BIOGERONTOLOGY

Melting of DNA Double Strand after Binding to Geroprotective Tetrapeptide

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Experimental relationship between the hyperchromic effect of DNA [poly(dA-dT):poly(dA-dT)] interacting with Ala-Glu-Asp-Gly peptide is presented by a saturation isotherm. The free DNA double strand is melting (the strands separate) at 69.5°C and at higher energy expenditures (enthalpy increase by 976.4 kJ/mol b.p.) in comparison with melting of the DNA-peptide complex (28°C and 444.6 kJ/mol b.p.). The detected regularities of melting of duplex DNA and the thermodynamic parameters of this process indicate the natural mechanism of interaction between DNA and regulatory peptides underlying functioning of the living matter.

Key Words: *DNA; tetrapeptide; complex formation; double strand melting*

The Ala-Glu-Asp-Gly tetrapeptide (TP) is characterized by remarkable geroprotective activity; due to this TP, cultured somatic cell surpass the Hayflick limit [1,2]. Physiological activity of TP is based on its involvement in transcription activation via interaction with duplex DNA on the gene promotor site [3].

Gel chromatography showed that TP formed a stable complex with high-molecular-weight synthetic duplex DNA $(TATATA)_n$. This was associated with a hyperchromic effect in the UV band (increase in optical density at 260 nm), which attests to separation of the DNA double strand [4]. In other words, the regulatory oligopeptide can bind to the complementary site on the gene promotor site, causing local separation of strands and thus initiating the process of gene transcription by RNA polymerase. This was proven by initiation of telomerase gene by the peptide in somatic cells [5].

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The phenomenon of strand separation is also observed during thermal denaturation of duplex DNA at high temperature [8]. Recently this phenomenon was used in studies of selective interactions between base pairs (b.p.) in short (10-15 b.p.) double strands [9]. In addition, the DNA melting curve is used for evaluating the energy strength of bonds in DNA complex with nuclear proteins of high mobility group enriched with glutamic and asparaginic amino acid residues [7,12].

We studied the relationship between the hyperchromic effect and TP concentration. Melting of DNA-TP or DNA-glutamic acid (GA) complexes in comparison with melting of free duplex DNA was studied at the peptide concentration providing the threshold saturation of DNA by the peptide.

MATERIALS AND METHODS

The following preparations were used in the study: synthetic nucleic acid (poly(dA-dT):poly(dA-dT); Sigma; later called DNA here) with an optical den-

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sity of 20 opt. density units/mg at 260 nm (double strand); TP synthesized at St. Petersburg Institute of Bioregulation and Gerontology, containing no low molecular weight electrolytes; GA (Serva).

After swelling in distilled water at 6°C, DNA preparation was dissolved in 0.1 n NaCl to a concentration of 18-20 μg/ml; pH of the resultant solution was 7.1. Synthetic peptide without salts was dissolved in 0.1 n NaCl and added (10, 20, or 50 μl) to 2 ml DNA solution.

UV bands of DNA and peptide solutions and their mixtures were measured at peptide concentrations of $(1\text{-}70)\times10^{-6}$ M on a Specord M40 spectrophotometer at 25°C. Since the molecular weight of DNA preparation ($S_{20,w}$=12) was at least by 2 orders of magnitude higher than the molecular weight of the peptide, the concentration of DNA was expressed in b. p. moles in solution, which constituted $(28.5\text{-}32)\times10^{-6}$ M.

Conformation transition of DNA and its complexes was studied using a Shimadzu UV-1700 spectrophotometer with cuvettes warmed in a thermostat; the solutions were warmed at a rate of 2°C/min. The concentration of DNA in all systems was 5.3 μg/ml, the content of GA and TP corresponding to the proportion: 6 amino acid or peptide molecules per 10 b.p.

Experimental curves and calculations were processed by the routine method with consideration for basal curves. Thermodynamic functions of the double strand separation process were calculated routinely by the shape of melting curves (melting temperature at the peak half-height and the slope of the curve to the temperature axis) [6,11].

RESULTS

The concentration dependence of the hyperchromic effect on the number of peptide molecules added to 10 b.p. DNA was presented by a saturation isotherm (Fig. 1). The initial segment of the isotherm reflects selective binding of the peptide to DNA, while the maximum values of the derivative determine the range of concentrations (6 peptide molecules per 10 b.p.) for the strongest multi-point binding of DNA with the peptide. The interaction of 2 polymeric molecules is realized due to hydrophobic binding of alanine and thymine methyl groups and due to polar interactions of 3 carboxyl groups of the peptide with 3 adenines, namely, with proton-accepting ^{7}N atom and with proton-donating amino groups at ^{6}C. This binding causes local separation of the double strand at the adjacent sites of DNA. The energy needed for this process is compensated for by potent polar interactions of nucleic

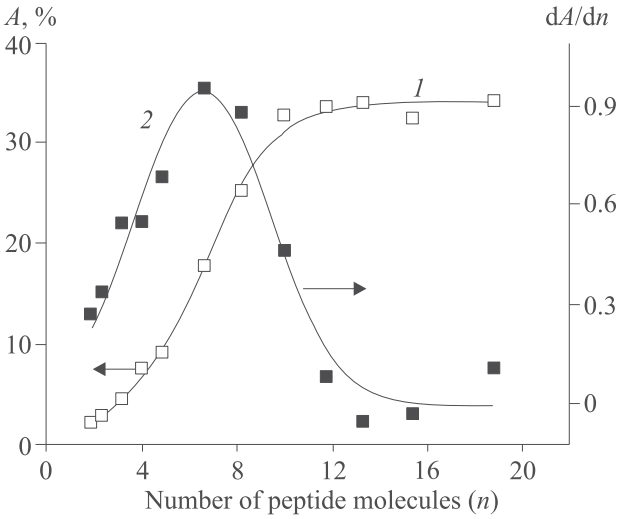

Fig. 1. Hyperchromic effect (*A*) depending on the number of peptide molecules (*n*) added per 10 b. p. of DNA (*1*) and the hyperchromic effect dA/d*n* derivative depending on the number of added peptide molecules (*2*).

bases with side groups of glutamic and asparaginic amino acids in the TP structure.

Separation of the free DNA double strand, also called melting of the strand or "helix-coil" conformation transition can also be induced by heating and is paralleled by a hyperchromic effect [10] (Fig. 2).

A very sharp conformation transition of free DNA is worthy of note. Presumably, it is determined by high cooperativity of the process, proportional to the DNA molecular weight [10].

The effect of heat capacity on enthalpy (enthalpic component of peptide-DNA interaction) was evaluated by published data on the molecular heat capacity of DNA for the studied temperature intervals ΔC_P=312.6 J/mol×K [6]. Increase in enthalpy

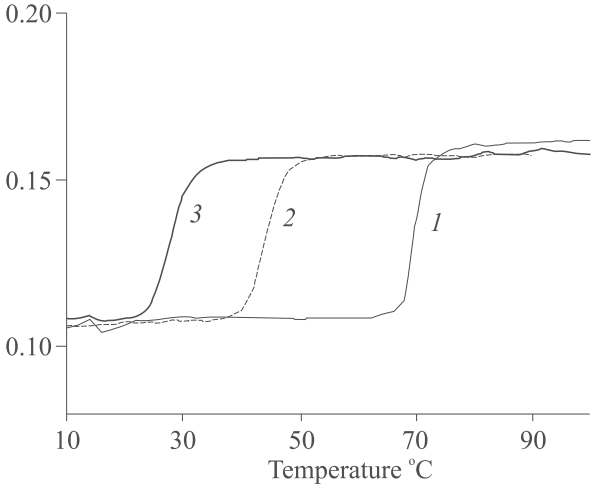

Fig. 2. Melting curves duplex DNA (poly(dA-dT):poly(dA-dT) (*1*) and its complexes with GA (*2*) and TP (*3*). Ordinate: optical density at 260 nm.

TABLE 1. Thermodynamic Functions of DNA Double Strand Separation

Object of study	Melting temperature, °C	Free energy (ΔG), kJ/mol b. p.	Want-Hoff's enthalpy, kJ/mol b. p.	Entropic constituent (TDS), kJ/mol b. p.
DNA	69.5	-36.0	976.4	1012
DNA+GA	44	-32.5	487.8	520.
DNA+TP	28	-30.9	444.6	475.5

at the expense of heat capacity was estimated as the product $\Delta C_P \times (T_m\text{-}25)$, where T_m is DNA melting temperature, which was less than 1.5% of estimated van't-Hoff enthalpy (Table 1).

High positive values of enthalpy and entropy constituent of melting of duplex DNA are determined not only by destruction of hydrogen bonds between the bases of two strands, but also by high value of stacking interactions between the base nucleotide pairs [13]. In systems where duplex DNA is bound to GA or TP the strand melting is characterized by lesser changes in free energy and almost 2-fold lower enthalpy and entropy values of the process. This indicates that separation of DNA strands at normal temperatures is not denaturing and points to a thermodynamically easier way of duplex restoration after separation due to GA or TP presence in the system.

A particularly important factor is detection of the lowest temperature of melting for DNA-TP complex, adequate to the thermal regimen of biochemical processes in the majority of living organisms. It is noteworthy that in order to analyze telomerase activity and evaluate the effect of TP on it, human pulmonary fibroblasts were incubated at 30°C for 30 min [2,5]. This led to a 2.5-fold increase in the mean length of telomeres and a 42.5% increase in the number of mitoses in comparison with the control were detected for the first time; this largely explains TP capacity to prolong the mean life span of animals [1,3].

Hence, the study of the regularities of duplex DNA melting and thermodynamic parameters of this process point to natural mechanism of interactions between DNA and regulatory peptides underlying the functioning of the living matter.

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