Effect of Chemical Modification on the Rate of Renaturation of Deoxyribonucleic Acid. Deaminated and Glyoxalated Deoxyribonucleic Acid[†]

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ABSTRACT: Two model systems for DNA base mismatching have been studied. Deaminated DNA was investigated as a model for mismatches other than a purine–purine mismatch. Glyoxalated DNA was studied as a model for the purine–purine case. The melting temperature of glyoxalated DNA is found to be decreased by 1.1 ± 0.2° per mol of glyoxal bound per 100 base pairs. The renaturation rate of deaminated DNA is reduced by a factor of 2 when the melting temperature is lowered by 23°, corresponding to 33% mismatching. The renaturation rate of glyoxalated DNA is reduced by a factor of 2 when the melting temperature is lowered by 17°, corresponding to 16% mismatching. A theory is derived for modified DNA which accounts for the observed results.

We wish to discuss the effects of mismatch and chemical modification of nucleic acid bases on the thermal stability of duplex DNA and the rate of renaturation to form duplex DNA. Mismatch has been considered (Southern, 1971; Sutton and McCallum, 1971) as an explanation for the discrepancy between the kinetic complexity of mouse satellite DNA (Britten and Kohne, 1968; Waring and Britten, 1966) and the sequence complexity suggested by the results of pyrimidine tract analysis (Southern, 1970). The effects of base mismatches and modified bases on the renaturation of nucleic acids are of interest for several reasons. First, we may consider the question raised above. Can the effect of mismatch on renaturation rate be correlated with the amount of mixmatch present and, if so, is the effect of mismatch on the rate of renaturation sufficiently large to render rate data a useful diagnostic method for determining the amount of mismatch or nonhomology present in DNA such as mouse, or other, satellite DNA? Second, the amount of mismatch or genetic divergence which can be tolerated in homology studies, such as those of Davis and Hyman (1971), by the formation of heteroduplexes of DNA strands from related organisms will be limited by the amount of mismatch above which stable specific heteroduplexes cannot be formed for either equilibrium or kinetic reasons. Finally, the amount of chemical modification which can be tolerated by DNA without effecting DNA renaturation very much is of interest for setting the useful limits of *in vitro* chemical labeling methods which introduce radioisotopes or other probes into DNA.

Deaminated DNA (Kotaka and Baldwin, 1964; Laird *et al.*, 1969) provides an excellent model system for studying the effect on renaturation rate caused by purine to purine and pyrimidine to pyrimidine conversions. However, a mismatch caused by replacement of a pyrimidine with a purine might be expected to produce a greater destabilizing effect due to the greater dislocation caused by the purine–purine interaction and might result in different kinetic behavior. A good model system for studying the steric effect of mismatches involving purine–purine interactions is that of DNA containing glyoxalated quanine residues. Glyoxalation introduces an extra ring into the potential G·C base pair and should result in a steric hindrance to helix formation similar to that of a purine–purine interaction (Nakaya *et al.*, 1968; Staehlin, 1959; Shapiro *et al.*, 1969; Broude *et al.*, 1967; Broude and Budowsky, 1971).

The effect of HNO_2 on nucleic acids has been studied extensively (Schuster and Schramm, 1958; Carbon, 1965; Geiduschek, 1961; Becker *et al.*, 1964) leading to the result that the base-pairing specificities of A, C, and G are changed (Michelson and Monny, 1966; Singer and Fraenkel-Conrat, 1969; Bessman *et al.*, 1958) such that they no longer pair with their complement and that for each per cent of base pairs modified, the melting temperature of the DNA is lowered by 0.7° (Kotaka and Baldwin, 1964; Laird *et al.*, 1969).

In this work, we study the effects of these two types of modification on the thermal stability of duplex DNA, and the rate of renaturation to form duplex DNA. We have extended the theory of Wetmur and Davidson (1968) to include the possibility of modification and to account for the observed results.

Materials and Methods

DNA Preparation. A stock solution of λb2b5c phage and a slant of *Escherichia coli* K12 W3110 were kindly provided by Professor J. C. Wang. Phage were prepared by infection of a logarithmically growing *E. coli* culture ($A_{650} \approx 0.4$) in tryptone broth plus 0.001 M $CaCl_2$ with a multiplicity of two phage per bacterium. After lysis, 1 ml of $CHCl_3$ was added per 100 ml of culture and 1 mol of solid NaCl was added per l. of culture. The phage were purified by differential centrifugation and banding in CsCl ($d = 1.50$ g/cm^3), 0.01 M Tris-OH plus HCl (pH 8.0), and 0.0025 M $MgSO_4$ in a Beckman SW-50 rotor at 40,000 rpm for 24 hr at 5°. The phage band was removed with a syringe and dialyzed at 4° against 0.01 M Tris-OH plus HCl (pH 8.0), 0.0025 M $MgSO_4$, and 0.15 M NaCl.

λb2b5c DNA was prepared by the phenol extraction procedure of Mandell and Hershey (1960) followed by repeated chloroform extractions. The DNA was extensively sonicated using a Bronson sonic power sonifier (four times for 2 min at

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power level 4 at 0°) to yield a single-stranded molecular weight of 10^6. Sonicated DNA was used to prevent molecular weight changes from occurring during modification reactions. The DNA was exhaustively dialyzed at 4° against 0.4 M NaCl, 0.01 M PO_4, and 0.0001 M EDTA (pH 6.8).

Reagents. Reagent grade $NaNO_2$ was obtained from Mallinckrodt Chemical Co. A 40% aqueous glyoxal solution was obtained from Eastman Chemicals. These reagents were used without additional purification. Optical grade CsCl was obtained from Harshaw Chemical Co.

HNO_2 Treatment of DNA. Deaminated $\lambda_{b_2b_5c}$ DNA was prepared by heating a solution of 1.0 ml of 0.1 mg/ml sonicated DNA, 1.2 M $NaNO_2$, 50% dimethyl sulfoxide to 80°, and adding 0.2 ml of 2.0 M trisodium citrate (pH 3.06) also at 80°. This resulted in a solution with pH 4.1 with a DNA melting temperature of 69°. After the desired reaction times, the reaction was stopped by adding 0.5 ml of 1.0 M NaOH and quenching in an ice bath. The deaminated DNA was then dialyzed against 0.4 M NaCl, 0.01 M PO_4, and 0.0001 M EDTA (pH 6.8). The DNA could be renatured by heating to 75° for 30 min and allowing the solution to cool slowly to room temperature over an interval of 3 hr.

To demonstrate that the DNA was not degraded to any significant extent by the reaction in acidic conditions, a blank was run for the same length of time as the most extensively modified DNA (60 sec at 80°) with NaCl substituted for $NaNO_2$. Neither the renaturation rate or the T_m of the DNA blank was effected by this treatment, demonstrating that no detectable chain scission or depurination had occurred.

Glyoxal Treatment of DNA. Glyoxalated DNA with the desired ratio of glyoxalated to unglyoxalated G residues was prepared by either controlling the amount of adduct initially formed or by controlling the alkaline removal of glyoxal from fully glyoxalated DNA. A 1.0-ml reaction mixture containing 50 μg of sonicated λb2b5c DNA, 50% dimethyl sulfoxide, 0.1 M NaH_2PO_4, 0.1 M Na_2HPO_4 (pH 6.8), and the desired amount of glyoxal, 1.1×10^{-3} to 1.7×10^{-2} M, was heated at 80° in a regulated water bath for 5 min. The reactions were quenched by cooling the solutions in an ice-water bath. Glyoxal was removed from glyoxalated DNA by treatment with 0.2 M Tris-OH plus HCl (pH 8.0) at 45° for 76 hr with aliquots removed every 8–10 hr the solutions were dialyzed at 4° against 0.4 M NaCl–0.01 M PO_4 (pH 6.0).

The same renaturation rate was obtained for a given melting temperature change regardless of which method had been used to prepare the adduct, indicating that no detectable degradation occurred under the most severe reaction conditions.

Melting Temperatures. Modified λ b2b5c DNA samples and a sample of unmodified DNA were dialyzed exhaustively at 4° against 0.0001 M EDTA–0.01 M H_3PO_4 + NaOH to pH 6.8 for deaminated DNA samples and pH 6.0 for glyoxalated DNA samples. The samples were melted in stoppered 1-cm path-length square cuvets in a Beckman Acta III spectrophotometer with a temperature programmer to monitor the absorbance of four cells at 260 nm and the temperature of the solutions. A recirculating Haake bath was equipped with a motor to rotate the thermostat control of the contact thermometer. The rate of temperature increase was 0.5°/min.

To check the stability of the glyoxalated DNA at pH 6.0, the T_m of a solution of glyoxalated DNA with a melting temperature decrease of 24° ($\Delta T_m = -24°$) was determined after renaturation kinetics measurements had been performed on the sample several times, that is, after the sample had been at 100° for 30 min, 70° for 3 hr and 4° for 2 days. The melting temperature decrease was still 24°. The adduct is stable under the conditions of this study. Cooling curves were measured to determine the extent of cross-linking by HNO_2 or glyoxal, if any. No cross-linking was observed.

Renaturation Kinetics. A DNA solution of A_{260} = 0.2–0.25 in 0.4 M Na^+–0.01 M PO_4 (pH 6.0) for glyoxalated DNA and pH 6.8 for deaminated DNA was placed in a 1.5-ml, 1-cm path-length, water-jacketed fused silica spectrophotometer cell and heated to 100° with a recirculating Haake bath to melt the DNA. After 2 min at 100°, the water-jacketed cell was rapidly switched to a second recirculating Haake bath regulated at the temperature desired for renaturation. The absorbance at 260 nm was recorded on a Cary 15 spectrophotometer, using a 0.0–0.1 full-scale absorbance slide-wire, as a function of time. After the renaturation reaction had been monitored, the cell was switched back to the 100° Haake bath. The spectrum of denatured DNA was determined using a 0.0–1.0 full-scale absorbance slide-wire and a solvent blank. This value was used for calculating the rate. The renaturation and melting cycle was repeated several times for each measurement.

Quantitation of the Glyoxal Adduct. ^{14}C-labeled glyoxal, with a specific activity of 5 Ci/mol, was custom synthesized by Mallinckrodt Chemical Co., St. Louis, Mo. The conditions for the formation of the glyoxal–DNA adduct and for the determination of the melting temperature were the same as previously described.

Theoretical

We shall use the formulation of Wetmur and Davidson (1968) for considering renaturation rates. If [P] is the nucleotide concentration of denatured DNA in solution, then the rate of renaturation is given by

$$\frac{-\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} = \frac{k}{2}[\mathrm{P}]^2 \qquad (1)$$

where t is time and k is the experimental second-order rate constant. Rates may be calculated from measurements of absorbance, A, as a function of time including time zero (denatured DNA) and time ∞ (native DNA) using

$$\frac{A_0 - A_\infty}{A_t - A_\infty} = \frac{k[\mathrm{P_T}]t}{2} + 1 \qquad (2)$$

where $[\mathrm{P_T}]$ is the total DNA nucleotide concentration.

A nucleation rate constant, k_N, may be related to the experimental second-order rate constant by

$$k = k_N\frac{L_s}{N} \qquad (3)$$

where L_s is the length, in nucleotides, of the shorter of two reacting denatured DNA strands and N is the complexity of the DNA in units of base pairs per unique genetic sequence.

We may use the sequential base-pairing model for calculating k_N and, hence, k. Let $k_{i-1,i}$ be the rate constant for formation of the ith base pair and $k_{i,i-1}$ be the reverse rate constant. Allow a preequilibrium, where the complementary bases diffuse into and out of a small volume element, to be described by an equilibrium constant, S_0, where

$$S_0 = k_{-1,0}/k_{0,-1} \qquad (4)$$

Any excluded volume effect will be contained in this preequilibrium. Wetmur (1971) has shown that

$$S_0 \sim L_s^{-1/2} \qquad (5)$$

According to the sequential model, the nucleation rate constant is given by

$$k_{\mathrm{N}} = \frac{1}{\sum_{=0}^{L_s}\left\{\prod_{i=0}^{j-1} k_{i,i-1} \Big/ \prod_{i=0}^{j} k_{i-1,i}\right\}} \tag{6}$$

or

$$k_{\mathrm{N}} = \frac{S_0}{\frac{1}{k_{0,-1}} + \frac{1}{k_{01}} + \frac{k_{10}}{k_{01}k_{12}} + \frac{k_{10}k_{21}}{k_{01}k_{12}k_{23}} \cdots} \tag{7}$$

We may define the equilibrium constant, S_i, for formation of the ith base pair to be given by the product of an equilibrium constant for helix propagation, s, and a factor due to stacking, modification or mismatch, σ_i, so that

$$\frac{k_{i-1,i}}{k_{i,i-1}} = \sigma_i s = S_i \tag{8}$$

for i greater than or equal to 1. σ_1 may be replaced by σ, the parameter resulting from the absence of stacking in the formation of the first base pair. Further, let the forward and reverse rate constants be related to a general forward rate constant, k_f, by

$$k_{i-1,i} = k_f f(i) \tag{9a}$$

$$k_{i,i-1} = k_f f(i)/S_i \tag{9b}$$

Then, for the general case

$$k_{\mathrm{N}} = \frac{S_0 k_f}{\frac{k_f}{k_{0,-1}} + \frac{k_f}{k_{01}} + \sum_{i=2}^{L_s}\left(\frac{1}{f(i)}\prod_{j=2}^{i}\frac{1}{S_{j-1}}\right)} \tag{10}$$

As the series converges, L_s may be taken to be infinity. If we assume now that the reaction is not diffusion controlled and that the dissociation of the first base pair is more rapid than the others, then

$$k = \frac{S_0 k_f L_s/N}{\sum_{i=2}^{\infty}\left(\frac{1}{f(i)}\prod_{j=2}^{i}\frac{1}{S_{j-1}}\right)} \tag{11}$$

The maximum rate, $k_{\max}$, in the absence of any modifications will be given by eq 11 with all $f(i)$ equal to 1, S_1 equal to σs and all subsequent S_i equal to s.

We shall now consider a model where the first two modification steps are explicitly calculated and the subsequent steps are considered by an averaging technique. We begin by looking at the region of nucleation. Consider a region of base pairs of length $M + 2$ with a modified base or mismatch at positions 1 and $M + 2$. Let p be the probability of such a modification or mismatch per base pair. The probability of occurrence of such an $(M + 2)$mer is $(1 - p)^M p^2$. There are M starting points for renaturation within such a region. The probability, $P(M)$, of a reaction beginning in an $(M + 2)$mer is given by

$$P(M) = M(1 - p)^M p^2 \tag{12}$$

If we sum over all possible lengths

$$\sum_{M=0}^{\infty} P(M) = 1 - p \tag{13}$$

or, overall, the probability of a reaction is reduced from 1 to $1 - p$ due to the exclusion of any modified site as a nucleation site. Consider, for example, one modification per eight base pairs, on the average. With p equal to 0.125, we now ask how many reactions begin in regions containing at least 11 unmodified base pairs. We find

$$\sum_{M}^{\infty} P(M) = (1 - p)^M((M - 1)p + 1) =$$

$$(0.875)^{11}(10 \cdot 0.125 + 1) = 0.52 \tag{14}$$

or, even at a coverage of 1 in 8, over half of the starting sequences contain at least 11 unmodified bases.

Now, we consider a second modification at M^1 bases away from the first, leaving a region of $M^1 - 1$ pairable base pairs. The probability, $P(M^1)$, of such a sequence is given by

$$P(M^1) = (1 - p)^{M^1-1} p \tag{15}$$

If we sum over all possible lengths

$$\sum_{M^1=1}^{\infty} P(M^1) = 1 \tag{16}$$

or, we have accounted for all possibilities.

We define the following terms

$$T_{\mathrm{m}} = \text{melting temperature with no modification} \tag{17a}$$

$$T_{\mathrm{r}} = \text{renaturation temperature} \tag{17b}$$

$$\Delta T_{\mathrm{m}} = T_{\mathrm{m}} \text{ change due to modification} \tag{17c}$$

$$s = e^{-\Delta H(T_{\mathrm{m}}-T_{\mathrm{r}})/RT_{\mathrm{m}}T_{\mathrm{r}}} \tag{17d}$$

$$\bar{s} = e^{-\Delta H(T_{\mathrm{m}}-T_{\mathrm{r}}+\Delta T_{\mathrm{m}})/R(T_{\mathrm{m}}+\Delta T_{\mathrm{m}})T_{\mathrm{r}}} \tag{17e}$$

where ΔH, the enthalpy of base-pair formation, is about -8000 cal/mol, R is the gas constant, and $\bar{s}$ is the average helix propagation equilibrium constant including modified or mismatched base pairs.

We may calculate σ_i for modification or mismatch at pair i from

$$\sigma_i = \sigma^1 = (\bar{s}/s)^{1/P} \tag{18}$$

where σ^1 is assumed to be the same for all modifications. The value of σ^1 is not necessarily the same as a nucleation parameter for begining a new helix following a long region of unpaired bases. The value of σ^1 is experimentally determined from the relation of p to ΔT_{m}. Physically reasonable values of $f(i)$, equal to f for all modifications, are limited to the range

$$\sigma^1 \leq f \leq 1 \tag{19}$$

as, otherwise, we would imply that the modification accelerated the pairing rate or retarded the dissociation rate.

We may calculate the terms in the denominator of eq 11 with modifications at 1, $M + 2$, and $M + M^1 + 2$ by taking advantage of the series

$$\sum_{1}^{n}\left(\frac{1}{S}\right)^n = \frac{1}{S - 1}\left(1 - \left(\frac{1}{S}\right)^n\right) \tag{20a}$$

and

$$\sum_{0}^{n}\left(\frac{1}{S}\right)^n = \frac{1}{S - 1}\left(S - \left(\frac{1}{S}\right)^n\right) \tag{20b}$$

and isolating the terms resulting from the mispaired bases

$$k = \frac{S_0 k_f L_s \sigma(s - 1)}{N} \sum_{M=1}^{\infty} \sum_{M^1=1}^{\infty} \frac{p^3 M(1 - p)^{M+M^1-1}}{D} \tag{21a}$$

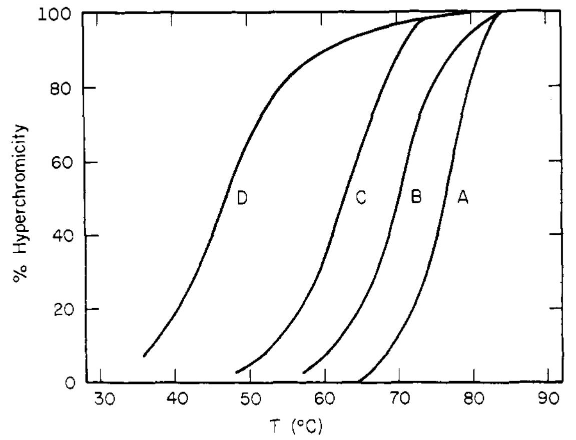

FIGURE 1: Melting curves for HNO_2-modified λb_2b_5c DNA. Unmodified DNA is shown in curve A. Curves B, C, and D show increasing degrees of modifications with ΔT_m of −7, −14, and −31°, respectively.

where

$$D = 1 + \frac{1}{s^{M-2}}\left(\frac{s-1}{f} - s + \frac{1}{\sigma^1}\right) + \frac{1}{\sigma^1 s^{M+M^1-2}} \times \left(\frac{s-1}{f} - s + \frac{1}{\sigma^1}\,\frac{\bar{s}\,(s-1)}{s\,(\bar{s}-1)}\right) \quad (21b)$$

The per cent relative rate, 100 k/k_{max}, is given by

$$\% \text{ relative rate} = \sum_{M=1}^{\infty}\sum_{M^1=1}^{\infty} \frac{100p^3M(1-p)^{M+M^1-1}}{D} \quad (22)$$

Results and Discussion

Modified DNA was prepared as described in the Methods section. Appropriate control experiments were performed to assure that no observed change in T_m or renaturation rate could be attributed to depurination and/or chain scission.

Some of the melting curves obtained for deaminated λb_2b_5c DNA are presented in Figure 1. The normalized change of absorbance at 260 nm (% hyperchromicity) is plotted as a function of temperature. Curve A is for unmodified DNA, which has a T_m of 77°. Curves B, C, and D are for samples with increasing degrees of modification with melting temperature depressions of 7, 14, and 31°, respectively. The relative rate of renaturation in 0.4 M Na^+, presented as the percent of the maximum rate obtained for unmodified DNA (*i.e.*, 100 $k_{modified}/k_{unmodified}$, 75°), for DNAs of varying degrees of modifictaion, is plotted as a function of melting temperature depression in Figure 2. The experimental values obtained are represented by the points. All the rates presented in Figure 2 were determined at 67°. The solid curve drawn through the points is the theoretical plot given by eq 22 using parameters described below.

As can be seen from Figure 2, the renaturation rate is still within experimental uncertainty of that for unmodified DNA up to a ΔT_m of −14°, which corresponds to having 20% of the base pairs mismatched. Even at 44% mismatch (sample with ΔT_m = −31°; curve B, Figure 3) the optimal renaturation rate, which occurs between 50 and 60°, is still 30% of the optimal rate for unmodified DNA.

The relative rate of renaturation of λb_2b_5c DNA, presented as the fraction of the maximum rate obtained for unmodified DNA, is plotted as a function of temperature in Figure 3.

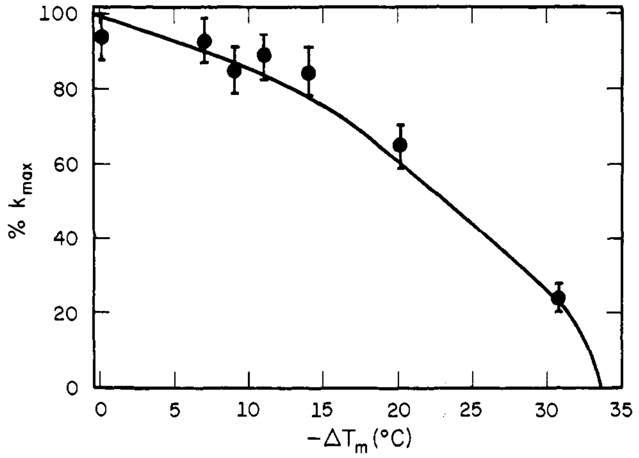

FIGURE 2: The relative rate of renaturation of HNO_2-modified λb_2b_5c DNA, as the per cent of the rate of unmodified DNA, as a function of the change in the melting temperature resulting from modification. The continuous curve shows the result of the theoretical calculation described in the text.

Curve A is for unmodified DNA (ΔT_m = 0). Curve B is for HNO_2-treated DNA with ΔT_m = −31°, the most heavily deaminated sample studied, as shown in Figure 2. Curve C is for glyoxal modified DNA with ΔT_m = −24°, the most heavily glyoxalated sample studied, as shown in Figure 6.

Some of the melting curves obtained for glyoxalated λb_2b_5c DNA are presented in Figure 4. Curve A is for unmodified DNA, which has a T_m of 77°. Curves B, C, D, and E are for DNA with increasing degrees of modification, which have melting temperature depressions of 7, 12, 17, and 24°, respectively. The dependence of ΔT_m on the number of moles of glyoxal bound per mole of DNA nucleotide is shown in Figure 5. As can be seen, the ΔT_m/per cent base pairs modified is 1.1 ± 0.2°. Assuming that the interaction of the glyoxalated guanine with its potential base-pairing cytosine is a reasonable model for a purine–purine interaction and that the interaction of a deaminated base with its potential base-pairing partner is a reasonable model for a purine–pyrimidine or a pyrimidine–pyrimidine interaction, then for a real hetero-

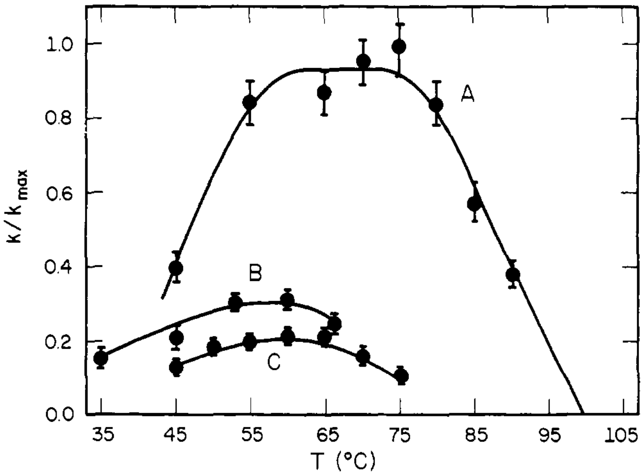

FIGURE 3: The relative rate of renaturation of λb_2b_5c DNA, as the fraction of the rate of unmodified DNA at the optimum temperature, as a function of temperature. Unmodified DNA is shown in curve A. Curve B shows heavily HNO_2-modified DNA; ΔT_m = −31°. Curve C shows heavily glyoxal modified DNA; ΔT_m = −24°.

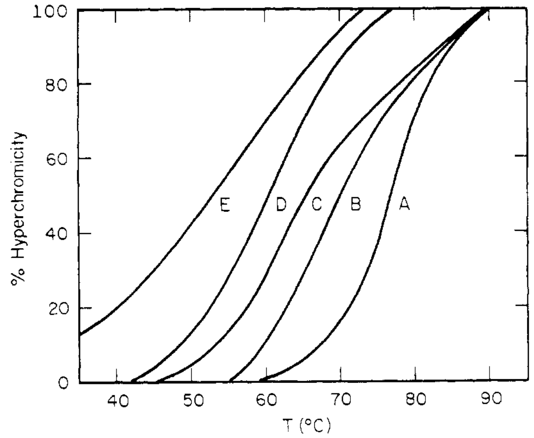

FIGURE 4: Melting curves for glyoxal-modified λb_2b_5c DNA. Unmodified DNA is shown in curve A. Curves B, C, D, and E show increasing degrees of modification with ΔT_m of −7, −12, −17, and −24°, respectively.

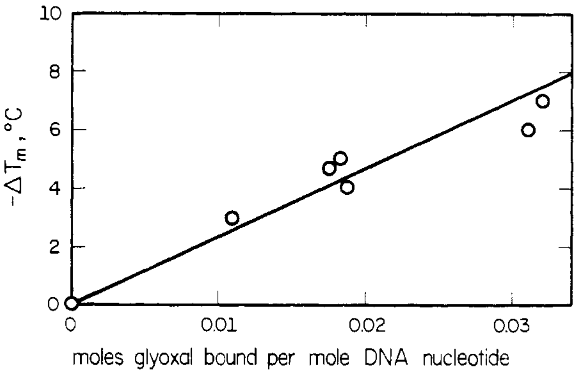

FIGURE 5: The change in melting temperature, ΔT_m(°C), of λb_2b_5c DNA as a function of moles of glyoxal bound per mole of DNA nucleotide.

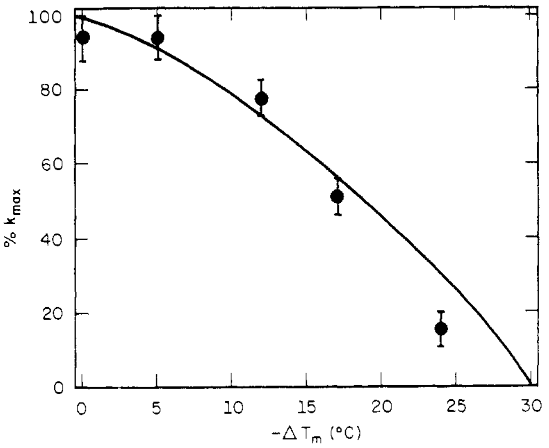

FIGURE 6: The relative rate of renaturation of glyoxal modified λb_2b_5c DNA, as the per cent of the rate of unmodified DNA, as a function of the change in the melting temperature resulting from modification. The continuous curve shows the results of the theoretical calculation described in the text.

duplex in which there would be a statistical assortment of such interactions, the expected dependence of ΔT_m on per cent mismatch is about 0.9°/per cent mismatch.

The relative rates of renaturation of the glyoxalated DNA samples as the percentage of the rate for unmodified DNA at the optimal temperature are represented by the points in Figure 6. All of the rates presented in Figure 6 were determined at 70°. The continuous curve is the theoretical plot obtained from eq 22 using parameters described below. In order for the rate to be reduced to one-half that of unmodified DNA, about 15% of the base pairs must be modified ($\Delta T_m = -17°$).

The theoretical curves in Figures 2 and 6 were obtained using a value of σ^1 of 0.1. The value of f makes very little difference. For deaminated DNA, the rates were calculated by assuming that both G·C and A·T base pairs could be modified. A value of $\sigma^1 = 0.1$ corresponds to a reduction in melting temperature of 0.7–0.8°/% mismatch. For glyoxalated DNA, the rates were calculated assuming that only GC base pairs could be modified. A value of $\sigma^1 = 0.1$ corresponds to a reduction in melting temperature of 1.0–1.2°% mismatch. The theory thus gives good agreement with both equilibrium (melting temperature) and kinetic data (renaturation rates) with a minimum input of one parameter.

Sutton and McCallum (1971) separated mouse satellite DNA into fractions according to thermal stability and attempted to correlate differences in thermal stability with differences in renaturation rate. They suggest that there may be a causal relationship between the observed amount of mismatch, as judged by thermal stability, and the observed renaturation rates. However, an implicit assumption central to this argument is that the "ancestral sequence complexity" is the same for the four fractions studied. This assumption has not been tested. Small differences in the ancestral sequence complexity of the fractions could easily account for the observed rate differences. Our results suggest that for degrees of mismatch which give ΔT_m of less than 8° (less than 10% mismatch), as was the case for all the fractions of mouse satellite DNA studied by Sutton and McCallum (1971), the rate of renaturation is not going to be altered by more than 20%. This implies that the factor of 2 to 3 in the renaturation rate obtained by them for a T_m difference of about 4° is to be attributed to an effect other than base mismatch, such as a factor of 2 to 3 range of difference in the ancestral sequence complexities of the fractions studied.

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Phosphate Transfer from Adenosine Triphosphate in a Model System†

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ABSTRACT: In 70% dimethyl sulfoxide there is a rapid transfer of the terminal phosphate of ATP to either water or inorganic phosphate. The hydrolysis of ATP requires the simultaneous presence of Mg^{2+}, a dicarboxylic acid and arsenate. The transfer reaction requires only Mg^{2+} but is stimulated by a dicarboxylic acid. The product of the transfer reaction appears to be exclusively inorganic pyrophosphate. These reactions may serve as model systems for phosphate transfer and ATPase activity in oxidative and photosynthetic phosphorylation.

The numerous hypothetical mechanisms and model reactions that have been devised to explain oxidative phosphorylation were reviewed by Lardy and Ferguson (1969). In most model systems an oxidation that takes place in a nonaqueous solvent is utilized for the formation of ATP or of pyrophosphate. Several of the models require formation of a phosphate ester which undergoes oxidation. The fact that in mitochondria the energy of oxidation can be preserved in the absence of phosphate (*cf.* Racker, 1965) makes these models unattractive. A more attractive model utilizes the oxidation of hemochrome with imidazole as the phosphoryl carrier (Brinigar *et al.*, 1967). Tu and Wang (1970) described a system phosphorylating imidazole in water, followed by a transfer of phosphate to AMP in dry *N,N*-dimethylacetamide. An important model reaction which takes place in aqueous solution is the formation of acid anhydrides during the oxidation of thioethers to sulfoxide (Higuchi and Gensch, 1966a,b). It is of interest to note that dicarboxylic acids facilitated the formation of the sulfoxide.

Lowenstein (1958a,b) described a phosphate transfer from ATP to inorganic phosphate catalyzed in aqueous solution at pH 9 by Ca^{2+} or Mn^{2+}. Mg^{2+} was ineffective and the reaction was comparatively slow. Hopkins and Wang (1965) described a slow transfer of the phosphate group from ATP to AMP. This reaction took place in Me_2SO solution and was catalyzed by Na^+ or K^+ but not by Ca^{2+} or Mg^{2+}.

It is the purpose of this paper to report on a phosphotransfer reaction from ATP to either water or P_i; this model system bears some resemblance to reactions observed in membranes catalyzing electron transport linked phosphorylation.

Experimental Procedures

Materials. Dimethyl sulfoxide, ATP, ADP, Dowex-1, Tricine, Tris, and maleic acid were obtained from Sigma. Other reagents were of analytical grade. [γ-^{32}P]ATP was prepared as previously described (Nelson *et al.*, 1972). Crystallized yeast inorganic pyrophosphatase was a gift from Dr. L. Heppel (Heppel, 1955). Mitochondrial ATPase was prepared as previously described (Horstman and Racker, 1970).

Assay of Transfer Reaction to P_i. In a final volume of 0.5 ml, 5 μmol of ATP, 5 μmol of $MgCl_2$, and 2.5 μmol of $Na^{32}P_i$ were adjusted to pH 8 with NaOH and buffer as specified in the legends to the figures. The reaction was started by addition of 1 ml of dimethyl sulfoxide with vigorous stirring. After 10 min incubation at 37°, the reaction was terminated by addition of 1 ml of H_2O or 0.1 ml of 30% trichloroacetic acid. Addition of 4 ml of 1.2% ammonium molybdate in 1 N HCl and 7 ml of a water-saturated solution of isobutyl alcohol–benzene–acetone (5:5:1) was followed by vigorous mixing in a Vortex shaker for 20 sec. The extraction was repeated twice with 7 ml of the solvent, and 1 ml of the water phase was dried and counted in a Nuclear Chicago flow counter.

Assay of ATPase. ATP hydrolysis was measured with [γ-^{32}P]ATP in the presence of sodium arsenate instead of NaP_i under the same conditions used for the transfer reaction. A sample (1 ml) of the solvent phase of the first extraction was dried and counted.

Results

Hydrolysis of ATP in a Model System. Solutions of ATP in the presence of $MgCl_2$, Tricine-maleate (pH 8), and arsenate were stable at 37° in aqueous solution for at least 4 hr. As can be seen from Figure 1, the addition of Me_2SO resulted in the rapid liberation of the γ phosphate of ATP (Figure 1) provided its concentration exceeded 50%. About 70% Me_2SO

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