Secondary Structure Differences between Normal and Tumour DNA Studied by UV-Spectrophotometry

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Abstract. Some differences between the structure of tumour and normal DNA were studied. An increase of the methylation degree of the tumour DNA was observed (by 42% as compared with normal DNA). Changes between normal and tumour DNA were found in the melting temperature (T_m), the melting interval (ΔT) and the shape of the differential melting curves (DMC). In tumour DNA, two types of regions with changed thermostability were observed differing in length. The first type regions contain more than 20–30 pairs of nucleotides and manifest themselves on DNA DMCs as a low-temperature plateau. The other type, much shorter, cause a shift of DNA DMCs towards lower temperatures. The value obtained for the concentration of tumour DNA regions with changed thermostability per one pair of nucleotides is 4.4×10^{-4}. The *in vivo* influences of three alkylating preparations on the structure differences of both kinds of DNAs were studied. The preparations tested lowered the content of 5-mC in tumour DNA, altered the total concentration of DNA regions with changed thermostability, and effectively suppressed these regions containing 20–30 pairs of nucleotides. Also, a correlation was found between the effect of the preparations studied on tumour DNA and their ability to suppress the growth of Sarcoma 45.

Key words: Sarcoma 45 — Normal and tumour DNA — 5-Methylcytosine — Changed thermostability DNA regions

Introduction

The studies of differences between normal and tumour cell structures are of special importance from the viewpoint of the understanding of the nature of cell tumour transformations. In a review Galloway (1989) has shown that all tumour transformations are due to genetic damage of the DNA. In connection with this, differences that appear on the level of DNA macromolecule are of great significance. Some recent results of the research into the local DNA structure have been reported by

Paleček (1991) and Paleček et al. (1992).

Numerous investigations into differences in the physicochemical properties of tumour compared with normal DNA have shown changes in both primary and secondary structures of tumour DNA.

The alterations in the chemical structure of the DNA macromolecules (Balmain et al. 1989; Rao et al. 1989; Vanyushin et al. 1970) suggest that the content of GC-pairs as well as the DNA methylation degree can change during the cell transformation. At the same time, the only product of enzymatic methylation of DNA is 5-methylcytosine (5-mC). Changes in the physical characteristics of the secondary structure of tumour DNA compared with normal DNA have been reported (Andronikashvili 1987). Using the kinetic formaldehyde method we could to find higher numbers of defects in the secondary structure of tumour DNA (Zhizhina et al. 1982). The occurrence of defects in the secondary structure of tumour DNA is not a consequence of ezymatic degradation of DNA due to the process of isolation.

To elucidate the possible changes in the DNA structure at tumour transformation, the nucleotide content of DNA and the differences in the DNA stabilization temperature were studied.

The knowledge on the influence of antitumour preparations on normal and tumour DNA properties is of a great significance (Procenko and Bulkina 1985; Zuck 1987). In the present work we studied the *in vivo* influence of three antitumour alkylating preparations on the changes in tumour and normal DNA structures.

Materials and Methods

Tumour DNA was isolated from Sarcoma 45 and normal DNA from the livers of healthy rats. Male rats weighing 100–120 g were used. Both DNAs were isolated by the method according to Marmur and Doty (1962) repeatedly deproteinized by phenol, pH 8, and by chloroform: isoamylalcohol (24 : 1). The protein concentration was determined by the Lowry method (Lowry et al. 1951). To determine the nucleotide content including 5-mC, the samples were dried at 105 °C and hydrolyzed to bases (99% methanoic acid, 175 °C, for 30 minutes). The bases were separated by thin-layer chromatography and their content specified by spectrophotometric methods. The used antitumour alkylating preparations from the group of diethyleneimides alkoxy-benzyl-pyrimidine-amidophosphoric acids (preparation 1) and from the group of homopolypeptid analogues of sarcolysine (preparation 2) were synthesized at the Institute of Organic Chemistry, Armenian Academy of Sciences, Yerevan. For comparison, the influence of currently used sarcolysine (preparation 3) was studied. The preparations were administered to rats for 8 consecutive days (once a day) at optimal therapeutic doses (60 mg/kg, 20 mg/kg and 2.5 mg/kg for samples 1,2 and 3, respectively).

The DNA melting process was studied in 0.1 SSC buffer, standard saline citrate (1 SSC = 0.15 mol/l NaCl + 0.015 mol/l Na citrate, pH 7.3). The melting curves were obtained using a UNICAM SP-8000 spectrophotometer with hermetically closed cells at continual mode of heating of the DNA solutions at a speed Mof 0.25 °C/min. The accuracy

of the temperature determination was 0.05 °C and that of the optical density measurements 0.002. The hyperchromicity for all DNA samples was 36–38%.

As the structure specificities of tumour DNA were manifested weakly on the melting curves, we decided to use DMC calculations. The original melting curve was normalized and approximated by a third order polynomial using the least-square method for 5 points. The DMC were obtained by numerical differentiation of the experimental melting curves. The calculations of DMC and their decomposition into the Gauss components were carried out on EC 1022 and EC 1030 computers.

Results

The data obtained by the analysis of the base composition of normal and tumour DNA are shown in Table 1; they represent average values of 5 to 10 measurements for both DNAs. It is clear that the two kinds of DNA do not differ in their contents of AT pairs. Large differences between the DNA samples were found in the content of 5-mC, the level of which is increased by 42% in tumour DNA.

Table 1. The base content, the melting temperature and the melting interval of normal and tumour DNA

DNA	AT mol %	5-mC mol %	T_m °C	ΔT °C
Normal	55.4	1.02	67.9	6.0
Tumour	55.1	1.45	67.7	6.5

Table 1 shows the characteristics of the melting curves for both DNAs (T_m and ΔT). T_m for tumour DNA is by 0.2 °C smaller and ΔT is by 0.5 °C wider in comparison with normal DNA.

DMCs are shown in Fig. 1. One can clearly see the difference between the DNAs studied. On the DMC of tumour DNA an additional low-temperature plateau can be seen (in the temperature interval 54–63 °C). The differences concerned the height, shape, and position of the characteristic DMC peaks. The peaks were shifted by 0.2 °C to lower temperatures.

The contents of 5-mC in normal and tumour DNA before and after the application of antitumour preparations are given in Table 2, the corresponding differential melting curves are in Figs. 2–4.

In vivo application of preparation 1 to healthy animals lead to an increase of methylation degree of normal DNA (Tab. 2). There were no substantial differences in both DNA DMCs before and after the application of preparation 1 (Fig. 2).

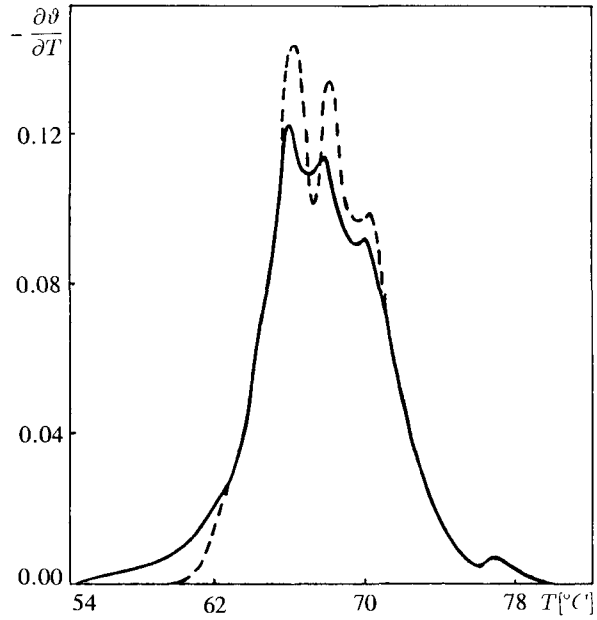

Figure 1. The differential melting curves for normal (dashed line) and tumour (solid line) DNA.

Table 2. The 5-mC content (mol %) in DNA before and after the application of an alkylating preparation

	Normal DNA	Tumour DNA
Without preparation	1.02	1.45
Preparation 1	1.29	0.98
Preparation 2	1.40	0.87
Preparation 3	1.47	1.16

Contrary to normal DNA, the methylation level in tumour DNA was decreased after preparation 1. The content of 5-mC practically reached the original values. Preparation 1 affects to a great extent the character of the DMC shape in tumour DNA (Fig. 3). At first, the characteristic low-temperature plateau of the tumour DNA disappears. Secondly, the whole curve is shifted to a lower temperature region (Fig. 1). It follows from the above that preparation 1 practically does not influence the temperature stabilization of normal but considerably destabilizes tumour DNA.

Table 2 suggests that preparations 2 and 3 decrease the level of 5-mC in tumour DNA by 40% and 20%. The shape of tumour DNA DMC after *in vivo* application of these preparations was close to that of the normal DNA DMC. A comparison

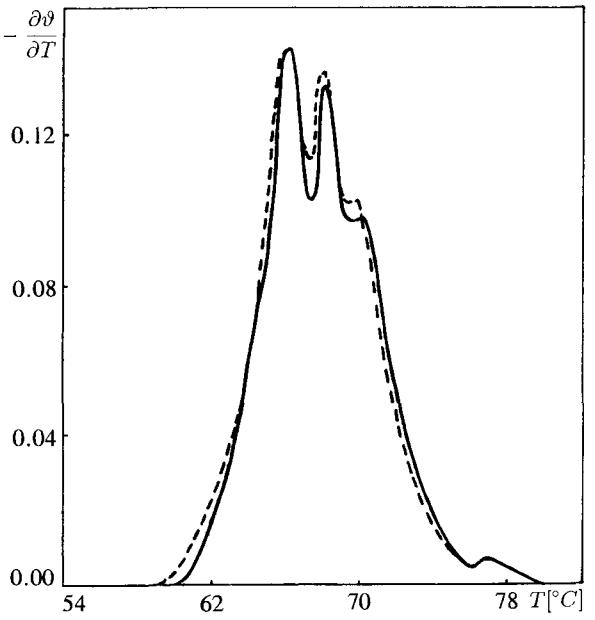

Figure 2. The differential melting curves for normal DNA before (solid line) and after (dashed line) *in vivo* administration of preparation 1.

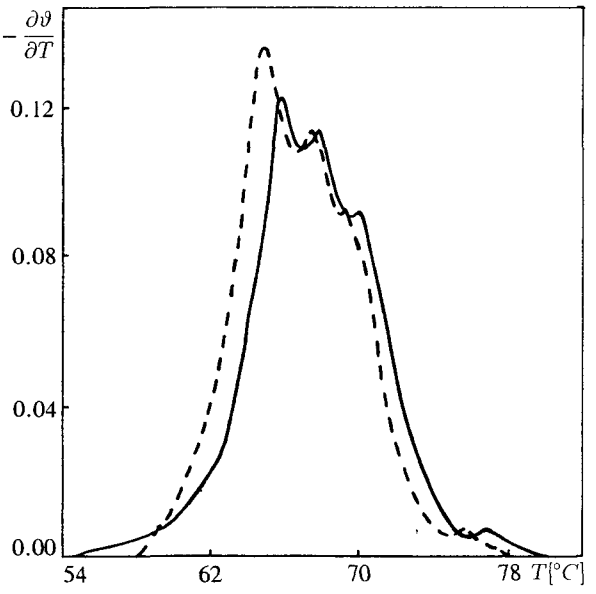

Figure 3. The differential melting curves for tumour DNA before (solid line) and after (dashed line) *in vivo* administration of preparation 1.

of tumour DNA DMCs after the application of preparation 2 and 3 shows (Fig. 4) that preparation 2 "restores" the DMC shape – as far as the length of the low-

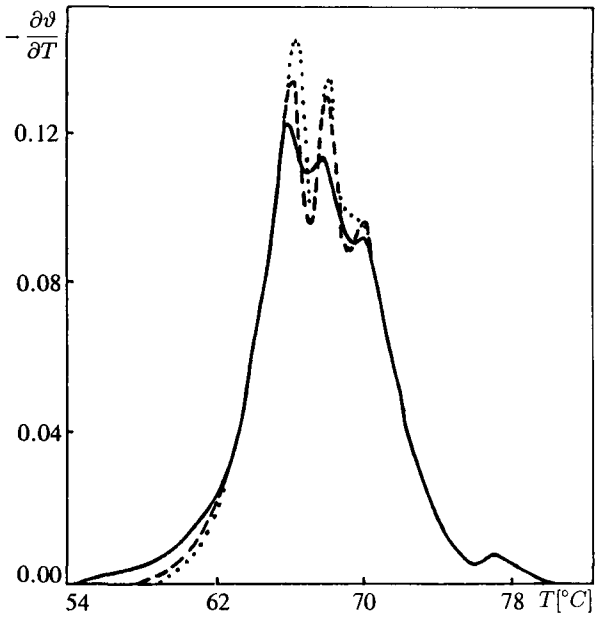

Figure 4. The differential melting curves for tumour DNA before (solid line) and after (dashed line) *in vivo* administration of preparation 2 and preparation 3 (dotted line).

temperature plateau and the heights of the characteristic peaks are concerned – less than does preparation 3.

Investigation of the effects of the preparations studied on the growth of Sarcoma 45 showed that preparation 1 suppressed the tumour growth by 91%, preparation 2 by 88%, and preparation 3 by 72%.

Comparing the data on alterations of the methylation levels for all three preparations studied and their ability to suppress sarcoma growth, a correlation was found between preparation 2 and 3. No such correlation was observed for preparation 1. This may be explained by the existence in the preparation of groups other than alkylating. These groups can contribute to the reduction of tumour DNA methylation.

Discussion

The study of differences between normal and tumour DNA has shown increased content of 5-mC and thermostability changes of tumour DNA (Tab. 1). Two main questions arise from the foregoing analysis: What is the influence of the methylation degree of tumour DNA on its total stability, and what is the cause for the thermostability decrease of tumour DNA?

Modified regions of DNA have been reported to affect DNA stability (at all values of stabilization or destabilization energies) (Ahrem et al. 1977a,b). These

authors have shown that the change of DNA melting temperature is proportional to the concentration of these regions.

The change of the melting temperature of tumour DNA corresponds to an increase in the 5-mC content, and can be calculated as

$$T_z = X_2(T_{G-5mC} - T_{GC}),$$

where X_2 is the relative content of the guanine-5methylcytosine (G-5mC) pairs in the DNA, and T_{G-5mC} (T_{GC}) is the melting temperature of the G-5mC (GC) pairs.

For the determination of the melting temperature T_{G-5mC} we used T_m for DNA from *Xanthomonas Phage* XP-12. In this DNA all cytosines are completely replaced by 5-mC. The melting temperature of this DNA (T_m = 83.2 °C in 0.012 mol/l [Na^+]) is by 6.1 °C higher than the expected T_m for DNA with the same content of AT-pairs (Ehrlich et al. 1975).

The melting temperature T_{G-5mC} was calculated from

$$T_m = T_{AT} + X_2\,(T_{G-5mC} - T_{AT}),$$

where T_{AT} is the melting temperature of AT pairs.

The value of the melting temperature for G-5mC was calculated as $T_{(G-5mC)}$ = 103.5 °C in 0.1 SSC.The transition towards the ion conditions of 0.1 SSC was done according to the Frank-Kamenetskii formula (private communication)

$$T_m = 82,1 + X_2\,(40 - 1,4\log[Na^+]) + 17,3\log[Na^+].$$

The difference of the melting temperature of tumour DNA, in comparison with normal DNA, does not exceed 0.04 °C, which is within the experimental error.

Similarly, we can show (when considering the block structure of DNAs studied) that the increase of the 5-mC content in tumour DNA extends the melting interval by 0.05 °C in comparison with normal DNA.

It follows from the above that the increase of the 5-mC content in tumour DNA does not affect the shape of the DNA melting curve, because the changes in the melting temperature and the interval do not exceed experimental error. However the increase of the 5-mC content in tumour DNA leads to substantial local stabilization, because the melting temperature $T_{(G-5mC)}$ is by 8.6 °C higher than the melting temperature T_{GC} in 0.1 SSC. Methylation of DNA is one way for obtaining stable changes of the local gene structure. This is manifested by the changed accessibility of DNA and by the inhibition of the transcription process (Cedar 1988).

A decrease in the length of the DNA chain is known to lower T_m and to extend ΔT (Crothers et al. 1965; Lazurkin et al. 1970). The observed changes of these parameters characterizing the DNA melting due to fragmentation are explained by

the absence of interactions at the level of the base pairs at the ends of the spiral sections. It is natural that these changes of T_m and ΔT do not occur only up on DNA fragmentation, but also as a result of the arisal of single- and distorted double-stranded DNA regions or open DNA structures (Paleček 1991). From the above discussion it follows that changes of T_m and ΔT can serve the determination of regions with changed thermostability. The concentration of these regions in tumour DNA was calculated from the dependence of the DNA melting temperature on the length of the macromolecule chain (Crothers et al. 1965). The value obtained for one nucleotide pair is 4.4×10^{-4}. This agrees with the values of defect concentration as determined by the formaldehyde method for DNA macromolecules of different sorts of tumour transformations (Zhizhina et al. 1982).

As shown in Fig. 1, the differences between tumour and normal DNAs are clear-cut. The low-temperature plateau found on the DMC for tumour DNA suggests the presence of characteristic longer destabilized sections, with lengths of at least a few tens base pairs (20 30 pairs). This deduction is possible on the basis of earlier data (Ahrem et al. 1977a,b). In accordance with the data, shorter regions of changed thermostability cannot cause the appearance of a plateau on DMC. Shorter regions of changed thermostability induce a shift in the melting temperature (T_m) of tumour DNA, decreasing it by 0.2 °C. Also, the characteristic peaks of DMC are shifted by the same values towards lower temperatures. This allows the conclusion that there are much more shorter regions of changed thermostability in tumour DNA a few pairs long. The increased ΔT of tumour DNA results in an extension of DMC and in a decrease of the height of the characteristic DMC peaks. The integral of the curve is normalized to unity. Since the increase in ΔT is connected with the presence of DNA structure defects a proportional decrease of the height of characteristic DMC peaks serves as a measure of the concentration of regions with changed thermostability.

We can see from Table 2 and Figs. 2–4 that *in vivo* application of antitumour alkylating preparations results in a decrease of 5-mC content in tumour DNA and in changes of its thermostability. These changes can be explained by the interaction mechanism of alkylating preparations with tumour DNA (Babin et al. 1979; Procenko and Bulkina 1985). During the interaction of the alkylating preparations with tumour DNA, an anomalous alkylation of purine bases (first of all guanine) occurs. It is followed by the DNA depurination and by the appearance of single-stranded defects (Babin et al. 1979). In the course of the correction of these defects in the cell by the repair system, 5-mC is probably excluded. A decrease of 5-mC content in tumour DNA was observed after the application of antitumour preparations. The whole DMC shift towards lower temperatures is due to the antitumour preparation replacing longer regions of changed thermostability with other, relatively short destabilized regions. These regions then induce a shift of the whole DMC. As one can see from Fig. 3, this is the case with preparation 1.

On the basis of the interaction mechanism of the alkylating preparations with tumour DNA, one can conclude that preparation 2 causes more destabilization of tumour DNA than does sarcolysine, as judged by the lowered 5-mC content. The DMC shape obtained after the administration of preparation 2 (Fig. 4) suggests that there are more destabilized regions (probably in reparation places) occuring after preparation 2 than in the case of preparation 3.

A comparison of the data on the ability of the different preparations to suppress the growth of sarcoma 45 and the amounts of regions of changed thermostability in tumour DNA after reparation reveals some correlations. Regions of changed thermostability in the DNA hinder reduplication and transcription processes and result in the suppression of the tumour growth. It can be supposed that reduced methylation and structural defects are mechanisms underlying the antitumour activity of the alkylating preparations studied, coming to expression during DNA reparation process (see the DMC).

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