BIOGERONTOLOGY

Effect of the Peptide Bronchogen (Ala-Asp-Glu-Leu) on DNA Thermostability

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Thermodynamic parameters of DNA melting in the presence of a peptide bronchogen in various concentrations were estimated on a differential scanning microcalorimeter. Bronchogen was shown to serve as a DNA-stabilizing agent. Bronchogen increased the melting temperature of DNA from calf thymus and mouse liver by 3.1°C in a narrow range of *r* (molar ratio of bronchogen/DNA b.p., 0.01-0.055). A further increase in *r* was not accompanied by changes in the melting temperature. The complex melting enthalpy (ΔH_{melt}) remained unchanged in this range of *r* (0.01-1.0). ΔH_{melt} for DNA from the thymus and mouse liver was 11.4 and 12.7 cal/g, respectively. Our results indicate that bronchogen is not an adenine–thymine-specific or guanine–cytosine-specific ligand. The type of binding is considered as strong and occasional. The binding occurs with both strands of DNA (mainly with nitrogen bases).

Key Words: *microcalorimeter; thermostability; DNA; peptide*

Studying the interaction of low-molecular-weight bioactive compounds exhibiting sequence-specific binding with DNA sites is an important pharmacological problem. These investigations involve a variety of physical, chemical, and biological methods [1,8].

Biological activity and mechanisms of binding of these ligands with DNA can be evaluated by studying the thermodynamic parameters for melting of complexes that consist of the test compounds and DNA (in solutions) [3,6].

New water-soluble low-molecular-weight peptide compounds were recently synthesized. They are extensively used in genomics and in medical practice [1,2,4]. A new peptide bronchogen (Ala-Asp-Glu-Leu) stimulates proliferation ad functional activity of major cell structures in tissue culture. This agent produces a trophic and stabilizing effect on morphological characteristics and regeneration of the differentiated tissue. A clinical study showed that bronchogen is efficient in combination therapy of patients with respiratory diseases. Moreover, bronchogen is successively used for prevention of bronchial disorders in elderly and old people.

Much success was achieved in bronchogen therapy of patients. For many medicinal agents, the inclusion complexes of product-DNA were shown to differ *in vitro* and *in vivo*. Therefore, it is important to study *in vitro* interaction of bronchogen with DNA.

Thermodynamic stability of the DNA-bronchogen complex was measured on a high-precision differential scanning microcalorimeter (DSM). This work was designed to evaluate whether bronchogen serves

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as a DNA-stabilizing or DNA-destabilizing agent. It was interesting to estimate whether bronchogen binds specifically to adenine–thymine or guanine–cytosine. Moreover, we studied whether bronchogen has a modulatory effect on relative thermostability of these pairs or specifically binds to DNA duplex.

MATERIALS AND METHODS

Measurements were performed on DSM (sensitivity 10^{-7} W, measuring cell 0.2 cm^3, heating rate 0.5°C/min). The temperature range was 20-150°C. The thermal parameters of DNA melting were evaluated. The melting temperature (T_{melt}), width of the melting range (ΔT_{melt}), and melting enthalpy (ΔH_{melt}) did not exceed 0.2°C, 0.1°C, and 0.8 cal/g, respectively [5,7]. T_{melt}, ΔT_{melt}, and ΔH_{melt} were calculated as the mean values of 5 measurements. The study was conducted with highly pure DNA from calf thymus and albino mouse liver (protein <0.5%, RNA <0.2%, molecular weight >15 MDa, hyperchromic effect 39%). The concentrations of DNA and bronchogen were 0.45-0.5 mg/ml and 10^{-6}-10^{-4} M, respectively.

RESULTS

Figure 1 shows the microcalorimetric curves of heat absorption for DNA from calf thymus and albino mouse liver at various ratios of 1 M bronchogen and 1 M DNA b.p. Under various conditions, the increase in *r* (molar ratio of bronchogen/DNA b.p.) from 0.01 to 0.055 was accompanied by an increase in the melting temperature of the major fraction of calf thymus DNA (T_{melt}=78.5°C), satellite fraction (T_{melt}=84°C, curve *a*), and major fraction of liver DNA (T_{melt}=71°C at 0.015 M NaCl, T_{melt}=85.0°C at 0.15 M NaCl) by 3.1°C. Further increase in *r* from 0.055 to 1.0 was not accompanied by an increase in the melting temperature of these DNA. The width of the melting range ($\delta(\Delta T_{melt})$) decreased ($0.055<r<0.1$) after addition of bronchogen. The changes for calf thymus DNA and mouse liver DNA were 0.9 and 0.85°, respectively, at a bronchogen/DNA b.p. molar ratio of 0.055 (0.015 and 0.15 M NaCl). Further increase in the concentration of bronchogen did not change the width of the melting range. Figure 2 shows the dependence of changes in T_{melt} of DNAs on *r*.

The stability of diluted solutions of DNA from various organs was shown to increase linearly with increasing the number of guanine-cytosine pairs and concentration of neutral salts (from 0.5 mM to 0.5 M) at neutral pH. Melting temperature of the satellite fraction containing a greater number of guanine-cytosine pairs (as compared to the major fraction after addition of bronchogen) increased similarly to that of the major fraction (by 3.1°C, Figs. 1 and 2). These data show that DNA-stabilizing ligand bronchogen does not exhibit specificity for adenine-thymine and guanine-cytosine. Differences in the relative thermostability of these pairs persist after bronchogen treatment. The increase in T_{melt} of the major fraction, satellite fraction of calf thymus DNA, and mouse liver DNA (Fig. 2) and the decrease in the width of the melting range (narrow range of *r*, 0.01-0.055) indicate that this type of bin-

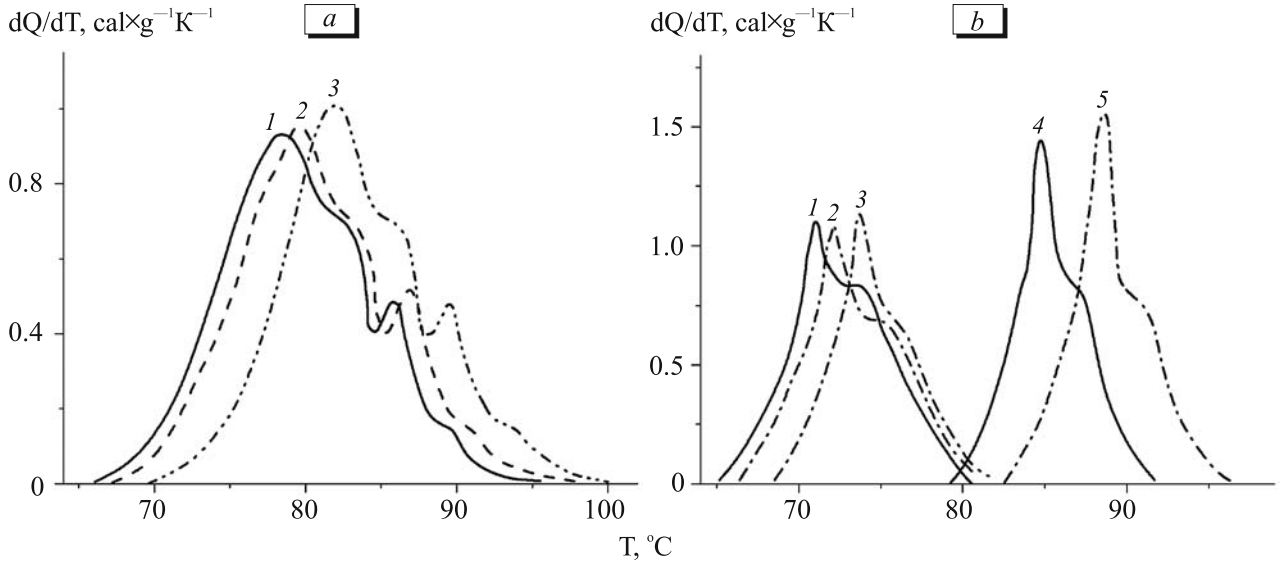

Fig. 1. Microcalorimetric curves of DNA melting, calculated per 1 g dry weight at various values of *r* (heating rate 0.5°C/min). (*a*) Calf thymus DNA (pH 7.02, 20 mM phosphate buffer, DNA concentration 0.045%, 92-94 μg DNA per ampoule). Curves: *1*, *r*=0; *2*, *r*=0.03; *3*, *r*=0.1. (*b*) Albino mouse liver DNA (pH 7.02, DNA concentration 0.05%, 100-102 μg DNA per ampoule). Curves: *1*, *r*=0; *2*, *r*=0.05; *3*, *r*=1.0 (0.0015 M citrate buffer, 0.015 M NaCl). Curves: *4*, *r*=0; *5*, *r*=0.1 (0.015 M citrate buffer, 0.15 M NaCl).

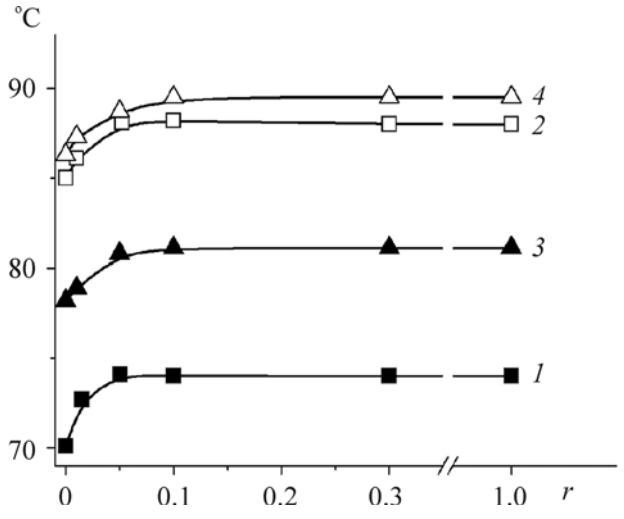

Fig. 2. Dependence of DNA melting temperature on *r* at pH 7.02. Major fraction (*1*); satellite fraction of calf thymus DNA (*2*). Major fraction (fine peak) of mouse liver DNA in solutions: (*3*) 0.015 M NaCl, 0.0015 M citrate buffer; (*4*) 0.15 M NaCl, 0.015 M citrate buffer.

ding can be considered as strong and occasional. The binding occurs with both strands of DNA. These data are consistent with the results of experimental and theoretical studies. As regards the small ligands that are characterized by reversible binding and do not interact with each other at the surface of DNA, the decrease in T_{melt} and increase in ΔT_{melt} of DNA reflect binding of ligands to a single strand. By contrast, the increase in T_{melt} and decrease in ΔT_{melt} are typical of binding to both strands (as observed in our study). The increase in *r* from 0.01 to 1.0 was accompanied by similar increase in the melting temperature (by 3.1°C) of DNA in 0.15 and 0.015 M NaCl. Therefore, bronchogen mainly binds to nitrogen bases of DNA.

We conclude that bronchogen stabilizes DNA and binds to rare sequences of a double strand. Rare binding sites are saturated at a bronchogen/DNA b.p. molar ratio of 0.05. Hence, these sites are located along the double strand of DNA at a distance of 50 b.p.

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