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# GERONTOLOGY

# Molecular Mechanism of Interaction between Oligopeptides and Double-Stranded DNA

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Formation of Ala-Glu-Asp-Gly peptide complex with duplex DNA [poly(dA-dT):poly(dA-dT)] at neutral pH and ambient temperature was studied experimentally. Peptide binding to duplex DNA was associated with hyperchromic effect indicating local separation of the double helix. Energies of paired interactions between amino acid residues and nucleotide bases were compared with the energy of bond between two bases in the nucleotide pair (dA-dT). A new concept of interaction between two types of information-carrying molecules is put forward. This interaction underlies triggering of protein synthesis mechanism and can explain the emergence of life on the Earth.

**Key Words:** *peptide; double helix; complex formation; hyperchromatic effect; protein synthesis*

Chromatin activation in cells of elderly subjects triggered by selective binding of regulatory peptides and transcription factors to the gene promotor sites was studied in detail [3,4,8,9]. The main task of these studies was to establish the correspondence of DNA nucleotide sequence on the gene promotor site to the amino acid sequence of the regulatory peptide at the site of their interaction [12-15]. We previously developed a structural model of complementarity between a certain nucleotide sequence at the gene promotor site (recognition block) and amino acid sequence of the oligopeptide specifically bound to the recognition block. The specific feature of this model is that functional groups of the oligopeptide are complementarily bound in the large groove of DNA molecule to both strands simultaneously. For example, a model of complementary binding of Ala-Glu-Asp-Gly synthetic peptide (epithalon) to ATTTG nucleotide sequence within the leading chain of duplex DNA repeated many times at the promotor sites of telomerase and RNA polymerase II genes is presented [5,8,10].

The aim of this study was experimental validation of the fact of formation of the regulatory oligopeptide complex with duplex DNA, evaluate the concentration ratios of the components in this complex, and, based on published data on paired nucleotide-amino acid interactions, evaluate the energy parameters of selective interaction between tetrapeptide and double-stranded DNA in the studied system.

## MATERIALS AND METHODS

Synthetic nucleic acid preparations were used: [poly(dA-dT):poly(dA-dT)] (double stranded), poly(dT) and poly(dA) (single stranded) (Calbiochem), synthetic Ala-Glu-Asp-Gly peptide (epithalon), obtained at St. Petersburg Institute of Bioregulation and Gerontology, and ninhydrin (for detecting the presence of peptide).

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Complex formation was carried out in a saline at ambient temperature by adding tetrapeptide to the solution of double- or single- stranded DNA. The formation of the peptide-DNA complex was controlled by gel chromatography on Sephadex G-25 (Pharmacia; 1×35 cm column; solution flow rate 6 ml/h). The resultant high-molecular-weight fractions were lyophilized and analyzed by thin layer chromatography on Silufol plates (Kavaliere).

Conformation changes in DNA during binding to the peptide were studied by UV spectrometry. To this end, the peptide and DNA solutions were mixed so that the final concentrations of DNA in the solution were 20-25 μg/ml, those of the peptide 1.0-1.4 μg/ml. Complex formation was realized at ultralow (nanomolar) concentrations of the reacting components. The error in measurements of concentrations and values did not surpass 10%.

## RESULTS

Molecular weights of nucleic acid preparations were about 140 kD. Molecular weights of DNA and peptide differed by more than two orders of magnitude. Results of gel chromatography of duplex DNA, peptide, and their mixture are presented (Fig.

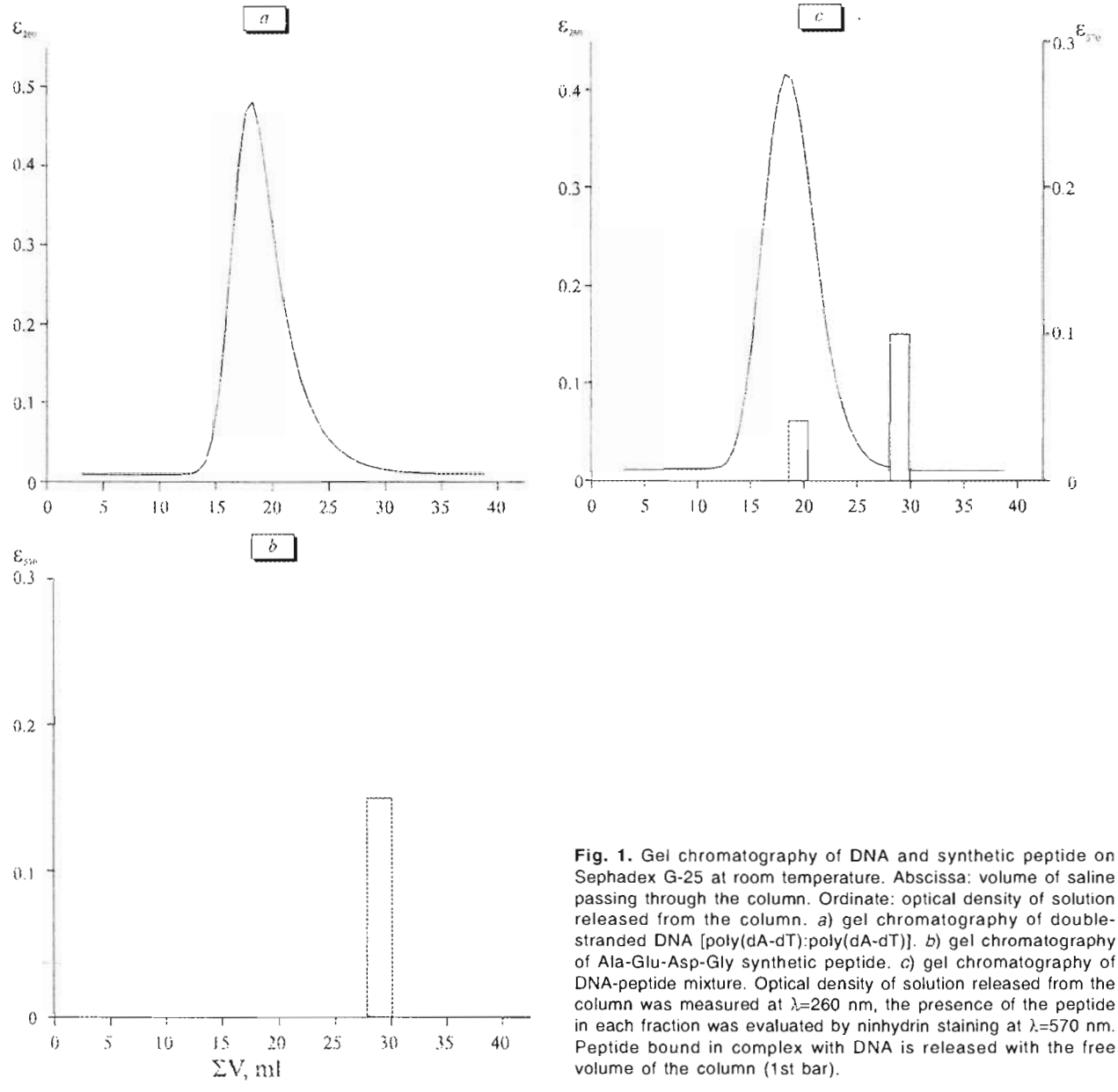


**Fig. 1.** Gel chromatography of DNA and synthetic peptide on Sephadex G-25 at room temperature. Abscissa: volume of saline passing through the column. Ordinate: optical density of solution released from the column. *a*) gel chromatography of double-stranded DNA [poly(dA-dT):poly(dA-dT)]. *b*) gel chromatography of Ala-Glu-Asp-Gly synthetic peptide. *c*) gel chromatography of DNA-peptide mixture. Optical density of solution released from the column was measured at λ=260 nm, the presence of the peptide in each fraction was evaluated by ninhydrin staining at λ=570 nm. Peptide bound in complex with DNA is released with the free volume of the column (1st bar).

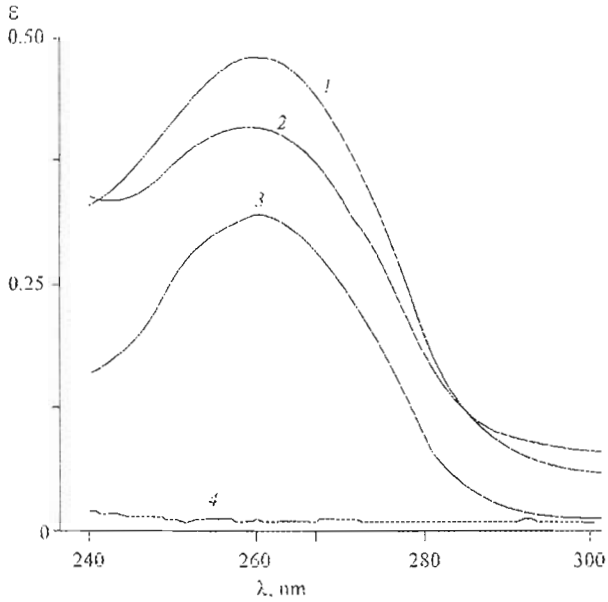


**Fig. 2.** UV spectra of DNA mixtures with peptide at different ratio of the numbers of nucleotide pairs and peptide molecules (*1* — 15:1; *2* — 9:1), solutions of double-stranded DNA [poly(dA-dT):poly(dA-dT)] (*3*) and Ala-Glu-Asp-Gly peptide (*4*).

1). DNA was released from the column with the free volume $V_0$=19 ml, peptide with retention volume 1.5 $V_0$=29 ml. The fact that gel chromatography of the peptide-double stranded [poly(dA-dT):poly(dA-dT)] mixture partially detected the peptide in the DNA peak indicates the formation of the peptide-double stranded DNA complex. Excess of peptide not bound in the complex was released from the column with its retention volume (29 ml). By concentrating the peak, the equilibrium content of nucleotide pairs (n. p.) per peptide molecule in the complex was evaluated as 17 n. p.

UV spectra of solutions containing double stranded DNA and peptide revealed an increase in optical density of DNA at 260 nm ($\varepsilon_{260}$) as a result of regulatory peptide binding (hyperchromatic effect). The maximum absorption wavelength was not shifted. UV spectra of peptide-DNA complexes at different ratios of the components (Fig. 2, *1*, *2*) and free DNA double helix (Fig. 2, *3*) are presented. The regulatory peptide (Fig. 2, *4*) exhibited no optical density in the studied wavelength range, and hence, the hyperchromatic effect can be attributed to local separation of DNA strands as a result of peptide binding. No hyperchromatic effect was observed when solutions of poly(dT) or poly(dA) (single stranded DNA) were mixed with the studied peptide.

The values of hyperchromatic effect are presented for double stranded DNA complex with the peptide at different ratios of molar concentrations of dA-dT nucleotide pairs and the peptide (Table 1).

Preparation poly[(dA-dT):poly(dA-dT)] is a synthetic analog of the known binding site for transcription factors (TATA box) at the promotor region of many genes [11,15]. We previously showed that due to its molecular size, Ala-Glu-Asp-Gly peptide in unfolded β-conformation can fit into the large groove of the DNA double helix along its axis [10], but not perpendicularly to it, as is proposed by models of binding of high molecular weight transcription factors [11,14]. The peptide has three carboxyl groups, terminal amino group, and lateral methyl group. The double-stranded [poly(dA-dT):poly(dA-dT)] exposes groups of nucleic bases capable of reacting with the peptide groups on the surface of the large groove. Adenine exposes two proton acceptor groups: amino group at $^6$C carbon atom and $^7$N nitrogen atom, while thymine exposes only methyl group. In a previously proposed structural model the proton donor carboxyl groups of the peptide can establish hydrogen bonds with proton acceptor groups of three adenines belonging to different chains of the double helix [5,9]. Hence, the studied tetrapeptide can form cross-links between six nucleotide pairs with the TATATA sequence in the leading DNA chain by extra hydrogen and one hydrophobic bonds.

Hyperchromatic effect (up to 30%) indicates partial destruction of hydrogen bonds between dA:dT nucleotide pair bases and local separation of double-stranded DNA. Initiation of gene transcription includes the stage of destruction of hydrogen bonds between nucleotide pairs and local separation of

**TABLE 1.** Hyperchromatic Effect in Ala-Glu-Asp-Gly Peptide Binding to [poly(dA-dT):poly(dA-dT)] Double Helix

| DNA concentration, n. p. | | Peptide concentration | | Ratio of components n. p./peptide, mol/mol | $\varepsilon_{260}$ | $\Delta\varepsilon_{260}$ | Hyperchromatic effect, % |
|---|---|---|---|---|---|---|---|
| μg/ml | nmol/ml | μg/ml | nmol/ml | | | | |
| 20 | 30.8 | 0 | 0 | — | 0.32 | — | — |
| 20 | 30.8 | 1.33 | 3.4 | 9.0 | 0.41 | 0.09 | 30 |
| 25 | 38.5 | 0 | 0 | — | 0.40 | — | — |
| 25 | 38.5 | 1.0 | 2.6 | 14.8 | 0.48 | 0.08 | 20 |

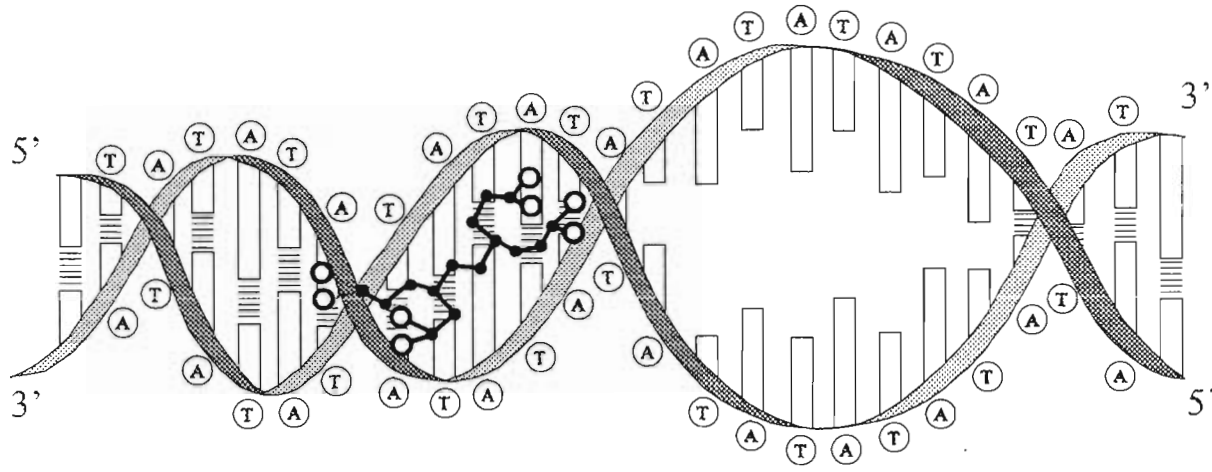


**Fig. 3.** Scheme of local separation of [poly(dA-dT):poly(dA-dT)] chains as a result of Ala-Glu-Asp-Gly regulatory peptide binding in the large groove of double-stranded DNA. ≡: hydrogen bonds between adenine and thymine (Watson—Crick model).

two DNA chains in the direction of the gene first exon [11]. A scheme of binding of regulatory peptidea in the large groove of the double helix is presented, as a result of which local and reversible separation of the double stranded DNA takes place (Fig. 3). It seems that such interactions can promote chromatin activation [4].

Spatial position of functional groups of the double-stranded DNA nucleic bases and Ala-Glu-Asp-Gly peptide permits these molecules participate in the multi-point complementary interaction [10]. The integral energy of this interaction can be evaluated using the data on enthalpy of paired interactions of amino acids and nucleotides participating in binding of Ala-Glu-Asp-Gly peptide and [poly (dA-dT):poly(dA-dT)] double helix (Table 2).

The total energy of binding of one pair of (dA:dT) bases in the Watson-Crick DNA double helix is 23.6 kJ/mol [2]. All rotation angles of double-stranded DNA are strictly interrelated and hence, conformational changes in DNA are regulated by a cooperative mechanism: cleavage of hydrogen bonds in one nucleotide pair facilitates this process in the neighboring pair. It seems that binding of the peptide to double helix is realized as an exothermal reaction, whose summary energy is sufficient for cleavage of hydrogen bonds between the bases of several neighboring nucleotide pairs. The resultant hyperchromatic effect indicates local separation of the double-stranded DNA.

According to the Jacob and Monod model [7], low-molecular-weight ligands, such as lactose and tryptophane, provide conformational changes in the repressors and thus cancel the block of gene transcription in the prokaryote cells. Presumably, the regulatory peptides in eucaryotic cells competitively substitute for components blocking gene transcription at the promotor sites. Local separation of double-stranded DNA promotes initiation of gene transcription by RNA polymerase II. Amino acid sequences of regulatory peptides are as a rule included in high-molecular-weight proteins and transcription factors as repeating blocks [6]. Ala-Glu-Asp-Gly amino acid sequence was detected 54 times in the Protein Sequence Database in cytostatin, prothymosine, parathymosine, troponins C and T, thyroglobulin, nerve cell adhesion factor, glyceraldehyde-3-phosphate dehydrogenase, and calmodulin binding proteins GAP-43 and P-57. It seems that endogenous regulation of protein synthesis suggests partial specific hydrolysis of high-molecular-weight precursor proteins with the release of regulatory peptides participating in initiation of gene transcription [6].

Hence, from the analysis of experimental results and our model of complementary interactions between peptides and DNA [5,9,10] we propose a new concept, according to which short peptides play the key role in the regulation of gene expres-

**TABLE 2.** Enthalpy Coefficients of Paired Interactions between Nucleotides and Amino Acids in Ala-Glu-Asp-Gly Peptide, kJ/mol[2]*

| Amino acid | Adenine | Thymine |
|---|---|---|
| Ala | -1.9 | -4.3 |
| Glu | -69.1 | -85.1 |
| Asp | -83.5 | -43.0 |
| Gly | -1.1 | -8.2 |
| Sum for tetrapeptide | -155.6 | -140.6 |

**Note.** *According to [1].

sion and hence, protein synthesis in the cells of different organisms. This mechanism of interaction between two types of information molecules underlies the emergence of protein synthesis, which, in the presence of favorable factors (temperature, water, gaseous composition, free amino acids, solar energy pulse, *etc.*) could lead to emergence of life on the Earth.

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