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MICROCALORIMETRIC INVESTIGATION OF INFLUENCE OF CANCEROGENIC AND ANTICANCEROGENIC COMPOUNDS ON NUCLEAR CHROMATIN OF TUMOR CELLS

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Abstract

The influence of mitoxantron (*M*) and benz(a)pyrene (BP) on chromatin DNA in composition of spleen and liver tissues and cells of BALB/c mice were studied using a high-sensitive differential scanning microcalorimeter. It was established that BP can cause a) the specific breaks in 'inactive' chromatin DNA chain and unfolding of the whole domain (a loop of chromatin) which should lead to uncontrolled genome activation; b) the breaks in the DNA double-helix creating short duplexes. *M* at low doses, interacting with 'naked' linker DNA of tumor restores the chromatin structure, at high doses or at repeated injections, *M* causes the disturbance of chromatin structure.

Keywords: cancerogenic and anticancerogenic compounds, microcalorimetry, tumor cells

Introduction

A certain material on thermal properties of chromatin in composition of animal tissue cells has been accumulated during the last decades [1–7]. These results gave the possibility to characterize the denaturation process of the whole chromatin and to show that this nucleoproteid complex, possessing hierarchic structure, 'melts' in a distinctly definite temperature range and shows a significantly high degree of reproduction. On the basis of analysis of heat denaturation process of tissues, cells, nuclei, chromatin, DNA [1–7] it became possible to characterize the separate stages of transition observed on these differential curves. This allows to put a question about the investigation of structural reconstructions of genetic material in composition of various tumor tissue cells to find out the slightest structural changes observing in tumor chromatin in comparison with norm on the level of tissues and cells.

Here we make an attempt to characterize an influence of anticancerogenic medicinal preparation mitoxantron (*M*) and cancerogenic compound benz(a)pyrene (BP) denaturation process of tumor chromatin being included into cell composition of higher organisms – eucaryot.

It should be noted that there are a lot of data on study of interaction mechanisms of *M* and BP in vitro [8, 9] but there are practically no data on the char-

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acter of interaction of *M* and BP compounds with chromatin DNA in vivo. The knowledge of these data is undoubtedly interesting because adducts, formed in vivo and in vitro, are different for many preparations. Therefore, we believe that the study of denaturation process of *M*-DNA and BP-DNA complexes in vivo is of certain interest as in fundamental so in applied (for medicine and pharmacology) sense.

Experimental

Measurements were performed using a differential scanning microcalorimeter designed for studies of both dilute biopolymer solutions and complex biological systems [10, 11]. Microcalorimeter sensitivity is 0.4 J sec^{-1}, measuring cell volume – 0.04–0.5 ml, heating rate – 5–30 K min^{-1} and temperature range of measurements – 20–120°C. Errors in determination of T_d, ΔT_d and Q_d did not exceed 1.0, 0.5 and 10.5 J g^{-1}, respectively, for chromatin and 0.15, 0.05 K and 4.0 J g^{-1}, respectively, for DNA.

Administration of Rauscher leukemic strain, *M*, BP to BALB/c mice, isolation of spleen, chromatin and DNA from normal and tumor tissues, definition of nucleic acid content in preparations under investigation were done according to [4, 7]. Protein content in DNA preparations did not exceed 0.5%. DNA from calf thymus was supplied by SERVA company (FRG).

Results

In Fig. 1 the excess of heat capacity $\Delta C_p = dQ/dT$ has been replotted as a function of temperature for BALB/c mouse liver cells and chromatin suspension. Comparison of curves showed that chromatin within liver cells denaturated in a way similar to free chromatin with transition parameters $T_d^1 = 69 \pm 1.0$°C, $T_d^2 = 80 \pm 1.0$°C, $\Delta T_d^1 = 6.0 \pm 0.5$°C, $\Delta T_d^2 = 7.5 \pm 0.5$°C and the heat denaturation values $Q_d^1 = 22 \pm 10$ J g^{-1} DNA, $Q_d^2 = 73 \pm 10$ J g^{-1} DNA [1–4]. It may be seen that the entire heat absorption curve (Curve 2) shifted down along the temperature scale. The maximum near 56°C, which, according to [7], corresponds to denaturation of RNP complex also shifted towards low temperatures by 5 K. Thus, the thermal stability of RNP complex decreased markedly. The most important, however, was a marked redistribution of heat between the heat absorption stages with $T_d^1 = 68$°C and $T_d^2 = 80$°C without significant changes in Q_d. Specifically, Q_d^1 rose up to 46.84±4.0 J g^{-1} DNA whereas Q_d^2 fell down to 46.0±4.0 J g^{-1} DNA.

Earlier, based on the analysis of melting curves of chromatin (300 Å fiber) [13, 14], chromatin depleted of H1 histone (100 Å fiber) [12] and nucleosomic particles [13, 14, 6] obtaining by spectroscopic methods, it has been concluded that at low ionic strength of solution the high-temperature peak ($T_d = 80$°C) of thermal transition of chromatin corresponds to denaturation of its compact part – nucleosomic core particle. But the low-temperature peaks at 55 and 69°C correspond to denaturation of internucleosomic – linker DNA, the latter being the 'active' chromatin [12].

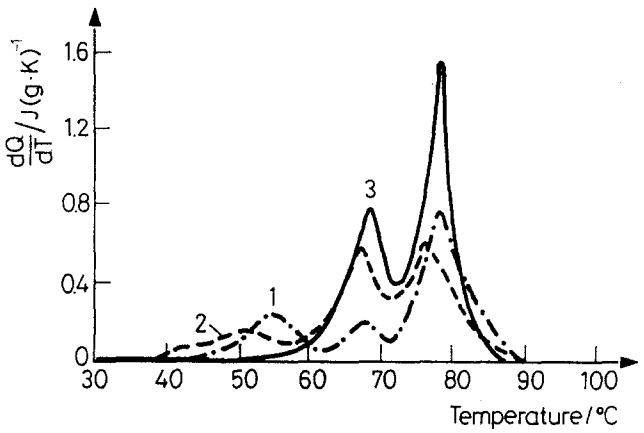

Fig. 1 Heat absorption curves as a function of temperature (dQ/dT, cal g^{-1} K^{-1}) of BALB/c mouse liver cells [1, 2] in Hank's solution (*pH* 7.2, the quantity of suspension is 410 mg, M_{DNA} = 1.8 mg, the heating rate (v) is 0.004°C sec^{-1}) and chromatin suspension (3) (sucrose 0.25 *M*, Tris 10 *mM*, $MgCl_2$ 3 *mM*, *pH* 7.6, the quantity of suspension is 400 mg, M_{DNA} = 1.6 mg, protein/DNA = 1.9, v = 0.004°C sec^{-1}): 1 – liver cells untreated with BP; 2 – liver cells treated with BP (r = 0.002); 3 – chromatin suspension

On the basis of the above considerations and experimental data shown in Fig. 1, it should be concluded that decrease in T_d^2 and increase in ΔT_d^2 as compared to T_d^1 and ΔT_d^1, with insignificant changes in integral Q_d suggest that BP has a greater influence on DNA of the high-stable domain (inactive chromatin) than on that of thermolabile one (active chromatin).

In Fig. 2 the excess of heat capacity $\Delta C_p = dQ/dT$ has been replotted as a function of temperature for BALB/c mouse spleen tissue before and after the administration of *M*. As it can be seen the process of denaturation of chromatin in composition of normal tissue cells has also two stages – two domains, but the structural domains denaturate at 73±1.0°C and 82±1.0°C. The integral heat of chromatin denaturation is 96±10 J g^{-1} DNA. In the case of tumor tissue denaturation (Curve 2) we observe more complex picture: an additional heat absorption with T_d^0 = 57±1.0°C, which can be already registered on the third day after Rauscher virus injection,is observed on the heat absorption curve. As it is seen, in the case of leukemia about 30% of 'active' chromatin structure underwent significant decompactization that is expressed in the decrease of Q_d^1 from 36.5 J g^{-1} DNA to 22.0 J g^{-1} DNA.

Therefore, we came to the conclusion that the heat absorption peak at 57°C, in the case of leukemic mouse spleen tissue heating, expresses the melting process of DNA linker regions which are not well protected from H1 histones. In this Figure it is also seen a shift of heat absorption high temperature peak to high temperatures by 2°C without noticeable change of Q_d^2.

We observe this phenomenon earlier in the case of C3HA mouse ascitic hepatoma and cells of human bone-marrow [4, 15]. It was supposed that it was

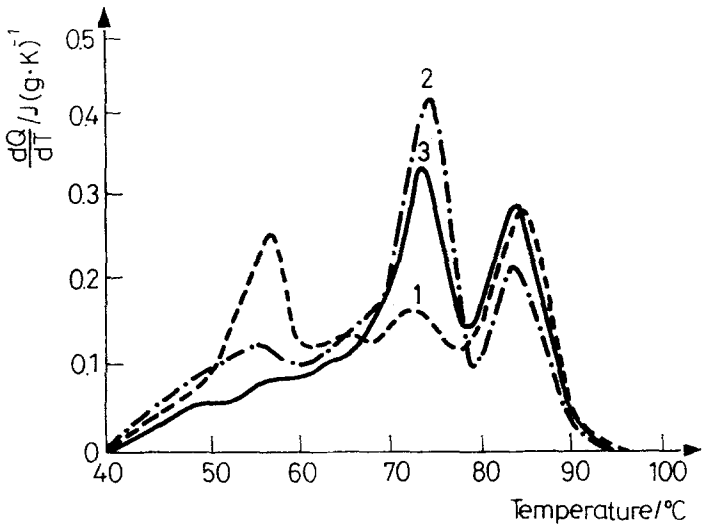

Fig. 2 Heat absorption curves as a function of temperature (dQ/dT, cal g^{-1} K^{-1}) of BALB/c mouse spleen tissue: 1 – leukemic tissue; 2 – leukemic tissue, but 24 h before the experiment, the mice were injected intraperitoneally 0.3 μg of *M* per gram of tissue; 3 – normal tissue. The tissue mass is 255 mg, $\nu = 0.004$°C sec^{-1}

connected with compactization of chromatin in cell composition due to the formation of DNA-protein–metal complex [16].

The injection of *M* intraperitoneally to leukemic mice (Fig. 2) leads to significant changes of heat absorption curve profile. In particular, at injection of 0.3 μg of *M* on 1 g of animal tissue, the peak intensity decreases at 57°C, the peak intensity at 73°C increases and the peak intensity with T_d^2 about 85°C shifts to low temperatures by 2.5°C. So, the profile of heat absorption curve (Curve 2) of leukemic spleen tissue of mice after injection of *M* became similar to the profile of normal mouse spleen tissue heat absorption curve (Curve 3).

It was interesting to find out what causes the decrease of denaturation temperature of leukemic chromatin in cell composition after cell treatment with *M* and BP. It could be supposed that its decrease was connected with decrease of DNA thermostability as a result of its structure change or with change of histone binding strength or non-histone proteins with leukemic DNA. With this aim we carried out microcalorimetric study of chromatin and DNA solutions isolated from BALB/c mice previously injected with *M* and BP.

Figure 3 shows T_d, ΔT_d and Q_d dependences of chromatin solutions of BALB/c mouse spleen on the number of *M* injections. You see that T_d for both stages has tendency to decrease approaching to denaturation temperature of norm after the third injection. But increase of injection quantity causes sharp thermostability decrease of both chromatin fractions that is obviously connected with strong structural changes of chromatin.

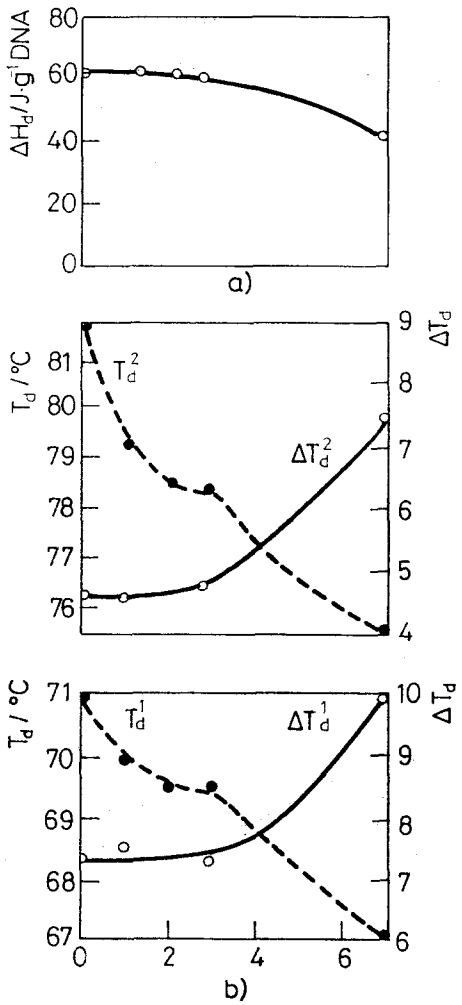

Fig. 3 Dependences of denaturation temperature, T_d, denaturation range width, ΔT_d, determined at half-height of heat absorption peak, and denaturation heat, Q_d, on the number of *M* injections (0.3 μg M/g tissue, Tris 10^{-2}, FMS 0.2, *pH* 8.0)

In Fig. 4 the excess of heat capacity $\Delta C_p = dQ/dT$ has been replotted as a function of temperature for DNA isolated from BALB/c mouse spleen chromatin. The first curve corresponds to the melting of healthy spleen DNA. The second – to leukemic spleen DNA, the third – to the spleen DNA isolated from BALB/c mice previously injected seven times with 0.3 μg of *M* per gram of tissue. It is seen that the melting temperature of tumor DNA isolated after seven injections of *M* (Curve 3) is by 2° lower in comparison with DNA without *M*.

The quite other picture is observed in the case of BP and *M* addition to DNA solutions.

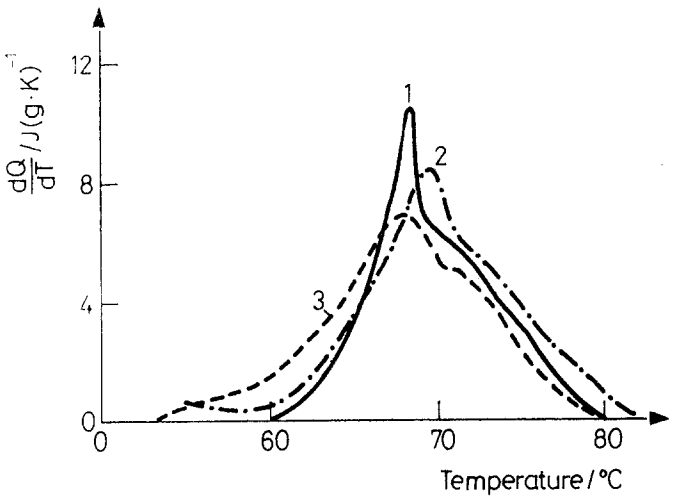

Fig. 4 Heat absorption curves as a function of temperature (dQ/dT, cal g^{-1} K^{-1}) of BALB/c mouse spleen DNA (SSC 0.1, *pH* 7.2, the quantity of solution is 410 mg, c =0.12%, v =0.004°C sec^{-1})

In Fig. 5 the excess of heat capacity $\Delta C_p = dQ/dT$ has been replotted as a function of temperature for calf thymus DNA at different concentrations of BP. It is seen that the process of DNA melting in the absence of BP (Curve 1) has several stages with transition temperatures T_m^1=60°C, T_m^2 =70°C, T_m^3 =78.5°C. The maximum at the end of the curve corresponds to the melting of satellite fraction of DNA. The melting heat calculated form the area under the peak is equal to 44±2.4 J g^{-1}. The increase of BP and *M* concentrations (Fig. 5, Curves 2–5, Table) in DNA solutions at constant *pH* 7.2 leads to the shift of the melting curve towards higher temperatures and to the broadening of heat ab-

Table 1 Dependence of temperature and melting enthalpy of thymus DNA on quantity of mitoxantron in solution

Mitoxantron/bp	T_m^1 /°C	T_m^1 /°C	T_m^2 /°C	T_m^3 /°C	Q_m/cal g^{-1}
0	69.5	–	73.3	77.5	11.5
0.0001	69.3	–	73.5	78.0	11.2
0.0008	70.5	–	73.2	77.1	11.5
0.005	72.4	–	77.0	81.5	12.0
0.06	73.8	–	80.1	86.2	12.5
0.14	76.0	84.9	–	–	11.0
0.28	76.0	84.5	–	–	10.8

T_m^1 and T_m^1 are melting temperatures of the main stage
T_m^2 and T_m^3 are melting temperatures of the satellite fractions
Q_m – total melting heat.

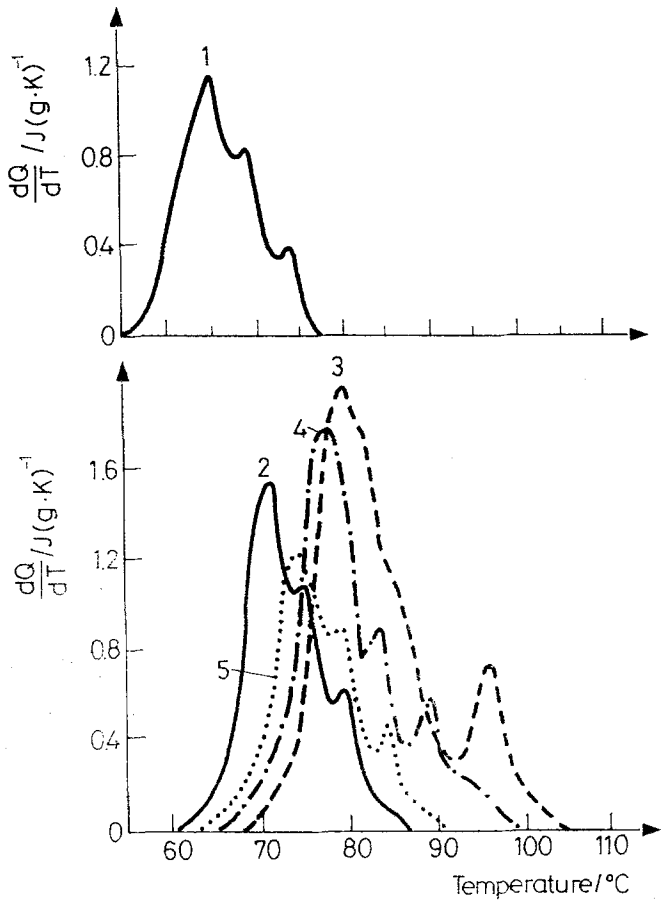

Fig. 5 Heat absorption curves as a function of temperature (dQ/dT, cal g^{-1} K^{-1}) of calf thymus DNA solutions in the presence and absence of BP (SSC 0.1, *pH* 7.2, the quantity of solution is 410 mg, c =0.1%, v =0.004°C sec^{-1}): 1 – r =0; 2 – r =0.001; 3 – r =0.0033; 4 – r =0.066; 5 – r =0.001

sorption peaks; the peak corresponding to the satellite fraction shifts to higher temperatures more intensively than the peak of the main stage. Taking into consideration that the satellite fraction of thymus DNA is more rich with GC-pairs (GC ~ 50%) in comparison with the main stage (GC ~ 42%), we can conclude that more strong stabilization of GC-pairs in comparison with AT-pairs occurs at increase of BP concentrations ($0< r <0.003$) and M concentrations ($0.001< r <0.1$) (Table) (r is the molar ratio of BP and M per one mole of DNA bp). The further increase of r causes the decrease of Q_m and T_m in the case of bp but Q_m and T_m practically do not change in the case of M (Fig. 6, Table).

Figure 7 shows that BP injected in vivo effectively acts on DNA at low doses (Curve 2). It is accompanied by a decrease in T_d by 2°C and in Q_d by 15% as

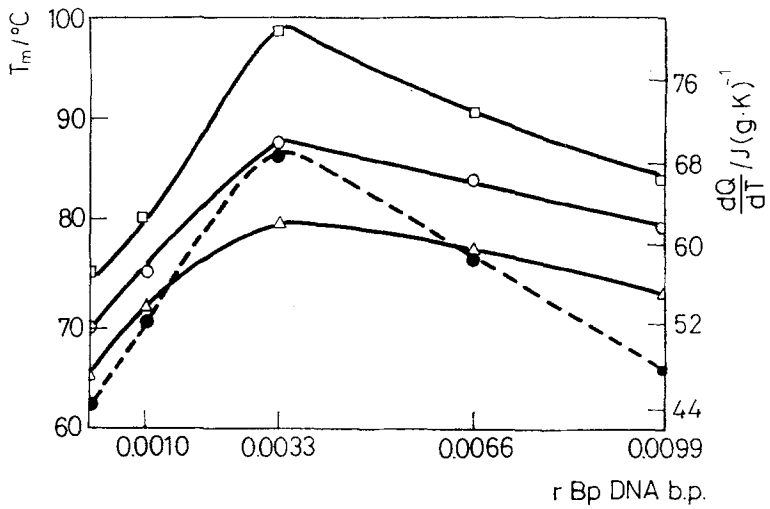

Fig. 6 Dependences of melting temperature and melting enthalpy, T_m, ΔH_m, of calf thymus DNA solutions on BP concentration (r) (SSC 0.1, pH 7.2, c =0.1%): o and Δ – T_m of the main fraction (GC-pair content about 50%); □ – T_m^3 of the satellite fraction (GC-pair content about 50%); • – integral ΔH_m

well as an increase in ΔT_d by 25% as compared to DNA in norm. Such behaviour of melting process definitely signifies that there are defects in the DNA double helix. Besides, Fig. 7 shows the low heat absorption in the temperature range of 45–60°C.

It should be mentioned that the low heat absorption preceding the process of global desintegration of the double helix had been observed earlier only for tumor DNA [15].

Discussion

The stronger dependence of $T_d^3 = f(r)$ as compared to $T_d^{1,2} = f(r)$ suggests that BP and M predominantly stabilize GC-pairs as compared to AT-pairs of DNA. The rise in melting enthalpy (Q_d by 65% at $r \sim 0.003$) as compared to DNA without ligands (Fig. 6) is a good evidence in favour of the existence of strong and rare binding of BP with double helix that agrees well with data [17]. The bell-like dependences of $T_d = f(r)$ and, therefore, $Q_d = f(r)$ confirm the fact that BP is connected with bp in a cooperative way; at $r < 0.0033$ the binding constant of BP with the helical form is more as compared to denaturated one; at $r > 0.0033$ (Fig. 6) on the contrary, the binding constant is more in denaturated form as compared to the helical one, it corresponds to the modern notion on DNA melting in the presence of ligands [18].

In the case of melting of DNA isolated from spleen and liver of BALB/c mice pretreated with M and BP, the opposite picture is observed. In both cases

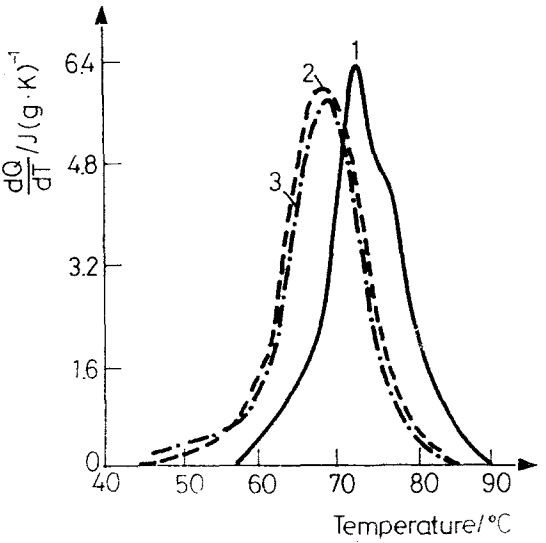

Fig. 7 Heat absorption curves as a function of temperature (dQ/dT, cal g^{-1} K^{-1}) of DNA isolated from BALB/c mouse liver cells (SSC 0.1, *pH* 7.2, the quantity of solution is 410 mg, c =0.11%, v =0.004°C sec^{-1}) before (Curve 1) and after (Curves 2, 3) BP injection to mice in vivo; Curve 2–0.9 μg BP/g tissue; Curve 3–4.0 μg BP/g tissue

T_d decreased by about 2.5°C, ΔT_d rose by about 2°C and Q_d decreased by 15–20%. Such changes in the parameters of melting process obviously show that the administration of BP and *M* in vivo produce defects in the DNA double helix (Figs 4, 7).

In the first approximation one assumes that the value of Q_d of DNA does not depend on temperature [18], the ratio of areas of low temperature absorption and the main stage will give share of melted out sites and short melted fragments of DNA falling at the temperature range of 45–60°C which is equal to 2–3% (Fig. 7).

Thus, it may be concluded that administration of BP in vivo causes a significant damage (2–3%) of DNA and only insignificant damage of the main part of DNA which is manifested by a slight decrease in T_d^2 (~2°) of the main stage.

On the basis of the above considerations and experimental data shown in Fig. 1, it should be concluded that the greater decrease of T_d^2 and increase of ΔT_d^2 as compared to T_d^1 with insignificant redistribution in Q_d, suggests that BP has a greater effect on DNA of inactive chromatin than on that of active one.

We think that BP has a double effect on DNA of chromatin:

a) BP breaks the double helix producing short duplexes in the solution which melt in the temperature range 45–60°C (Fig. 7);

b) BP may also cause the specific breaks in one strand of double chain of chromatin DNA in a way similar to the case of X-ray irradiation [20], unfold the domain (a loop of chromatin) and cause a significant genome activation.

At low doses *M* quite differently influences on chromatin in vivo as compared to BP. In the case of leukemia it interacts specifically with unprotected from histones, 'naked' linker DNA regions which appear in leukemic chromatin. It is supposed that aromatic rings intercalate between base pairs of 'naked' DNA [9] and long side groups interact with phosphate groups located in a large groove of DNA double helix, imitating the interaction of histones with DNA. As a result, the tumor chromatin structure is restored as it is observed in the case of increase of ionic strength of chromatin solution depleted of H1 histone [12]. This allows us to suppose that *M* at small doses in vivo does not cause the tumor cells destruction but promotes their transformation into normally functioning cells. As for influence of high doses or repeated injections of *M* on genetic material, in this case *M* destructs the structure of DNA of both structural domains.

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