Dynamic Structure of DNA Modified with the Carcinogen *N*-Acetoxy-*N*-2-acetylaminofluorene†

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ABSTRACT: Native calf-thymus DNA was allowed to react with *N*-acetoxy-*N*-2-acetylaminofluorene and the dynamic behavior of this carcinogen-reacted DNA was studied by means of the formaldehyde unwinding technique. In all cases, the initial rate of formaldehyde attack is higher for DNA reacted with the carcinogen than for native DNA. Thus, in addition to the "natural breathing" of the DNA duplex it is demonstrated that each fixed fluorene residue gives rise to weak points from which formaldehyde unwinding starts. The model we propose enables us to determine the size of these disorganized loops at temperatures lying between 30 and 10° below the melting temperature. Theoretical calculations showed that the destabilizing effect measured by direct helix–coil transition is consistent with the model obtained from pure kinetic data. Finally, we consider the possible biological consequences of permanently open sections in native DNA for chemical carcinogenesis.

It has been recently shown (Von Hippel and Wong, 1971; Utiyama and Doty, 1971) how it is possible to get very useful indications about the dynamic structure of nucleic acids by following the kinetics of the reaction of formaldehyde with the nucleic acid bases. This method belongs to the more general "chemical probe" technique (for details see Von Hippel and Wong, 1971). Hydrogen exchange can also be considered as a chemical probe reaction. It has been shown (Printz and Von Hippel, 1965; Von Hippel and Printz, 1965), using tritium exchange, that DNA is subject to local structural fluctuations called "breathing" of base pairs. In fact, the formaldehyde and the hydrogen exchange probes visualize different "open" forms. That seen by hydrogen exchange is "single strand stacked" (McConnell and Von Hippel, 1971) while the transition state reacting with HCHO involves total melting (unstacking) (P. H. Von Hippel, personal communication). Nevertheless, formaldehyde unwinding kinetics presents some advantages with regard to hydrogen exchange. As a matter of fact, the formaldehyde technique permits us to visualize these open regions in DNA, at temperatures inaccessible to the hydrogen exchange technique (this last method is limited to quite low temperature). Lazurkin *et al.* (1970) were using the same experimental procedure to detect the concentration of "weak" points in shear degraded DNA samples.

In this paper we made use of formaldehyde in order to check the dynamic behavior of DNA which has reacted with N-AcO-AAF,[1] a reactive metabolite of the strong carcinogen AAF (for details see the review of Miller, 1970), found in the rat liver. N-AcO-AAF reacts covalently on position C_8 of guanine in native DNA (Kriek *et al.*, 1967). We have studied the structural modifications of DNA after the binding of the fluorene residues by means of circular dichroism and melting curve analysis (Fuchs and Daune, 1972). We have been able to show that in native DNA the guanine moiety in the alkylation product rotates around the C_1'–N_9 bond, thus putting the fluorene ring in the space available between the two nearest neighbors of the G·C pair. Thereafter the fluorene ring is stacked coplanar with the adjacent bases. Grunberger *et al.* (1970) and Nelson *et al.* (1971) using oligoribonucleotides containing a G-AAF residue have reached analogous conclusions. Two remarks can be made: (i) the hydrogen bonds of the modified G·C plate are probably broken; (ii) the larger size of the fluorene ring gives rise to a local distortion and weakening of the double helix.

In this paper we demonstrate that the general destabilization of the DNA after carcinogen binding (viewed by a linear decrease of the T_m^{260} with an increase in the percentage of modified bases; see Fuchs and Daune, 1971; Kapuler and Michelson, 1971; Troll *et al.*, 1969) can be explained by the existence of disorganized loops around each G-AAF residue. The formaldehyde unwinding method permits us to determine the size of such loops and their thermodynamic parameters at temperatures lying between 30 and 10° below the melting temperature.

Material and Methods

All chemicals were reagent grade (Merck). Native DNA was prepared from calf thymus (Kay *et al.*, 1952) and had the following characteristics: hyperchromicity at 260 nm, 40%; $s_{20,w}$ = 21 S; ϵ_P^{260} 6420. Protein content was lower than 0.8% in weight in all samples. Denatured DNA was obtained by heating DNA samples in sealed vials up to 100° for 10 min. N-AcO-AAF has been synthetized as previously described (Lotlikar *et al.*, 1966). Reaction of DNA with N-AcO-AAF was performed according to Miller *et al.* (1966) with minor modifications (Fuchs and Daune, 1971). All noncovalently bound fluorene residues were removed by three extractions with an equal volume of ether, followed by either an extensive dialysis (three changes of the buffer) or by precipitation of the DNA at −20° with two volumes of ethanol.

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[1] Abbreviations used are: N-AcO-AAF, *N*-acetoxy-*N*-2-acetylaminofluorene; AAF, *N*-2-acetylaminofluorene; G-AAF, 8-(*N*-2-fluorenylacetamido)deoxyguanosine; T_m^{260}, melting temperature at 260 nm; ϵ_P^{260}, DNA specific extinction coefficient per mole of phosphorus at 260 nm; $H_m(\lambda_m)$ and $H_0(\lambda_m)$, the hyperchromicity due to the complete reaction and that due to complete denaturation of the wavelength λ_m, respectively; $H_0(\lambda_0)$, the hyperchromicity due either to complete denaturation or to complete reaction at the wavelength λ_0; $H_m(\lambda_m,t)$ and $H(\lambda_0,t)$, the hyperchromicity at time t for the wavelengths λ_m and λ_0, respectively.

TABLE I: Kinetic Measurements on Native Calf-Thymus DNA.

	Temp, °C				
	42.9	47.3	50.7	54.2	57.9
$(k_{nd})_0$, min^{-1}	0.0	0.0	0.0040	0.0300	0.1480
$(k_{nr})_0$, min^{-1}	0.0014	0.0051	0.0088	0.0216	0.0334
$(k_{dr})_0$, min^{-1}	0.0950	0.1430	0.1950	0.2700	0.3800
$H_0(\lambda_0)$	0.330	0.330	0.330	0.330	0.330
$H_0(\lambda_m)$	0.430	0.430	0.430	0.430	0.430
$H_m(\lambda_m)$	0.882	0.893	0.902	0.910	0.919

The melting curves of native and carcinogen-reacted DNA were obtained directly on a two-dimensional recorder (Wilhelm *et al.*, 1970). Sodium borate buffer (0.042 M) (pH 9) was used in all kinetic experiments. The percentage of modified bases in carcinogen-reacted DNA was determined according to our method (Fuchs and Daune, 1972).

The change in optical density with time was measured in a Cary 15 spectrophotometer equipped with a jacketted cell maintained at a constant temperature. Since the kinetics of reaction of formaldehyde with DNA is temperature dependent, solutions were separately equilibrated to the given temperature before mixing. The formaldehyde solutions were preheated in a thermostated syringe (Cornwall syringe equipped with a metal pipetting holder). The DNA sample was heated in a 1-cm path length quartz cuvet in the thermostated cell housing of the spectrophotometer. Temperature was kept constant within ±0.1° by means of a Haake thermostat. The rapid mixing of the two solutions was obtained by six to seven cycles of injection–aspiration. The mixing time never exceeded 15 sec and the beginning of the kinetics could be obtained with great precision and reproducibility. The measurements at two wavelengths were made in two separate experiments under otherwise the same conditions.

Reagent grade 37% formaldehyde (Merck) was used for all experiments without any further purification. However, it was pretreated according to the procedure described by Freifelder and Davison (1963). The final mixture contained 1.05 M HCHO, 0.042 M sodium borate buffer (pH 9), and 0.3–0.9 optical density (OD) unit at 260 nm of DNA.

According to Utiyama and Doty (1971) and Von Hippel and Wong (1971), we determined for native DNA, and each carcinogen-modified sample, the two specific wavelengths λ_0 and λ_m. We found in every case λ_0 251 nm, the wavelength for which the extinction coefficient of denaturated but unreacted DNA coincides with that of reacted DNA; λ_m 278 nm is the wavelength for which the hyperchromicity of reacted DNA exhibits a maximum. We measured $H_m(\lambda_m)$ and $H_0(\lambda_m)$, the hyperchromicity due to complete reaction and that due to complete denaturation at the wavelength λ_m, respectively, and $H_0(\lambda_0)$, the hyperchromicity due either to complete denaturation or to complete reaction at the wavelength λ_0. According to the method developed by Utiyama and Doty (1971), we calculated the fraction of base pairs in the native, denaturated but unreacted, and reacted forms as follows

$$H_0(\lambda_0, t) = \frac{\Delta OD(\lambda_0, t)}{OD(\lambda_0, 0)}$$

$$H_m(\lambda_m, t) = \frac{\Delta OD(\lambda_m, t)}{OD(\lambda_m, 0)}$$

$$X_n(t) = 1 - [H(\lambda_0, t)/H_0(\lambda_0)]$$

$$X_r(t) = \frac{H(\lambda_m, t) - KH(\lambda_0, t)}{H_m(\lambda_m) - H_0(\lambda_m)}$$

where

$$K = H_0(\lambda_m)/H_0(\lambda_0)$$

and

$$X_d(t) = 1 - X_n(t) - X_r(t)$$

The functions of time X_n, X_d, and X_r were calculated with the assistance of a computer program from the experimental increase of optical density at the wavelengths λ_0 and λ_m.

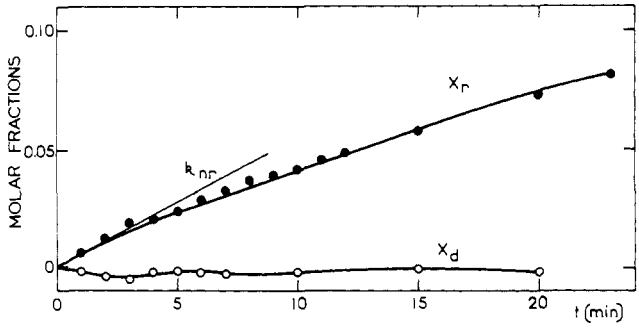

FIGURE 1: Variation with time of X_d and X_r on a native calf-thymus DNA sample at 47.3°. The open circles represent X_d, the fraction of base pairs that are in a denatured form, and the closed circles represent X_r, the fraction of bases that are reacted with formaldehyde. The reaction was performed in a mixture containing sodium borate buffer (0.042 M, pH 9) and formaldehyde (1.05 M).

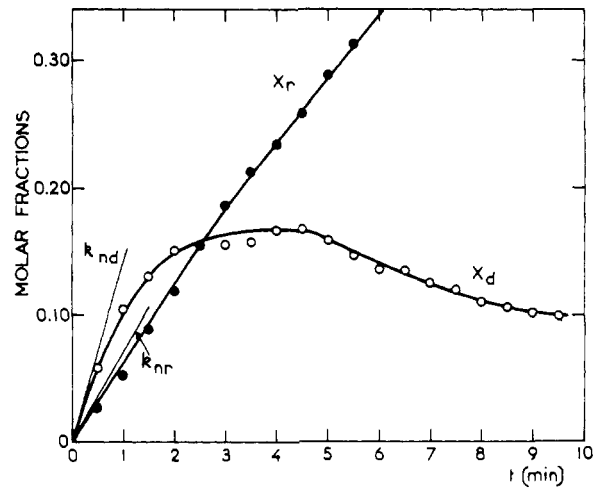

FIGURE 2: Variation with time of X_d (open circles) and X_r (closed circles) on a native calf-thymus DNA sample at 57.9°. The reaction was performed in a mixture containing sodium borate buffer (0.042 M, pH 9) and formaldehyde (1.05 M).

Results

Kinetic Experiments on Native Calf Thymus. We have measured the kinetics of native calf-thymus DNA at both the wavelengths λ_0 251 nm and λ_m 278 nm for various temperatures lying between T_m −30 and −10°. The parameters $H_0(\lambda_0)$, $H_0(\lambda_m)$, and $H_m(\lambda_m)$ were measured at each temperature. As shown in Table I, $H_0(\lambda_0)$ and $H_0(\lambda_m)$ are constant, whereas $H_m(\lambda_m)$ increases slowly with temperature. In Figures 1 and 2 are represented the calculated fractions $X_r(t)$ and $X_d(t)$ for native DNA at 47.3 and 57.9°. The initial slope of these curves gives us the initial rate constants $(k_{nr})_0$ and $(k_{nd})_0$ for X_r and X_d, respectively.

TABLE II: Kinetic Measurements on Carcinogen-Reacted DNA.[a]

	Temp, °C						
	39.4	42.5	45.0	47.7	49.5	53.7	55.3
$(k_{nd})_0$, min^{-1}	0.0	0.0	0.0087	0.0176	0.0320	0.1015	0.1100
$(k_{nr})_0$, min^{-1}	0.0056	0.0079	0.0087	0.0120	0.0165	0.0350	0.0650
$(k_{dr})_0$, min^{-1}	0.0660	0.0910	0.1150	0.1480	0.1750	0.2600	0.2950
$H_0(\lambda_0)$	0.314	0.314	0.314	0.314	0.314	0.314	0.314
$H_0(\lambda_m)$	0.380	0.380	0.380	0.380	0.380	0.380	0.380
$H_m(\lambda_m)$	0.752	0.759	0.765	0.772	0.777	0.788	0.792

[a] These measurements were performed on calf-thymus DNA having 1.7% of the total bases modified by the carcinogen.

$(k_{nr})_0$ is the initial rate constant of the reaction between formaldehyde and native DNA. In our experimental conditions where formaldehyde is present in great excess (1 M) we may consider $(k_{nr})_0$ as a pseudo-first-order rate constant. In the same way, $(k_{dr})_0$ is defined as the initial pseudo-first-order rate constant of the reaction between formaldehyde and fully denatured DNA. On the other hand, $(k_{nd})_0$ refers to the opening of base pairs in a "breathing unit," *i.e.*, that separate to permit reaction with formaldehyde; these parameters are given in Table I.

At temperatures lying between T_m −40 and −20° we always found a figure where the fraction of denaturated but unreacted bases X_d remains equal to zero over the course of the reaction (Figure 1). However, for temperatures lying between T_m −20° and T_m, we found that in the initial stage of the reaction there is a nonnegligible fraction of bases which is denaturated (unstacked but not yet reacted with formaldehyde (see Figure 2)). This fraction, X_d, comes back to zero after a time which decreases when temperature increases. This bell-shaped curve can be related to the creation of denatured bases induced by the reacted bases (Utiyama and Doty, 1971).

Kinetic Experiments on Carcinogen-Modified DNA. We used a DNA sample with 1.7% of modified bases. The kinetics of unwinding of this sample was followed at 251 and 278 nm over a temperature range lying between T_m −30 and −10°. The parameters $H_0(\lambda_0)$, $H_0(\lambda_m)$, and $H_m(\lambda_m)$ were measured at each temperature (Table II). Figures 3 and 4 represent the calculated fractions $X_r(t)$ and $X_d(t)$ at 45.0 and 55.3°. As shown in Table II, the initial rate constants $(k_{nd})_0$ and $(k_{nr})_0$ are in all experiments (at comparable temperatures) much greater for the carcinogen-reacted sample than for the native one. The remarks made about the shape of X_d in the case of native DNA remain quite valid with carcinogen-modified DNA. From a qualitative point of view, the fact that the initial rate constants are always higher with modified DNA indicates strongly that each carcinogen molecule induces in the DNA duplex a point of initiation to formaldehyde unwinding. Thus, it looks as if each fluorene residue destabilizes a certain number of base plates in its neighborhood, giving rise to centers, from which formaldehyde unwinding starts.

Measurement of Open Sequences in Native and Carcinogen-Reacted DNA. According to Von Hippel and Wong (1971), let us consider the following equilibrium

$$\text{closed regions (C)} \rightleftharpoons \text{open regions (O)} \qquad (1)$$

The initial rate of native DNA opening, as seen by formaldehyde, is equal to the initial rate for denatured DNA (under otherwise the same conditions of solvent and temperature) times the fraction (θ) of the open regions in native DNA

$$(k_{nd} + k_{nr})_0 = \theta(k_{dr})_0 \qquad (2)$$

In the first member of eq 2 we have to put the sum of k_{nd} and k_{nr}, the two rate constants both being involved in the opening of native DNA.

$(k_{nd})_0$ refers to the opening of bases before the chemical reaction has been achieved (the chemical reaction being the rate-limiting step; some open base pairs will react with

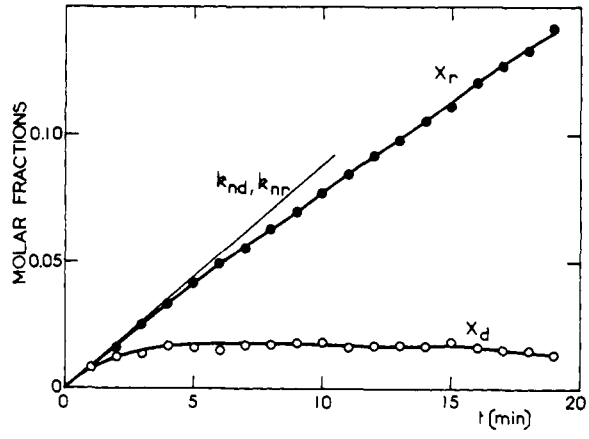

FIGURE 3: Variation with time of X_d (open circles) and X_r (closed circles) on a DNA reacted with N-AcO-AAF, at 45.0°; the fraction of modified bases was a = 0.017. The reaction was performed in a mixture containing sodium borate buffer (0.042 M, pH 9) and formaldehyde (1.05 M).

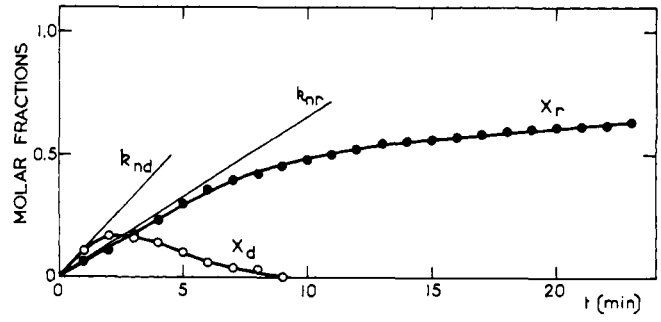

FIGURE 4: Variation with time of X_d (open circles) and X_r (closed circles) on a DNA reacted with N-AcO-AAF, at 55.3°; the fraction of modified bases was a = 0.017. The reaction was performed in a mixture containing sodium borate buffer (0.042 M, pH 9) and formaldehyde (1.05 M).

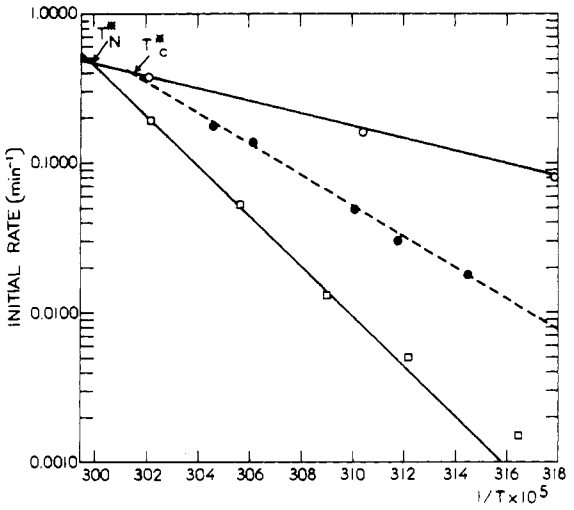

FIGURE 5: Arrhenius plots of the initial rate constants of attack by formaldehyde for native (squares), carcinogen-reacted (closed circles), and denatured DNA (open circles) in 0.042 M sodium borate buffer (pH 9, 1.05 M formaldehyde). The initial rate constants are plotted in a logarithmic scale *vs.* $1/T$. In the carcinogen-reacted DNA sample the fraction of modified bases was $a = 0.017$.

formaldehyde in a subsequent step). $(k_{nr})_0$ refers to the DNA opening followed by the chemical reaction with formaldehyde. The fraction of open sites is

$$\theta = (O)/[(O) + (C)]$$

which is approximately equal to

$$\theta \simeq (O)/(C) \quad (3)$$

in the conditions where DNA is mainly in the native form. On the other hand, if K represents the equilibrium constant for eq (1), we have

$$K = (O)/(C) \simeq \theta \quad (4)$$

Combining eq 2 and 4, one obtains

$$(k_{nr} + k_{nd})_0 = K(k_{dr})_0 \quad (5)$$

Equation 5 is only valid if (O) ≪ (C), *i.e.* if kinetic experiments are performed at temperatures much lower than the melting temperature.

We can remark that the sum $(k_{nd} + k_{nr})_0$ is determined directly from the slope of the experimental curve measured at 251 nm, as follows

$$(k_{nd} + k_{nr})_0 = \frac{\text{(initial slope at 251 nm)}}{H_0(\lambda_0) \times \mathrm{OD}(\lambda_0, 0)} \quad (6)$$

We measured the rate of formylation of native DNA and of carcinogen-reacted DNA at various temperatures, and plotted log $(k_{nd} + k_{nr})_0$ *vs.* $1/T$. These Arrhenius plots are drawn on the assumption that the plots are linear over the entire temperature range (Figure 5). This hypothesis seems to be valid as shown by Von Hippel and Wong (1971). The enthalpy of activation ΔH_n^* (or ΔH_c^*) of the reaction between formaldehyde and native (or carcinogen reacted) DNA is obtained from the slope of the straight lines given in Figure 5. We have also plotted log $(k_{dr})_0$ *vs.* $1/T$ in order to determine ΔH_d^*, the enthalpy of activation of the reaction between formaldehyde and denatured DNA. We found the following values: $\Delta H_d^* = 19.4$ kcal/mol, $\Delta H_n^* = 79$ kcal/mol, and $\Delta H_c^* = 48$ kcal/mol (for a sample containing 1.7% of modified bases).

From eq 5, in the case where (O) ≪ (C), it follows that

$$\Delta H_n = \Delta H_n^* - \Delta H_d^* \quad (7)$$

TABLE III: Calculation of the Number of Open Base Plates $x(T)$ Introduced by a Given Single Fluorene Derivative Bound to Native Calf-Thymus DNA.

Temp, °C	Fraction of Modified Bases	θ_n	θ_c	x
44.2	0.017	0.013	0.139	7–8
49.0	0.017	0.047	0.264	12–13
49.0	0.037	0.047	0.517	12–13
55.7	0.017	0.295	0.680	22–23

$$\Delta H_c = \Delta H_c^* - \Delta H_d^* \quad (8)$$

where ΔH_n and ΔH_c represent the equilibrium enthalpy change for the conformational reaction 1 for, respectively, nonreacted and carcinogen-reacted DNA. We found $\Delta H_n = 60$ kcal/mol and $\Delta H_c = 29$ kcal/mol for a DNA with 1.7% of modified bases.

Discussion

Thermodynamic Parameters in the Native DNA Opening Process; Equilibrium Enthalpy. The enthalpy of the melting process (involving unstacking of the base pairs) has been estimated to be ≃ 7 kcal/mol of base pairs in the case of calf-thymus DNA at pH 7 (Shiao and Sturtevant, 1973). Thus, from the above value of ΔH_n deduced from eq 7, we may estimate that the average number of base pairs in a "breathing unit" is equal to $\Delta H_n/7 \simeq 8$–9 base pairs in native calf-thymus DNA, over a temperature range lying between 10 and 35° below T_m. This value is in good agreement with the data of Von Hippel and Wong (1971). These authors found that the most probable cooperative opening unit seen by the formaldehyde probe is 9 ± 1 base pairs in length in native calf-thymus DNA (the solvent used in these experiments was 0.1 M phosphate buffer (pH 7.8)). It must be pointed out that this calculation is very simplified. In fact, the "breathing" process is cooperative and thus very difficult to interpret in a simple way.

Equilibrium Entropy. It is possible to estimate independently the size of an opening unit from the value of the equilibrium entropy change ΔS. In the classical relationship between K, ΔH, and ΔS (eq 9), K is obtained from values of

$$\ln K = -(\Delta H/RT) + (\Delta S/R) \quad (9)$$

kinetic constants at every temperature according to eq 5 and ΔH was determined from eq 7.

For the equilibrium native DNA ⇌ denatured DNA we found $\Delta S_n = 172$ eu. If the entropy of formation of a double helix is taken equal to 20 eu per base pair (Shiao and Sturtevant, 1973), we get another independent estimation of the most probable opening unit, *i.e.* 172/20 ≃ 8–9 base pairs in length. This number is in full agreement with that found previously from enthalpy measurements.

Carcinogen-Modified DNA: A Model of Local Disorganization. We measured over the same range of temperature the kinetics of unwinding at 251 and 278 nm of calf-thymus DNA having 1.7% of modified bases. If $\theta_n(T)$ represents the fraction of open base pairs in native DNA and $\theta_c(T)$ the corresponding relative amount in the carcinogen-reacted DNA at the same temperature T, one always observes that $\theta_c(T) > \theta_n(T)$. Thus, in addition to the "natural breathing" determined for native DNA, we have to admit the existence of open base pairs around each carcinogen-reacted guanine. If a is the fraction of modified base pairs, we may estimate

the average number $x(T)$ of open base pairs in the neighborhood of each carcinogen-reacted base as follows

$$x(T) = [\theta_c(T) - \theta_n(T)]/a \qquad (10)$$

The fraction a of reacted base pairs must be kept sufficiently low in order to avoid direct interaction between two neighbor loops ($a \leq 0.04$). In Table III are summarized the values of θ_n, θ_c, and x for three given temperatures. In order to verify that $x(T)$ is only dependent on temperature, we studied, at a given temperature, the kinetics of unwinding of DNA having a different fraction of reacted base pairs: a = 0.037. Thus, at 49°, *i.e.* 20° lower than the native DNA melting temperature, we can remark that the number x of open base plates in the vicinity of a covalently bound fluorene residue is independent of a and is equal to 12 base pairs (Table III).

This number $x(T)$ can therefore be considered as a characteristic parameter of the destabilization process induced by the carcinogen AAF. In a subsequent study we have obtained results showing a strong correlation between this parameter $x(T)$ and the carcinogen activity of different AAF derivatives (Fuchs and Daune, 1973).

As shown in Figure 5 the intersection temperature T^* of the straight line corresponding to native DNA, with the straight line corresponding to denatured DNA, is higher than the corresponding temperature relative to carcinogen-reacted DNA. As shown by Von Hippel and Wong (1971), this temperature gives a good indication of the melting temperature of native DNA in the presence of formaldehyde (which acts as a melting point depressant).

The intersection temperature T^* relative to native DNA was found equal to 60.5°. In the case of DNA having 1.7% of modified bases this intersection temperature was found equal to 58.9°, *i.e.* 1.6° lower than for native DNA. This decrease is in good agreement with the decrease observed by direct melting temperature measurements, since in the case of DNA modified with the carcinogen we observed a linear decrease of melting temperature with the amount of modified bases, the slope of the straight line being equal to −1.1°/% of modified bases (Fuchs and Daune, 1973). On the other hand, we can notice that 1 M formaldehyde depresses the melting temperature by about 10°.

At two given temperatures T_1 and T_2 according to eq 10, we can estimate the number $x(T_1)$ and $x(T_2)$ of open base pairs around an inserted fluorene ring. As shown in Table III, the parameter x is independent of the percentage of modified bases; thus according to the general Arrhenius relation (eq 11) we can calculate the equation of the

$$\ln k = \ln A - \frac{E}{RT} = \ln A - 1 - \frac{\Delta H^*}{RT} \qquad (11)$$

straight line (in an Arrhenius plot) we expect to find for a DNA having a percentage of modified bases equal to a. At temperature T_1 let us call k_{1c} and k_{1n} the initial rate constant measured by the slope of the kinetic curves followed at 251 nm (*i.e.*, defined in eq 6 by the sum $(k_{nd} + k_{nr})_0$) for carcinogen-reacted and native DNA, respectively. According to our hypothesis of additional opening induced by each fluorene nucleus, k_{1c} for a DNA having a per cent of modified bases is given as follows

$$k_{1c} = k_{1n} + k_{1d}x(T_1)a \qquad (12)$$

where k_{1d} is $(k_{dr})_0$ at temperature T_1. Equation 12 can be written at temperature T_2. Equation 11 is thus determined by the set of values $(1/T_1, \ln k_{1c})$ and $(1/T_2, \ln k_{2c})$. It follows the expressions of ΔH_c^* and $\ln A_c$ as a function of a by

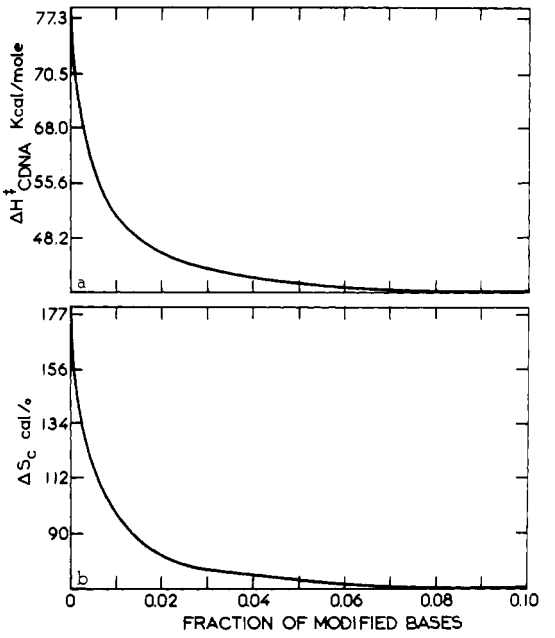

FIGURE 6: (a) Computer-calculated variation of ΔH_c^*, enthalpy of activation of the reaction between formaldehyde and carcinogen-reacted DNA, *vs.* the fraction of modified bases. (b) Computer-calculated variation of ΔS_c, equilibrium entropy change for the conformational reaction between carcinogen-reacted DNA and denatured DNA, *vs.* the fraction of modified bases.

combining eq 11 and 12. T^* (a) is defined as the reciprocal

$$\Delta H_c^*(a) = R\frac{T_2T_1}{T_1 - T_2} \ln \frac{k_{1n} + k_{1d}x(T_1)a}{k_{2n} + k_{2d}x(T_2)a} \qquad (13)$$

$$\ln A_c(a) = \ln (k_{1n} + k_{1d}x(T_1)a) + \frac{T_2}{T_1 - T_2} \ln \frac{k_{1n} + k_{1d}x(T_1)a}{k_{2n} + k_{2d}x(T_2)a} + 1 \qquad (14)$$

of the abcissa of the intersection point of the two straight lines in the Arrhenius plot

$$\ln k_C = \ln A_c(a) - 1 - \frac{\Delta H_c^*(a)}{RT} \qquad (15)$$

$$\ln k_D = \ln A_d - 1 - \frac{\Delta H_d^*}{RT} \qquad (16)$$

Equation 17 follows. The thermodynamic parameters ΔH_c

$$T^*(a) = \frac{\Delta H_c^*(a) - \Delta H_d^*}{R \ln A_c(a)/A_d} \qquad (17)$$

and ΔS_c for the conformational reaction 1 for carcinogen-reacted DNA may be calculated when the concentration of open base pairs (O) ≪ (C)

$$\Delta H_c(a) = \Delta H_c^*(a) - \Delta H_d^* \qquad (18)$$

and

$$\Delta S_c(a) = R \ln A_c(a)/A_d \qquad (19)$$

As shown in Figure 6, after a very fast initial decrease $\Delta H_c^*(a)$ and $\Delta S_c(a)$ tend to a constant value respectively equal to 40 kcal/mol and 70 eu. Thus, according to eq 18 and to the above given value for ΔH_d^*, the equilibrium enthalpy change for the conformational reaction between carcinogen-reacted DNA and denatured DNA drops to 20 kcal/mol for DNA having ≥5% of modified bases. However, we must be careful in interpreting these limit values, because they occur at ratios of modified bases where neighboring fluorene-induced loops may interact ($a > 0.05$). We

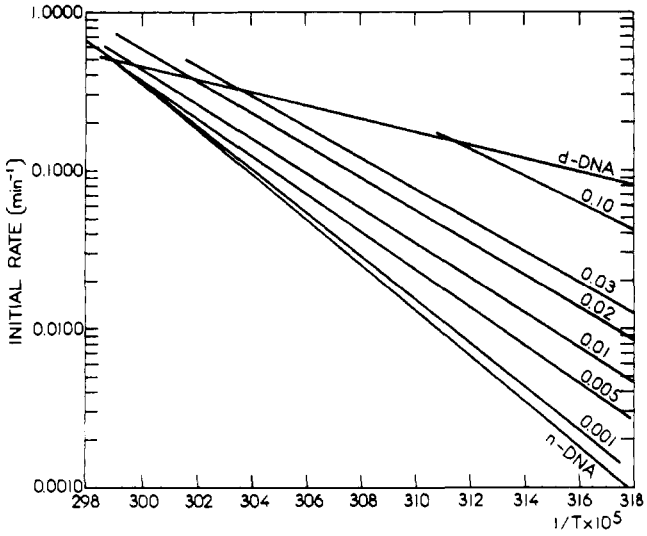

FIGURE 7: Computer-calculated Arrhenius plots for DNA reacted with N-AcO-AAF. The fractions of modified bases are written on each straight line.

have plotted the calculated Arrhenius lines (Figure 7) for various fractions of modified bases. The intersection temperatures have been drawn *vs.* a in Figure 8. $T_c^*(a)$ shows a decrease which can be approximated to a straight line; this fact is in good agreement with the linear decrease of the melting temperature as directly measured without formaldehyde. Moreover, there is a good correspondence in the value of the slope, since in the case of the melting temperature we found a decrease of 1.1°/% of modified bases (Fuchs and Daune, 1973) and in the case of $T_c^*(a)$ the slope is equal to −1.3°/% of modified bases.

In conclusion, the destabilizing effect measured by direct helix–coil transition is consistent with the model obtained from kinetic data. From analog experiments done with one carcinogenic and one noncarcinogenic analog of *N*-2-acetylaminofluorene we have concluded that local opening of DNA double helix only occurs with carcinogenic compounds, as opposed to the noncarcinogenic one (Fuchs and Daune, 1973). This fact has been correlated to the "molecular thickness" of the aromatic nucleus which can or cannot

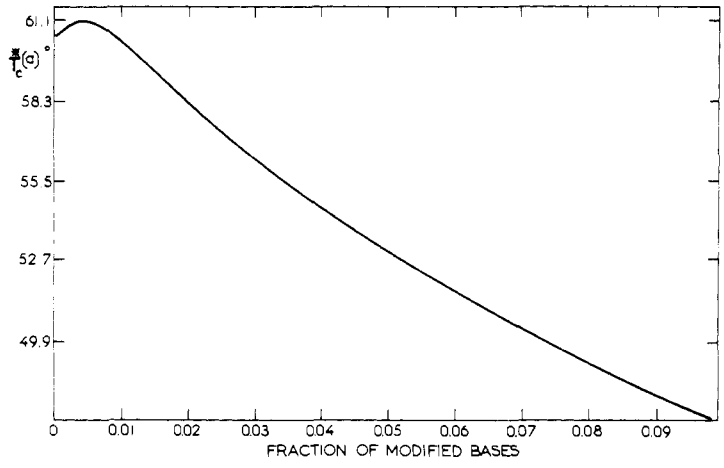

FIGURE 8: Computer-calculated variation of T_c^*, intersection temperature of the Arrhenius plot corresponding to a given carcinogen-reacted DNA sample, with the Arrhenius plot corresponding to denatured DNA, *vs.* the fraction of modified bases.

be inserted in place of guanine in the double helix giving or not giving rise to the local distortion. On the other hand, as shown by Kapuler and Michelson (1971) the reaction of the fluorene on C_8 is only possible if we admit the guanine in a transient open state (for steric reasons). Thus, the most reactive guanines in a given DNA molecule are those which are frequently implied in "breathing units" (maybe guanines isolated in A + T rich regions, where breathing is supposed to take place preferentially). Such regions, as outlined by Von Hippel and Wong (1971), "contain enough information, to play a role in biologically significant molecular recognition mechanisms." Thus, the permanent and local opening of such segments in native DNA of the magnitude given in Table III may disturb these specific interaction mechanisms (either by creating new and wrong interaction sites or by inhibiting the specific recognitions). Though the biological consequences are as yet quite unclear, the creation of a permanently open region could be viewed as the triggering event of chemical carcinogenesis by aromatic amides.

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