PHYSICAL STUDIES OF CHLOROACETALDEHYDE LABELLED FLUORESCENT DNA

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Summary

The reaction of chloroacetaldehyde with denatured DNA produces a fluorescent DNA where both the adenine and cytosine bases are modified. The rate of modification of DNA by chloroacetaldehyde was measured using the absorption spectrum shift. The depolarization and quantum yield of native DNA and denatured DNA were investigated as a function of temperature.

The melting points and the renaturation rates of a series of derivative DNA's were investigated. The melting point was decreased by 1.3°C for each base modified per 100 base pairs corresponding to a 2.8 Kcal destabilizing free energy per mismatched base pair. The renaturation rate of the derivative DNA is reduced by a factor 2 when the melting temperature is lowered by 13°C.

INTRODUCTION

The chemical modification of the base residues in nucleic acids is of importance for structural and functional investigation of RNA and DNA.[1] Hutton and Wetmur[2] have investigated the effects of mismatched base pairs due to deaminated DNA and glyoxalated DNA on DNA melting points and renaturation rates. In both cases, the renaturation rate was reduced by a factor of 2 when the melting point was lowered by around 20°C. Kochetkov[3] et al have found that chloroacetaldehyde reacts with 9-N-methyladenine and 1-N-methylcytosine in weakly acidic aqueous solution. Barrio et al[4] have investigated the flurorescent properties of adenosine and cytidine derivatives.

We have found that denatured DNA can react with chloroacetaldehyde to produce fluorescence properties similar to the monomers. The effects of mismatched base pairs, due to DNA modified with chloroacetaldehyde, on the melting point and renaturation rate have been investigated. The fluorescent properties of the derivative DNA have been studied.

MATERIALS AND METHODS

T2 DNA was sonicated and purified as described before.[2] Chloroacetalde-

hyde was a gift of Professor Barrio. The concentration of chloracetaldehyde was determined from NMR spectra.

The fluorescent derivative T2 DNA (εA, εC DNA) was prepared by incubating the following solution at 53°C: 4.0 ml $NaC_2H_3O_2$-$HC_2H_3O_2$ buffer, pH 4.5, 12 ml dimethylformamide, 2 ml DNA in H_2O, and 2 ml 0.17 M $ClCH_2CHO$. Under these conditions, about 1.5% of the A and C residues of T2 DNA are modified per 5 minute incubation. The fluorescent derivative T2 DNA was then dialysed against 0.4 M NaCl, 0.005 M NaH_2PO_4 plus NaOH, pH 7.15, 2 x 10^{-4} M EDTA. Native fluorescent derivative T2 DNA was obtained by incubating the denatured fluorescent derivative T2 DNA at 61°C overnight. The renaturation rates and melting points of modified T2 DNA were measured as described before.[2]

Technical fluorescence emission spectra were measured on a Hitachi Perkin-Elmer MPF -2A spectrophotometer. Excitation polarization spectra of fluorescence were measured on an analog-digital version of the instrument described by Weber and Bablouzian.[5] The quantum yield was calculated from the observed absorbance at 310 nm and the areas of the molecular emission spectra. Quinine sulfate in 0.1 N H_2SO_4 was used as a reference compound, assuming a quantum yield of 0.70.[6]

RESULTS AND DISCUSSION

(a) Chemistry and Kinetics of Modification

The absorption spectrum of completely modified T2 DNA is shown in Figure 1(a). The absorption minimum at 230 nm and absorption maximum at 257 nm for T2 DNA were shifted respectively to 240 nm and 266 nm. The modification percentage of T2 DNA was calculated from the shift of the absorption minimum wavelength. Figure 1(b) shows that the reaction of chloroacetaldehyde with DNA is a psuedo-first order reaction. The real second order kinetic rate constant may be calculated to be 3 x 10^{-3} sec^{-1} M^{-1} from Figure 1(b) at 53°C.

(b) Fluorescence Properties

The fluorescence emission spectra for native and denatured fluorescent derivative T2 DNA are shown in Figure 2. The spectra consist of two bands, one at 347 nm and one at 400 nm, corresponding to the modified cytosine and

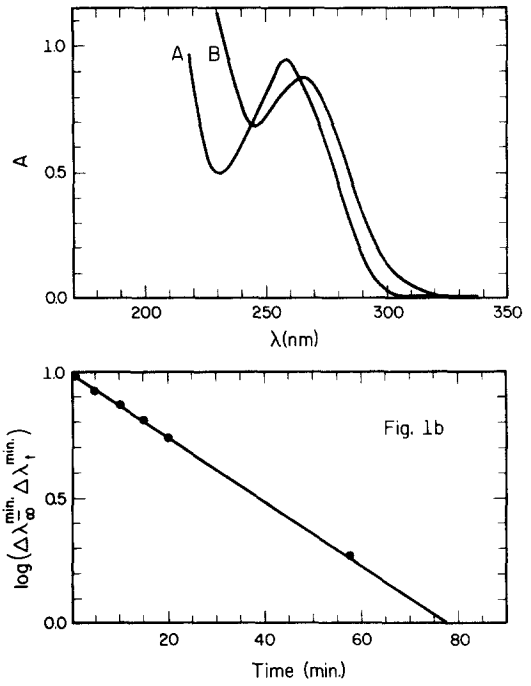

Figure 1 (a): Ultraviolet absorption spectra of A: T2 DNA, B: fluorescent derivative T2 DNA. The concentration of DNA is 1.3×10^{-4} M.
(b): A psuedo-first order rate plot for the modification of T2 DNA with chloroacetaldehyde. The solution is described in the text, except the chloroacetaldehyde concentration is ten times higher in this case.

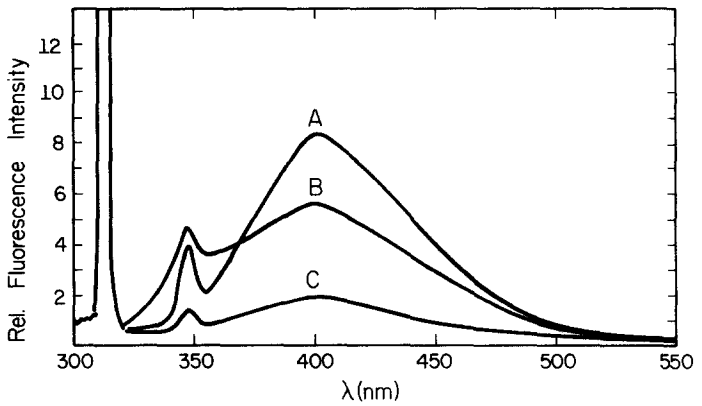

Figure 2: Technical fluorescence spectra of fluorescent derivative T2 DNA with about 15% of the base pairs modified. A: native fluorescent derivative T2 DNA at room temperature. B: denatured fluorescent derivative T2 DNA at room temperature. C: native fluorescent T2 DNA at 59°C.

adenine base residues (εC and εA) respectively. At a higher percentage of base modification, the fluorescence emission intensity at 347 nm for the ε-

cytosine residue does not increase linearly, in contrast to the ε-adenine residue band. This may be due to energy transfer from the excited state of ε-cytosine to ε-adenine base residues. We also find that the fluorescence intensity of ε-cytosine residues in denatured derivative DNA is higher than in native derivative DNA, while that of ε-adenine in denatured derivative DNA is lower than in native derivative DNA. This result may also be due to energy transfer. The fluorescence yield of derivative DNA proved to be very sensitive to temperature as may be seen in Figure 2. These results are similar to those of εA · HCl.[7]

The quantum yield for ε-adenine base residues of denatured T2 DNA is about 0.2 at neutral pH at 33°C. This quantum yield is a little smaller than that of εA · HCl. The value of the fluorescence polarization of native derivative DNA is 0.178 at room temperature with a 310nm excitation wavelength. At 59°C, the value of the polarization for native derivative DNA becomes 0.146, while that of denatured derivative DNA is 0.083. These results show that the polarization value may be used to detect the helix coil transition of labelled DNA alone or in the presence of other unlabelled DNA.

(c) Thermodynamic Studies of Fluorescent Derivative DNA

Fink and Crothers[8] have theoretically calculated the relation of the melting point of RNA or DNA with buldge defects in one strand. We will derive a similar result with a different approach for the case of mismatched base pairs, instead of only the buldge effect in one strand.

Let x be the fraction of base pairs modified. At small values of x, we may consider that only one isolated mismatched base pair exists. In other words, the case of two or more consecutive mismatched base pairs may be neglected. Then we may use equation (1),

$$\Delta G^{x} = (1 - x)\Delta G_0 + x\Delta G_1, \qquad (1)$$

where ΔG^{x} is the average free energy per base pair and ΔG_1 is the free energy of formation of a modified base whether or not such a pair really forms.

At the melting point, Tm^x, ΔG^x must be zero. Thus, we have equation (2)

$$(1 - x)\Delta G_0 + x\Delta G_1 = 0 \text{ at } Tm^x \quad . \tag{2}$$

As

$$\Delta G_0 = \Delta H_0 - Tm^x \Delta S_0$$

and

$$Tm^0 = \frac{\Delta H_0}{\Delta S_0} \quad , \tag{4}$$

we may obtain equation (5) from eqs. (2), (3), and (4)

$$Tm^x = Tm^0 + \frac{Tm^0}{\Delta H_0}(\Delta G_1 - \Delta G_0)x \quad . \tag{5}$$

This result is similar to that derived by Fink and Crothers.[8] A study of the melting point of modified DNA versus fraction of modification of base pairs will give the value of destabilizing free energy ($\Delta G_1 - \Delta G_0$) due to a mismatched base pair. The above argument is valid only if the change of G + C content is neglected in the range of a small fraction of modification of DNA.

Figure 3(a) shows the melting point of fluorescent derivative T2 DNA modified by chloroacetaldehyde versus the fraction of base pairs modified. The melting point was decreased by 1.3°C with one mismatched base pair per 100 base pairs, corresponding to a 2.9 Kcal destabilizing free energy. These results are similar to those for the buldge defect in one strand reported by Fink and Crothers.[8] The effects of mismatches and buldges appear to be almost the same.

(d) Renaturation Kinetics of Fluorescent Derivative DNA

Theoretical calculations of the effect of mismatches on renaturation rates have been performed by Hutton and Wetmur.[2] The results are shown below

$$\% \text{ relative rate} = \sum_{M=1}^{\infty} \sum_{M'=1}^{\infty} \frac{100x^3 M(1-x)^{M+M'-1}}{D} \tag{6}$$

where

$$D = 1 + \frac{1}{S^{M-2}}\left(\frac{S-1}{f} - S + \frac{1}{\sigma'} + \frac{1}{\sigma' S^{M+M'-2}}\left(\frac{S-1}{f} - S + \frac{1}{\sigma'}\frac{\bar{S}}{S}\cdot\frac{(S-1)}{(\bar{S}-1)}\right)\right. \tag{7}$$

and

$$S = e^{-\Delta H(Tm-Tr)/RTrTm} \quad ; \quad \bar{S} = e^{-\Delta H(Tm-Tr+\Delta Tm)/R(Tm+\Delta Tm)Tr} \tag{8}$$

$$\sigma' = \left(\frac{\bar{S}}{S}\right)^{\frac{1}{x}} \quad ; \quad \sigma' \leq f \leq 1 \quad \text{(The choice of f is unimportant.)} \tag{9}$$

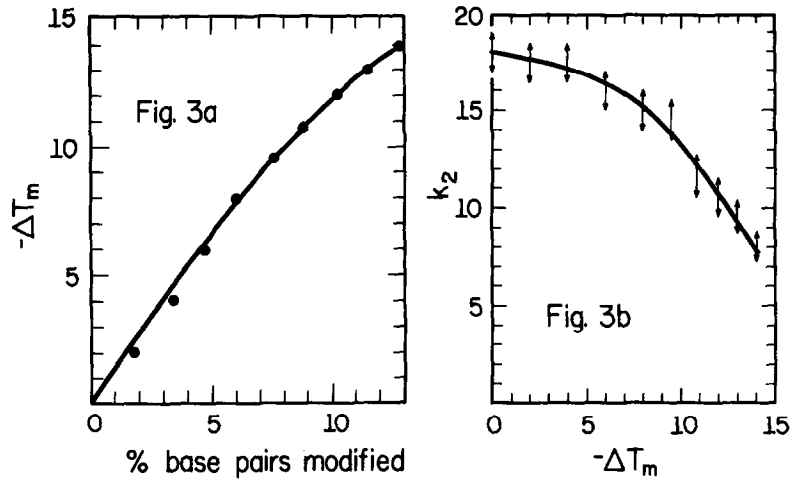

Figure 3 (a): The change in melting point ΔTm ($^{\circ}C$) of fluorescent derivative T2 DNA as a function of the percent of base pairs modified.
(b): The second order renaturation rate constant, k_2 (liters/moles-sec), of fluorescent derivative T2 DNA at 61°C in 0.4 M Na^+ as a function of the change in melting point, ΔTm ($^{\circ}C$).

Tr and Tm are the renaturation and melting point temperatures of unmodified DNA. ΔTm is the change in melting point due to mismatch.

σ' may be correlated with $(\Delta G_1 - \Delta G_0)$.

$$\Delta G_1 - \Delta G_0 = -RT \ln \sigma' \qquad (10)$$

The renaturation rate constant, k_2, of fluorescent derivative T2 DNA is shown in Figure 3(b) as a function of ΔTm. The renaturation rate constant is decreased by a factor of 2 when the melting temperature is lowered by 13°C, comparable to 11.5% mismatch. These results are similar to those predicted by eq. (6). The effects on Tm and k_2 of modification of DNA with glyoxal and chloroacetaldehyde are similar.

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REFERENCES

(1) Kochetokov, N. K. and Budowsky, E. I. (1969), Prog. Nuc. Acid Res. and Mol. Biol. 9, 403.

(2) Hutton, J. R. and Wetmur, J. G., Biochemistry (in press).
(3) Kochetkov, N. K., Shibaev, V. W., and Kost, A. A. (1971), Tetrahedron Lett., 1993
(4) Barrio, J. R., Secrist, J. A., III, and Leonard, N. J. (1972), Biochem. Biophys. Res. Commun. 46, 597.
(5) Weber, G. and Bablouzian, B. (1966), J. Biol. Chem. 241, 2558.
(6) Scott, T. G., Spencer, R. D., Leonard, N. J., and Weber, G. (1970), J. Amer. Chem. Soc. 92, 687.
(7) Secrist, J. A., III, Barrio, J. R., Leonard, N. J., and Weber, G. (1972), Biochemistry 11, 3499.
(8) Fink, T. R. and Crothers, D. M., J. Mol. Biol. (1972), 66, 1.