Kelly, T. J., and Smith, H. O. (1970), *J. Mol. Biol. 51*, 393–409.
Landy, A., Abelson, J., Goodman, H. M., and Smith, J. D. (1967), *J. Mol. Biol. 29*, 457–471.
Landy, A., Ross, W., and Foeller, C. (1974), *Nature (London)* (in press).
Linn, S., and Arber, W. (1968), *Proc. Nat. Acad. Sci. U. S. 59*, 1300–1306.
Meselson, M., and Yuan, R. (1968), *Nature (London) 217*, 1110–1114.
Meselson, M., Yuan, R., and Heywood, J. (1972), *Annu. Rev. Biochem. 41*, 447–466.
Middleton, J. H., Edgell, M. H., and Hutchison, C. A., III (1972), *J. Virol. 10*, 42–50.
Miller, R. C., Besmer, P., Khorana, H. G., Fiandt, M., and Szybalski, W. (1971), *J. Mol. Biol. 56*, 363–368.
Morrow, J. F., and Berg, P. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 3365–3369.
Mowbray, S. L., and Landy, A. (1974), *Proc. Nat. Acad. Sci. U. S.* (in press).
Peacock, A. C., and Dingman, C. W. (1968), *Biochemistry 7*, 668–674.
Roulland-Dussoix, D., and Boyer, H. W. (1969), *Biochim. Biophys. Acta 195*, 219–229.
Roy, P. H., and Smith, H. O. (1974), *J. Mol. Biol.* (in press).
Russell, R. L., Abelson, J. N., Landy, A., Gefter, M. L., and Smith, J. D. (1970), *J. Mol. Biol. 47*, 1–13.
Sharp, P. A., Sugden, B., and Sambrook, J. (1973), *Biochemistry 12*, 3055–3063.
Sinsheimer, R. L. (1959), *J. Mol. Biol. 1*, 43.
Smith, H. O., and Nathans, D. (1973), *J. Mol. Biol. 81*, 419–423.
Smith, H. O., and Wilcox, K. W. (1970), *J. Mol. Biol. 51*, 379–391.
Yoshimori, R. (1971), Ph.D. Thesis, University of California, San Francisco, Calif.

Physical Studies of *N*-Acetoxy-*N*-2-acetylaminofluorene-Modified Deoxyribonucleic Acid†

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ABSTRACT: Denatured T4 phage DNA was reacted with *N*-acetoxy-*N*-2-acetylaminofluorene. The rate of reaction was determined using the change in the melting temperature of renatured DNA as an indicator of the percentage of modification. The renaturation rates of a series of modified DNAs were investigated. This rate is reduced by a factor of 2 when the melting temperature is lowered 15°. The absorption at 305 nm, the negative circular dichroism band around 300 nm, the melting temperature change, and the buoyant density shift due to modification were investigated. From these data, we conclude that the melting temperature change due to 1% modified base pairs is 0.9°. Electric dichroism of modified DNA was studied in an alternating field. From the dichroism, we calculate the angle between the aminoacetylfluorene residue 305-nm transition moment and the DNA helix axis to be 60 ± 4° under conditions where the 305-nm band is optically active. The aminoacetylfluorene residue must be located in an asymmetric potential field outside of the DNA helix.

Previous studies have shown that *N*-2-acetylaminofluorene a carcinogen, when administered *in vivo*, is converted in rat liver to the reactive carcinogenic ester, *N*-2-acetylaminofluorene *N*-sulfate. N-AcO-AAF[1] is used as an analog for *N*-sulfate-*N*-2-acetylaminofluorene as the analog reacts slower with nucleophiles, hydrolyzes slower, but forms the same products (Cramer *et al.*, 1960; Miller *et al.*, 1961; Marroquin and Farber, 1962, 1965; Henshaw and Hiatt, 1963; Willard and Irving, 1964; Irving *et al.*, 1967; Kriek, 1968; Agarwal and Weinstein, 1970). The N-AcO-AAF reaction site on DNA has been demonstrated to be the C-8 position of guanine with AAF present in the product (Kriek *et al.*, 1967). Recent work has shown that adenine nucleotides and N-AcO-AAF may react to some extent, although with lower reactivity than guanine nucleotides (Miller and Miller, 1969; Marroquin and Coyote, 1970; Kapuler and Michelson, 1971; Kriek and Reitsema, 1971; Michelson *et al.*, 1972).

The effects of various mismatched base pairs and chemically modified bases on the thermal stability of nucleic acids have been investigated (Kotaka and Baldwin, 1964; Bautz and Bautz, 1969; Laird *et al.*, 1969; Uhlenbeck *et al.*, 1971; Fink and Crothers, 1972; Lee and Wetmur, 1973; Hutton and Wetmur, 1973; Gralla and Crothers, 1973). The effect of N-AcO-AAF modification of DNA on the melting temperature has been studied by Troll *et al.* (1969) and Fuchs and Daune (1971, 1972). Because of the methods used to separate the hydrolysis products of N-AcO-AAF from the modified DNA, the melting temperature change resulting from modification was substantially underestimated. Recently, Fuchs and Daune (1972) interpreted their results on the optical properties of AAF-DNA to indicate that the AAF residue was inserted into the DNA helix.

In this work, we present data on the time course of the reaction of N-AcO-AAF with denatured DNA, measurements of

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[1] Abbreviations used are: N-AcO-AAF, *N*-acetoxy-*N*-2-acetylaminofluorene; AAF, the *N*-2-acetylaminofluorene residue; AAF-DNA, the *N*-2-acetylaminofluorene residue covalently linked to DNA bases; CD, circular dichroism; ED, electric dichroism.

the physical properties of native AAF-DNA, together with a correlation between these properties and the fraction of DNA bases modified, and ED studies of AAF-DNA which indicate that the AAF residue is not inserted into the helix.

Materials and Methods

DNA Preparation. Bacteriophage T4M41 was a gift of Dr. D. A. Goldthwait. The phage were then grown according to a scaled-down version of the method described by Carroll *et al.* (1972). The phage were released from the concentrated infected cells by vigorous aeration in the presence of lysozyme and chloroform and were purified by two cycles of differential sedimentation. T4 DNA was prepared by phenol and chloroform extraction of the phage, dialysis, and sonication with a Bronson power sonifier.

Reagents. N-AcO-AAF was a generous gift from Professor J. A. Miller, McArdle Laboratory, University of Wisconsin. Proflavine and *d*-10-camphorsulfonic acid were obtained from Sigma and Aldrich, respectively. All other chemicals used were reagent grade.

N-AcO-AAF Treatment of DNA. T4 DNA (30 ml; 220 μg/ml), in 0.01 M NaCl–0.002 M sodium citrate buffer (pH 7.0), was denatured by heating for 5 min at 100° and quenched in ice. The DNA solution was mixed with 30.8 mg of N-AcO-AAF dissolved in 28 ml of 100% ethanol and incubated at 37°. At various time intervals, aliquots were removed, quenched in ice, and dialyzed at 4° for four changes (24 hr) against the same 50% ethanol solution as used for the reaction and then into the buffer solution alone. After dialysis, the solution was sedimented at 40,000*g* for 1 hr to remove aggregated DNA-AAF which causes light absorption or scattering at 340 nm (Kapular and Michelson, 1971) and to remove any aggregated reagent hydrolysis products.

Studies of the time course of the reaction were also carried out under the same conditions with varying N-AcO-AAF concentrations. Control DNA was carried through the procedure with no N-AcO-AAF and with incubation at 37° for the largest time used for any of the samples. Neither the melting temperature nor the renaturation rate of the control DNA was affected by the treatment.

Melting Temperatures. Modified and unmodified T4 DNA samples were exhaustively dialyzed at 4° against 0.4 M NaCl–0.002 M sodium citrate buffer (pH 7.0). The melting temperatures were taken in a Beckman Acta III spectrophotometer as described before (Hutton and Wetmur, 1973).

Renaturation Kinetics. Renaturation rates were measured as described before (Hutton and Wetmur, 1973). The renaturation temperature was 60°. Renaturation and melting cycles were repeated for each measurement.

Buoyant Densities. Buoyant densities were measured in neutral CsCl solutions in a Beckman Model E analytical ultracentrifuge using native *Escherichia coli* DNA as a reference.

Absorption Spectra. Absorption spectra were measured at 10° in a Cary 15 spectrophotometer.

Circular Dichroism. CD spectra were measured at 10° in a Jasco ORD/UVS with CD attachment. The CD spectra were calibrated with a 0.1% aqueous solution of *d*-10-camphorsulfonic acid (Yang and Samejima, 1969).

Electric Dichroism. Measurements of ED were carried out using the alternating field birefringence apparatus described elsewhere (Miller and Wetmur, 1974) with the analyzer and Fresnel rhomb removed and with the polarizer set at 0° with respect to the electric field; 7.7-kHz pulses of 10-msec duration were used. The root-mean-square field strength, kept constant throughout each spectrum, was 4–6 kV/cm. The melting temperature and circular dichroism and absorption spectra were measured before dialysis at 4°, 70–90 μg/ml of DNA, to 2×10^{-4} M NaEDTA buffer (pH 7.0). After dialysis, cold glycerol was added to 58% by weight as determined by measuring the refractive index of an aliquot at 20° using an Abbe 3-L refractometer. ED, CD, and absorption spectra were measured at 10°. The CD and absorption spectra agreed with the spectra measured before dialysis.

With the polarizer set parallel to the electric field, the electric dichroism, ΔA, is given by

$$\Delta A = (3/2)[\log (1 + \Delta I_{\parallel}/I_{\parallel})] \quad (1)$$

where $I_{\parallel}$ is the initial light intensity and $\Delta I_{\parallel}$ is the change in intensity upon application of the field. $I_{\parallel}$ and $\Delta I_{\parallel}$ are measured in volts at the output of the cathode follower and read off an oscilloscope tracing.

Calculation of the Orientation Angle. The angle, α, between the transition moment of the chromophore and the symmetry axis can be related to the reduced dichroism, $\Delta A/A$, where A is absorbance by the equation (Yoshioka and Watanabe, 1964)

$$\Delta A/A = (3/2)[3 \cos^2 \alpha - 1]\Phi \quad (2)$$

where Φ, the orientation, is the fractional orientation of the sample. Φ will vary from zero to one as the sample varies from random to perfect orientation. As the sample cannot be completely oriented, Φ must be calculated. If the chromophore of interest (AAF), *c*, is bound to DNA

$$\Phi(c) = \Phi(\text{DNA}) \quad (3)$$

We assume that $\alpha(\text{DNA}) = 90°$ and measure $\Delta A/A$ where $A(\text{DNA}) \gg A(c)$ and where the transitions involved in A(DNA) are in the plane of the bases. Φ is determined from eq 2. $\alpha(c)$ may then be determined by measuring $\Delta A/A$ where $A(c) \gg A(\text{DNA})$. For cases where the DNA and chromophore absorption spectra overlap, absorbance and ED values are determined by comparison with a reference sample of DNA alone.

Results and Discussion

Labeling Kinetics. The rate of reaction of N-AcO-AAF with DNA has been studied under one set of solvent conditions. The effect of initial N-AcO-AAF concentration on the progress of the reaction is shown in Figure 1a. The amount of modification is indicated by ΔT_m, the decrease in DNA melting temperature due to the modification. A fraction of base pairs modified, p, is shown below to be related to ΔT_m by

$$p = 0.011\Delta T_m \quad (4)$$

Three reactions need to be considered. The hydrolysis of N-AcO-AAF (F) with rate constant k_1. The reaction of guanine residues (G) with N-AcO-AAF with rate constant k_2, and the reaction of adenine residues with N-AcO-AAF with rate constant k_2'

$$-\text{d(G)}/\text{d}t = k_2(\text{G})(\text{F}) \quad (5a)$$

$$-\text{d(A)}/\text{d}t = k_2'(\text{A})(\text{F}) \quad (5b)$$

$$-\text{d(F)}/\text{d}t = k_1(\text{F}) + k_2(\text{G})(\text{F}) + k_2'(\text{A})(\text{F}) \quad (5c)$$

Combining eq 5a and 5b and integrating

$$(\text{G})/(\text{G}_0) = [(\text{A})/(\text{A}_0)]^{k_2/k_2'} \quad (6a)$$

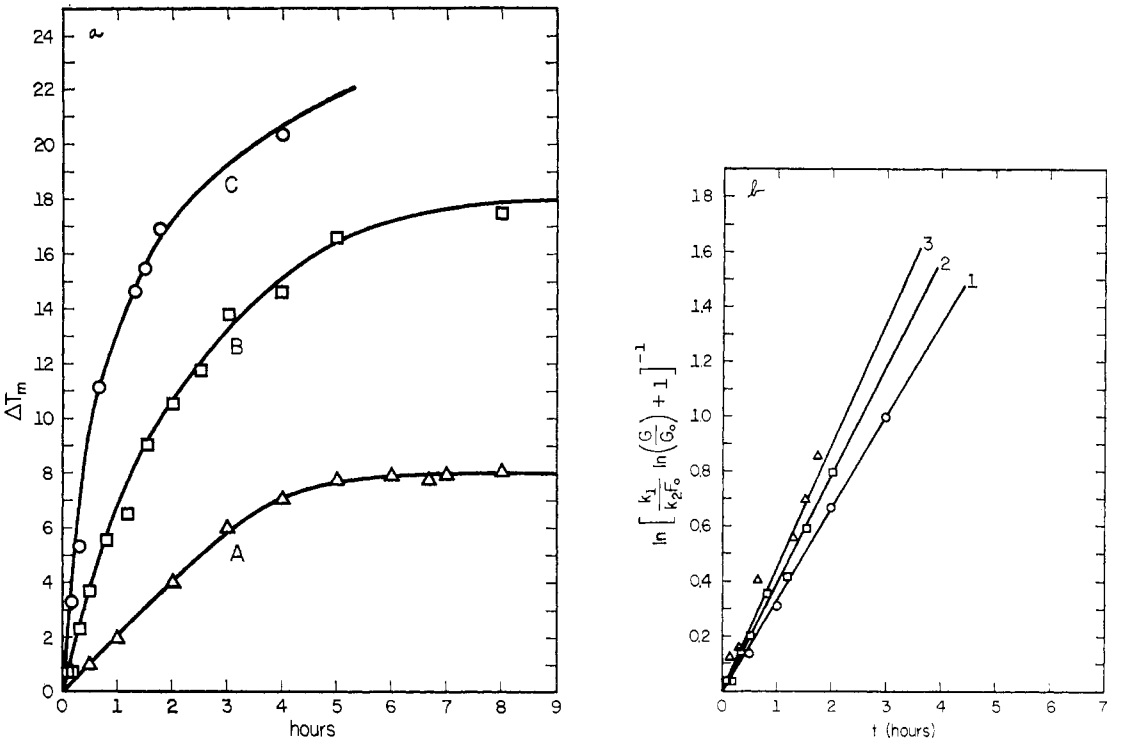

FIGURE 1: (a) The extent of modification of DNA by N-AcO-AAF, indicated by ΔT_m, the decrease in melting temperature due to modification, is shown as a function of time. The initial N-AcO-AAF concentrations were (A) 8.10×10^{-4} M, (B) 1.96×10^{-3} M, and (C) 3.35×10^{-3} M. The initial DNA nucleotide concentrations were (A) 1.32×10^{-4} M, (B) 3.23×10^{-4} M, and (C) 3.68×10^{-4} M. The solvent was 0.01 M NaCl–0.001 M citrate buffer (pH 7.0), 50% ethanol, 37°. (b) Rate plots for the reactions shown in part a: 1, C; 2, B; 3, A.

The limit of this result when time goes to infinity is

$$(G_\infty)/(G_0) = [(A_\infty)/(A_0)]^{k_2/k_2'} \tag{6b}$$

Kapuler and Michelson (1971) found that

$$\frac{[(G_0) - (G_\infty)]/(G_0)}{[(A_0) - (A_\infty)]/(A_0)} = \frac{0.45}{0.195} = 2.3 \tag{7}$$

which is in fairly good agreement with the result of Kriek and Reitsema (1971) that the relative reactivity of poly(G) and poly(A) with N-AcO-AAF is about 3. We will accept the literature value of 2.3 and investigate the resulting kinetic equations. Combining eq 6b and 7, we obtain

$$k_2' = 0.36k_2 \tag{8}$$

As T4 DNA has 34% G + C content, we know that

$$(A_0) = (0.66/0.34)(G_0) = 1.94(G_0) \tag{9}$$

Combining eq 5a, 5c, 6a, 8, and 9, we obtain

$$\frac{k_1}{k_2} = \frac{[(F_0) - (F)] - [(G_0) - (G)] - 1.94(G_0)(1 - [(G)/(G_0)]^{0.36})}{\ln [(G_0)/(G)]} \tag{10a}$$

When time goes to infinity, (F) goes to zero, or

$$\frac{k_1}{k_2} = \frac{(F_0) - [(G_0) - (G_\infty)] - 1.94(G_0)(1 - [(G_\infty)/(G_0)]^{0.36})}{\ln [(G_0)/(G_\infty)]} \tag{10b}$$

The relation between percentage modification and ΔT_m, eq 4 may be rewritten as

$$1 - 0.34[(G)/(G_0)] - 0.66[(G)/(G_0)]^{0.36} = 0.011\Delta T_m \tag{11a}$$

or

$$1 - 0.34[(G_\infty)/(G_0)] - 0.66[(G_\infty)/(G_0)]^{0.36} = 0.011\Delta T_m \tag{11b}$$

From the data in Figure 1a and making use of eq 10b and 11b, we may calculate k_1/k_2 for the three sets of data. The results are given in Table I. The three sets of data give essentially identical results. If we take the value for k_1/k_2, 5.1×10^{-3}, and use eq 5c, we see that the first-order hydrolysis is dominant for the consumption of (F) throughout all of the reactions studied. Thus

$$-d(F)/dt \approx k_1(F) \tag{12a}$$

TABLE I: Reaction Rate Data for N-AcO-AAF Plus DNA.

	(F_0)	(G_0)	$\Delta T_m(\infty)$ (°C)	k_1/k_2	k_1	k_2	k_2'
1	8.1×10^{-4}	2.3×10^{-5}	8	5.1×10^{-3}	0.9×10^{-4}	1.8×10^{-2}	6.5×10^{-3}
2	2.0×10^{-3}	5.7×10^{-5}	18	5.0×10^{-3}	1.1×10^{-4}	2.2×10^{-2}	7.9×10^{-3}
3	3.4×10^{-3}	6.5×10^{-5}	27	5.2×10^{-3}	1.2×10^{-4}	2.3×10^{-2}	8.3×10^{-3}

TABLE II: Melting Temperature Changes.

Source of Data	$\Delta T_m/100p$	Comments
Maher *et al.* (1968)	1.5	Only one data point
Troll *et al.* (1968)	0.33	Incomplete purification
Fuchs and Daune (1971)	0.41	Incomplete purification

When eq 12a is integrated and substituted into eq 5a, we have

$$\ln\left[\frac{k_1}{k_2(F_0)}\ln\frac{(G)}{(G_0)}+1\right]^{-1} = k_1 t \qquad (12b)$$

Using eq 11a and 12b and the data in Figure 1a, we obtain the rate plots shown in Figure 1b, which give values of k_1 which are proportional to k_2 and k_2'. The rate constants appear to have a small systematic error. All of the data is collected in Table I.

Melting Temperature Changes. The effect of modification of DNA, to produce AAF-DNA, on the melting temperature has been studied by Troll *et al.* (1968), Maher *et al.* (1968), and Fuchs and Daune (1972). Their results are summarized in Table II. ΔT_m is the number of degrees that the melting temperature is lowered as a result of modification. $100p$ is the percentage of base pairs modified. Maher *et al.* showed that extraction with chloroform or ether or dialysis against water did not remove impurities present following N-AcO-AAF labeling of DNA. Hence, the $\Delta T_m/100p$ values of Troll *et al.* (1968) and Fuchs and Daune (1971) are probably underestimated. The purification procedure used in this work leads to removal of most of the impurities. Banding in CsCl (Maher *et al.*, 1968) leads to the same result.

We measured the ratio of the 305-nm to 260-nm absorbances (A_{305}/A_{260}) for different values of ΔT_m. The data are shown in Figure 2. The ΔT_m corresponding to a given A_{305}/A_{260} is much larger than that reported by Troll *et al.* (1968) or Fuchs and Daune (1971). To further check this result, we looked at the CD spectra of AAF-DNA as a function of ΔT_m. The results are shown in Figure 3. The data of Fuchs and Daune (1971) are shown for comparison. The magnitude of the 300-nm negative band at a given ΔT_m agrees, within experimental error, with the data of Fuchs and Daune (1972), indicating that the excess absorbance they reported at 305 nm did not correspond to an optically active material. The magnitude of the CD band begins to drop off around $\Delta T_m = 15°$. This change is probably because of failure to completely

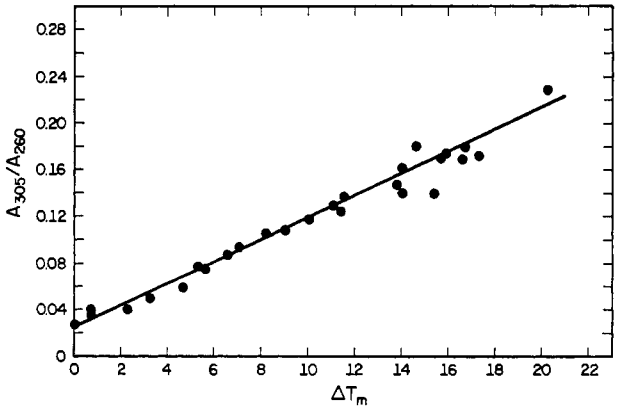

FIGURE 2: The correlation between melting temperature depression, ΔT_m, and absorbance of AAF-DNA at 305 nm.

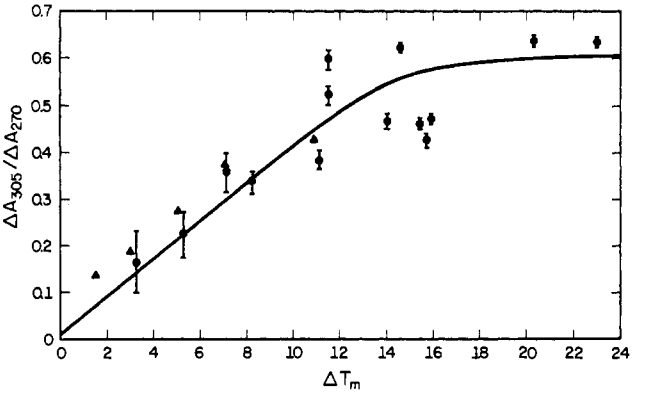

FIGURE 3: The correlation between melting temperature depression, ΔT_m, and the ratio of the circular dichroism of AAF-DNA at 305 and 270 nm ($\Delta A_{305}/\Delta A_{270}$): (△) data of Fuchs and Daune (1971); (●) data from this work.

renature the DNA, in agreement with renaturation rate data given below. The 300-nm CD band disappears when AAF-DNA is denatured, leading to a CD spectrum identical with unlabeled denatured DNA.

In order to calculate $\Delta T_m/p$, we measured the buoyant density of AAF-DNA as a function of ΔT_m. These data, as well as the data of Troll *et al.* (1968), are shown in Figure 4. These data are included, as ΔT_m, circular dichroism and buoyant density are correlated to AAF-DNA even if the absorbance spectra include the spectra of impurities. As expected, most of the data fall on a straight line. Using the result of Maher *et al.* (1968) that the buoyant density shift, $\Delta\rho$, is correlated with

$$\Delta\rho/100p = 2.85 \qquad (13)$$

we obtain

$$\Delta T_m/100p = 0.9 \qquad (14)$$

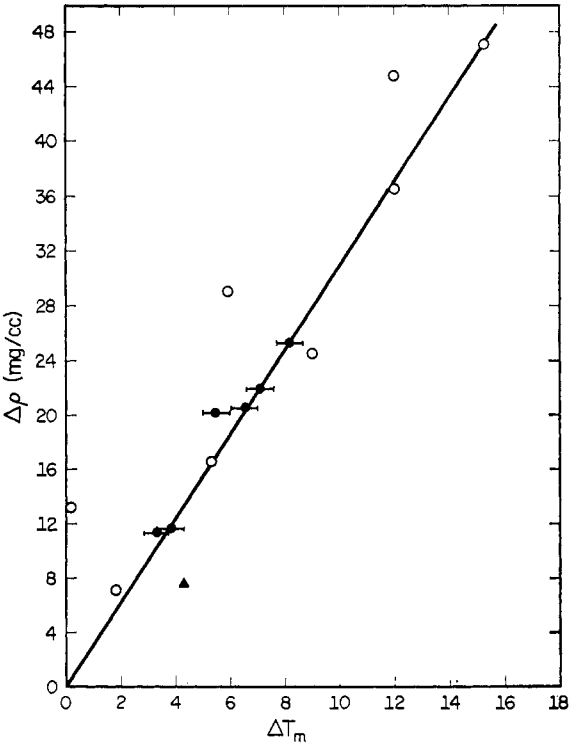

FIGURE 4: The correlation between melting temperature depression, ΔT_m, and the buoyant density change from unmodified DNA: (△) data of Maher *et al.* (1968); (○) data of Troll *et al.* (1968); (●) data from this work.

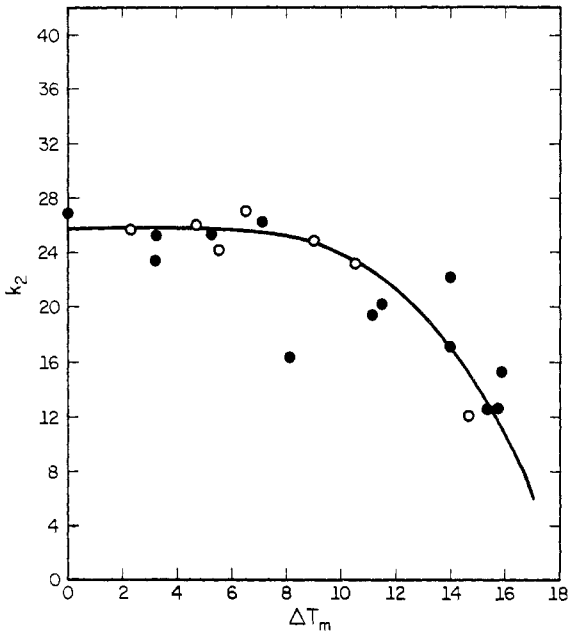

FIGURE 5: The correlation between melting temperature depression, ΔT_m, and the rate of renaturation of AAF-DNA. The rates were measured with T4 DNA in 0.4 M NaCl–0.002 M citrate buffer (pH 7.0) at 60°. Two sets of experiments are shown.

A simple calculation may also be made by assuming that the extinction coefficient of AAF-DNA at 305 nm is the same as that of free 8-(*N*-2-fluoreneacetylacetamino)deoxyguanine, which is 18,000 (Kapuler and Michelson, 1971), and correcting for DNA absorbance at 305 nm and, when determining DNA concentration, AAF absorbance at 260 nm. We find that

$$\Delta T_m/100p = 0.93 \quad (15)$$

We conclude that the 305-nm absorbance which we study by ED below is due to AAF-DNA and that we may relate the fraction of modification of base pairs, p, to the decrease in the melting temperature, ΔT_m, by

$$p = 0.011\Delta T_m \quad (16)$$

Melting Temperature Changes Resulting from Base Mispairing. The formation of the first base pair in a helix formation reaction may be considered to involve the product of two equilibrium steps, a volume parameter, v, involving diffusion into and out of a volume element, bringing the complementary base within a contact radius, a, and the equilibrium constant for formation of the first base pair, $\sigma^* s$, where s is the equilibrium constant for helix propagation and σ^* is the nucleation parameter introduced to account for the absence of one stacking interaction with the formation of the first base. The volume parameter, in l./mol, is

$$v = (4/3)\pi a^3(N_0/1000) \quad (17)$$

where N_0 is Avogadro's number. If a is chosen to be about 7 Å, then v is about 1. v contributes little to log $v\sigma^*$.

We may similarly consider the opening of a base pair in the middle of a long DNA helix to be described, to a first approximation, by an equilibrium constant, K_{eq}, where

$$K_{eq} = \sigma^*/s \quad (18)$$

Two stacking interactions are lost in this case, compared to one for opening one-end base pair. The free energy corresponding to this equilibrium would be

$$\Delta G_1 - \Delta G_0 = -RT \ln \sigma^*/s \quad (19)$$

where $\Delta G_1 - \Delta G_0$ is the difference in free energies of the open (ΔG_1) and closed (ΔG_0) configurations. If we take σ^* to be about 10^{-4}, then at 70°

$$\Delta G_1 - \Delta G_0 \approx 6 \text{ kcal/mol} \quad (20)$$

We will use this open base pair as a model for a mismatched base pair.

Thomas and Dancis (1973) have correctly pointed out that the weight fraction $W(M)$, of DNA of length less than or equal to M, with a mismatch randomly introduced at each end, is given by

$$W(\leq M) = 1 - (1 - p)^M(1 + pM) \quad (21)$$

which, for large M, may be approximated by

$$W(\leq M) = 1 - (1 + pM)e^{-pM} \quad (22)$$

The midpoint of the helix–coil transition would occur at

$$W(\leq M) = 0.5 \text{ or } pM = 1.7 \quad (23)$$

when compared to uniformly spaced mismatches. Using this result, we may compare random and nonrandom (uniform) mismatches by

$$\frac{\Delta T_m}{100p}(\text{random}) = \frac{1}{1.7}\frac{\Delta T_m}{100p}(\text{nonrandom}) \quad (24)$$

Lee and Wetmur (1973) calculated the relationship between ΔT_m and $\Delta G_1 - \Delta G_0$ for nonrandom cases to be

$$\Delta T_m/p = -(T_m^0/\Delta H^\circ)(\Delta G_1 - \Delta G_0) \quad (25)$$

where ΔH° is the enthalpy of base pair formation of about −8000 cal/mol. Adding eq 20, 24, and 25, we find that the introduction of a mispair into DNA might result in

$$\Delta T_m/100p = 1.5°/\% \text{ base pairs modified} \quad (26)$$

This result is similar to experimental results (Lee and Wetmur, 1973; Hutton and Wetmur, 1973) and is similar to the calculations of Gralla and Crothers (1973) although not at all in

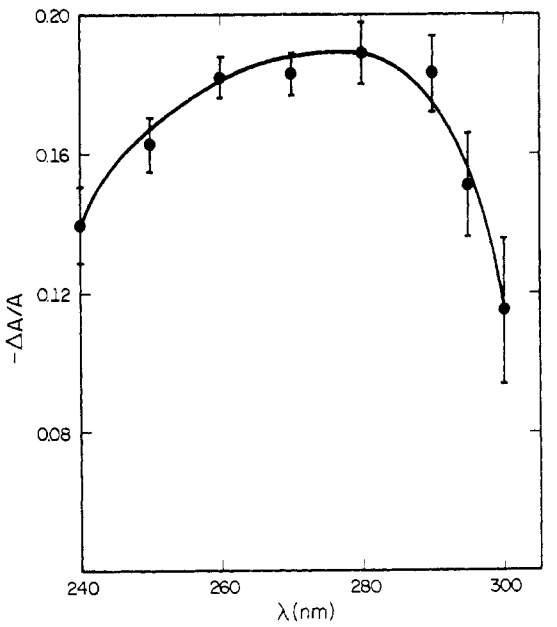

FIGURE 6: The reduced dichroism spectrum of sonicated T4 DNA with 12% of the base pairs modified with AAF. The DNA concentration was 95 μg/ml. The solvent was 58% glycerol–0.001 M Na_2EDTA plus NaOH (pH 7.0), 10°.

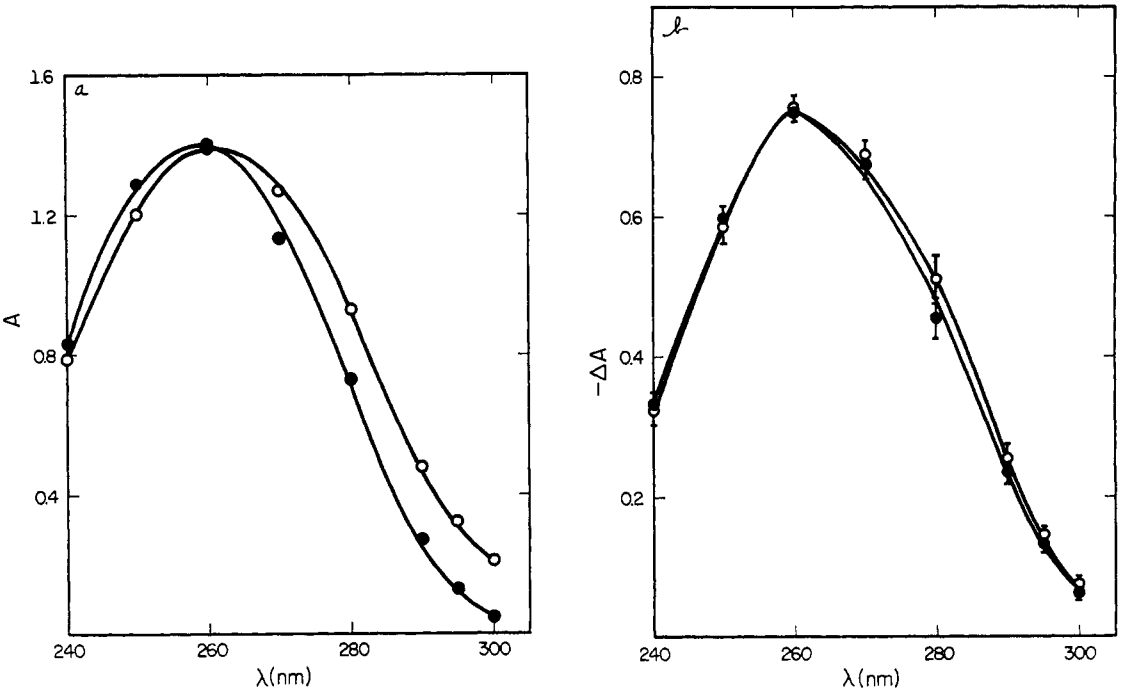

FIGURE 7: Absorbance (a) and electric dichroism (b) spectra of sonicated T4 DNA with the same solvent conditions as in Figure 6: (●) unmodified DNA; (○) DNA with 12% of the base pairs modified.

agreement with the model of Thomas and Dancis (1973) which compared chain cleavage with modification.

The experimental results of $\Delta T_m/100p$ for AAF-DNA fall slightly below the calculated or experimental values for G·C mismatches, but not so far below that the configuration of the G residue may be uniquely determined from thermodynamic arguments.

Renaturation Rates. The rate constant for renaturation, k_2, of AAF-DNA was measured as a function of ΔT_m. The data are shown in Figure 5. The renaturation rate is decreased by a factor of 2 when the melting temperature is lowered 15°. The shape of the k_2 *vs.* ΔT_m curve is the same as previous experimental and theoretical observations in other systems (Hutton and Wetmur, 1973; Lee and Wetmur, 1973). The drop in k_2 occurs at the same point as the failure to see increased CD at 300 nm as shown in Figure 3.

Circular Dichroism. The strength of the 300-nm CD band is linear in AAF residue concentration on native DNA and thus must not result from coupling to other residues. The 300-nm band depends on the existence of a helical DNA structure. This result implies that the AAF residue is held into a specific environment in the native AAF-DNA and is released to rotate in the denatured state (Nelson *et al.*, 1971). If the G·C base pair remains relatively intact, the AAF residue may be found, by model building, to be constrained along the helix winding angle. In this case, the $\Delta T_m/100p$ observed could be due to the strained nature of such a structure as well as to changes in the electronic configuration of the guanine residue due to C-8 substitution. If the G·C pair is opened, the AAF-substituted G would be free to take up many configurations, including the one suggested by Fuchs and Daune (1972), insertion of AAF into the helix. Of the three possibilities, the free AAF-substituted G seems to be the least likely to be consistent with the loss of optical activity following denaturation.

Electric Dichroism. To check that the orientation angle of AAF could be obtained by ED, we first determined α for proflavine bound to sonicated T4 DNA with a DNA : dye ratio of 25. At this binding ratio, the dye is essentially 100% intercalated (Lloyd *et al.*, 1968). From the absorbance and ED at 260 and 460 nm, α was calculated to be 86 ± 4°, as expected.

The ED spectrum of sonicated T4 DNA with 12% of the base pairs modified with AAF was then examined. The reduced ED spectrum is shown in Figure 6. Absorbance and ED spectra are shown for DNA and AAF-DNA in Figures7a and 7b, respectively. The dichroism of AAF-DNA at 300 nm is almost the same as that of DNA while the absorbance is substantially different, immediately showing that α is not 90°, as is seen for proflavine and would be expected if the AAF residue were inserted into the helix.

Assuming the absorption at 300 nm was due primarily to AAF, the absorbance at 260 nm due to AAF was calculated from the difference spectra of Fuchs and Daune (1972) and subtracted from the AAF-DNA absorbance to give the DNA absorbance at 260 nm. Assuming the absorbance of AAF-DNA and DNA at 260 nm are the same does not significantly change the results. The absorbance of AAF at 300 nm was then determined by subtracting the DNA absorbance at 300 nm, corresponding to the 260-nm absorbance, from the AAF-DNA absorbance. Using the ED data in Figure 7b, $\Delta A/A$ was calculated for the AAF residue at 300 nm and for DNA at 260 nm. Using eq 2 and again assuming the 260-nm transition moment has an α of 90°, the value of α for the AAF residue was found to be

$$\alpha_{AAF} = 60 \pm 4° \quad (27)$$

This result is consistent with the AAF residue being constrained to lie along the helix winding angle and not inserted into the DNA helix.

References

Agarwal, M. K., and Weinstein, I. B. (1970), *Biochemistry 9*, 503.

Bautz, E. K. F., and Bautz, F. A. (1964), *Proc. Nat. Acad. Sci. U. S. 52*, 1476.

Carroll, R. B., Neet, K. E., and Goldthwait, D. A. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 2741.
Cramer, J. W., Miller, J. A., and Miller, E. C. (1960), *J. Biol. Chem. 235*, 885.
Fink, T. R., and Crothers, D. M. (1972), *Biopolymers 11*, 127.
Fuchs, R., and Daune, M. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 14*, 206.
Fuchs, R., and Daune, M. (1972), *Biochemistry 11*, 2659.
Gralla, J., and Crothers, D. M. (1973), *J. Mol. Biol. 78*, 301.
Henshaw, E. C., and Hiatt, H. H. (1963), *Proc. Amer. Ass. Cancer Res. 4*, 27.
Hutton, J. R., and Wetmur, J. G. (1973), *Biochemistry 12*, 558.
Irving, C. C., Veazey, R. A., and Williard, R. F. (1967), *Cancer Res. 27*, 720.
Kapuler, A. M., and Michelson, A. M. (1971), *Biochim. Biophys. Acta 232*, 436.
Kotaka, T., and Baldwin, R. L. (1964), *J. Mol. Biol. 9*, 323.
Kriek, E. (1968), *Biochim. Biophys. Acta 161*, 273.
Kriek, E., Miller, J. A., Juhl, U., and Miller, E. C. (1967), *Biochemistry 6*, 177.
Kriek, E., and Reitsema, J. (1971), *Chem. Biol. Interactions 3*, 397.
Laird, C. D., McConaughy, B. L., and McCarthy, B. J. (1969), *Nature (London) 224*, 149.
Lee, C. H., and Wetmur, J. G. (1973), *Biochem. Biophys. Res. Commun. 50*, 879.
Lloyd, P. H., Prutton, R. N., and Peacocke, A. R. (1968), *Biochem. J. 107*, 353.
Maher, V. M., Miller, E. C., Miller, J. A., and Szybalski, W. (1968), *Mol. Pharmacol. 4*, 411.
Marroquin, F., and Coyote, N. (1970), *Chem. Biol. Interactions 2*, 151.
Marroquin, F., and Farber, E. (1962), *Biochim. Biophys. Acta 55*, 403.
Marroquin, F., and Farber, E. (1965), *Cancer Res. 25*, 1262.
Michelson, A. M., Kapuler, A. M., and Pochon, F. (1972), *Biochim. Biophys. Acta 262*, 441.
Miller, J. A., and Miller, E. C. (1969), *Progr. Exp. Tumor Res. 11*, 273.
Miller, E. C., Miller, J. A., and Hartmann, H. A. (1961), *Cancer Res. 21*, 815.
Miller, S. J., and Wetmur, J. G. (1974), *Biopolymers 13*, 115.
Nelson, J. H., Grunberger, D., Cantor, C. R., Weinstein, I. B. (1971), *J. Mol. Biol. 62*, 331.
Thomas, C. A., and Dancis, B. M. (1973), *J. Mol. Biol. 77*, 43.
Troll, W., Rinde, E., and Day, P. (1969), *Biochim. Biophys. Acta 174*, 211.
Uhlenbeck, O. C., Martin, F. H., and Doty, P. (1971), *J. Mol. Biol. 57*, 217.
Williard, R. F., and Irving, C. C. (1964), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 23*, 167.
Yang, J. T., and Samejima, T. (1969), *Progr. Nucl. Acid Res. Mol. Biol. 9*, 223.
Yoshioka, K., and Watanabe, H. (1969), *in* Physical Principles and Techniques in Protein Chemistry, Leach, S., Ed., Part A, Chapter 7, New York, N. Y., Academic Press, pp 335–367.