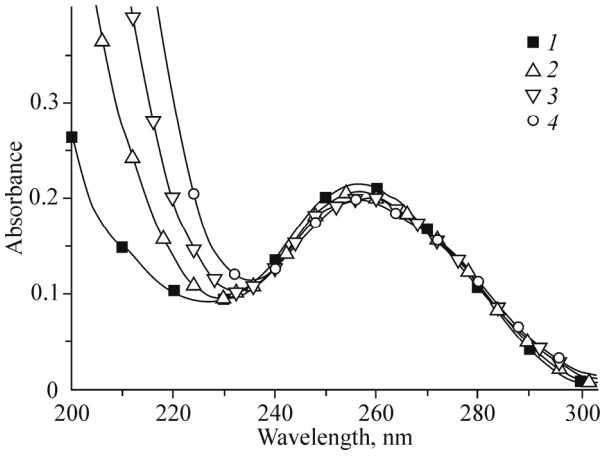

Fig. 3. Absorption spectra of the DNA complexes with the AEDL peptide in the 5 mM NaCl solution at the DNA concentration of 0.001% (*1*) and different peptide concentrations ($r = 2$ (*2*), $r = 5$ (*3*), $r = 10$ (*4*)).

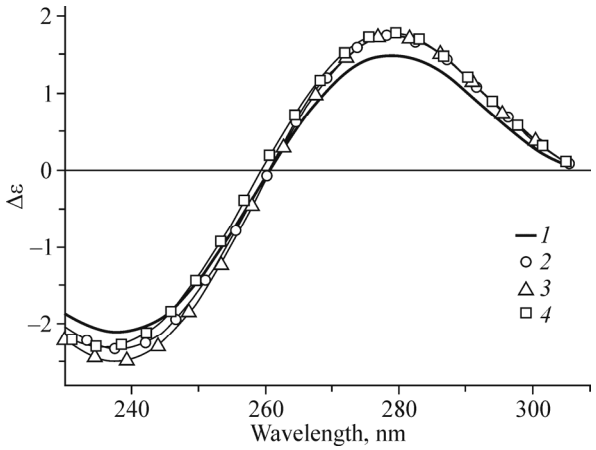

Fig. 4. Circular dichroism spectra of DNA and its complexes with the AEDL peptide in the 5 mM NaCl solution at the DNA concentration of 0.001% (*1*) and different peptide concentrations ($r = 1$ (*2*), $r = 5$ (*3*), $r = 15$ (*4*)), pH was fixed by a *tris*-acetate buffer.

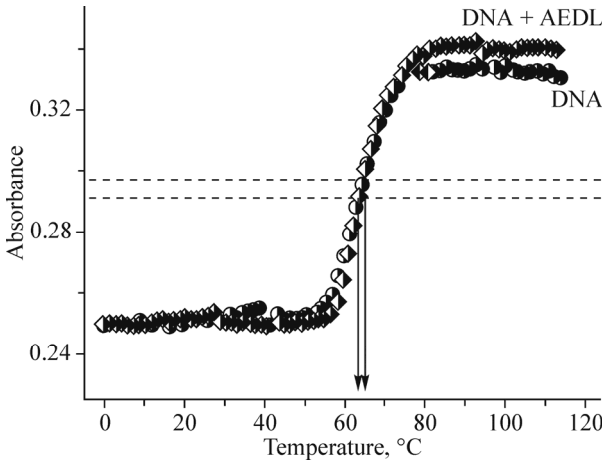

Fig. 5. Melting curves of the reference native thymic DNA and DNA in the presence of the AEDL peptide in the H_2O solution with an ionic strength of 5 mM NaCl.

To study the AEDL peptide effect on the stability of a DNA molecule, the DNA complex with the AEDL peptide was molten (Fig. 5). The DNA melting point was 65°C and in the presence of the peptide it was 62°C. Thus, under the AEDL peptide action, the DNA melting point does not change as compared to the reference. This also agrees with the spectral data discussed above.

To examine the AEDL tetrapeptide effect on the tertiary structure of the DNA molecule, low-gradient viscometry was used.

As evident from Fig. 6, in the AEDL peptide interaction with a DNA molecule the viscosity changes insignificantly. We cannot state that the macromolecule volume changes in the interaction, because these changes are within the experimental error. This result does not mean the absence of the AEDL peptide binding with DNA because other methods show the interaction. The experimental data also indicate the absence of destabilization of the DNA secondary structure in the interaction with the peptide.

Therefore, spectral changes together with the absence of a notable decrease in the macromolecule volume suggests that DNA nitrogenous bases are involved in binding with the peptide, but this binding does not destabilize the secondary

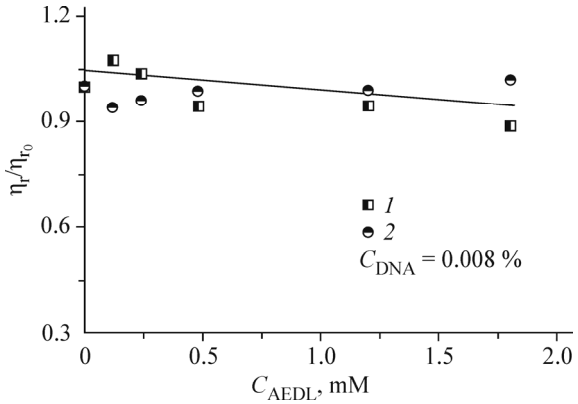

Fig. 6. Relative change in the reduced viscosity of the DNA solution in the presence of the AEDL peptide at different concentrations. The measurements were performed at the ionic strengths of 5 mM (*1*) and 0.15 M (*2*).

structure of the macromolecule and does not cause a decrease in the macromolecule rigidity. We think that the peptide is located in the DNA major furrow.

Our data allow us to conclude that the AEDL peptide interacts with a DNA macromolecule in solution and an increase in the low-molecular salt concentration hinders the interaction between the components. The collection of experimental results indicates that the peptide bonds with the DNA nitrogenous bases causing characteristic changes in its UV absorption spectrum. The presumed binding site of tetrapeptides with DNA is the guanine N7 atom in the major furrow. Therewith, the peptide binding does not destabilize the secondary structure of the macromolecule, as it follows from the analysis of the melting curves of DNA in the complex with the peptide. The peptide binding also does not have a significant effect on the tertiary structure of DNA in solution.

At this stage of the study, the obtained results experimentally confirm the possibility of the *in vivo* interaction between the AEDL peptide and DNA. However, the issue of determining nucleotide sequences (binding sites) of the peptide with DNA is still open. Our study also outlines a concentration range of the interaction components and opens the ways for further investigations of the molecular mechanisms of the *in vitro* interaction between the tetrapeptide and DNA using other methods.

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