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The Effect of Thymine Dimers on DNA:DNA Hybridization

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Synopsis

DNA from bacteriophage T7 was irradiated at long ultraviolet wavelengths in the presence of silver ions. Such treatment leads to selective production of thymine:thymine dimers in DNA. The DNA was melted and the renaturation rate was determined as a function of thymine dimer content and renaturation temperature. Under "normal" hybridization conditions little change in the renaturation rate was observed even when 30% of the thymine was dimerized. This result is consistent with the view that up to a 15% change in the primary sequence of DNA does not appreciably change the renaturation rate.

The kinetics of nucleic acid hybridization have been shown to be dependent on a variety of factors such as temperature, ionic strength, and guanine–cytosine content.[1] A long-standing question is to what extent a mismatch of bases (i.e., unconventional pairing of bases) will affect the renaturation rate.[1] Since the measurable parameter in the Wetmur and Davidson formulation[2] of renaturation kinetics K_N contains both a complexity correction factor and a putative mismatch correction factor, the estimation of complexity based solely on K_N might be questioned in cases in which mismatch is known to be present.[3,4]

As an example of the effect of mismatching, reannealed hybrids of the rapidly renaturing fraction of eukaryotic DNA show a decreased melting temperature after renaturation, indicating that the hybrid contains mismatched bases.[5] Direct sequence analysis of the guinea pig α satellite DNA suggests that mutational change in the primary DNA sequence is common and will lead to mismatch upon hybridization. Sutton and McCallum[4] reannealed mouse satellite DNA and subsequently separated the duplexes into four classes on the basis of differing melting temperature T_m. The rate of renaturation of these four classes was strongly dependent on their respective T_m's—an observation that led Southern to propose[3] that the lowered thermal stability was the result of mutational changes in some homogeneous primal sequence and that these changes reduced the rate of hybrid formation.

The system described in this paper was designed to determine whether a correction for mismatch should be applied to kinetic hybridization data. In this system the intrastrand thymine dimer was used to model a muta-

tional mismatch. The choice was justified by the knowledge that a dimer decreases the stability of the DNA duplex and that its action is at the level of primary structure. These are the minimum defining properties of mismatch. Brunk[6] and Shafranovkaya et al.[7] concluded by different methods that at low dimer concentrations the distribution of dimers in DNA is not random. Brunk showed that the longer pyrimidine tracts contained a greater than expected percentage of thymine as dimers. At a high level of dimerization the dimers are as random as the pyrimidine tracts in which they occur.[6]

The advantages of the thymine dimer as a specific lesion in DNA are numerous. Using silver ions, high yields of dimers are possible with negligible contribution from other photoproducts.[8] Double-stranded DNA can be used and therefore melting temperatures will show no effects due to self-sorting of altered strands. Introduction of the photoproduct is rapid and convenient, and the photoproducts have been well characterized and are fairly simple to assay.

Similar model systems have been used in attempts to determine the effect of mismatch on renaturation rate. Deamination has been used in order to model transition-type mutations.[9–11] Transversions have been modeled by using glyoxal to complete a third ring on guanosine residues[11] and by using chloroacetaldehyde to modify adenine and cytosine.[12] In all of these studies renaturation rate was not found to be very dependent on the presence of the alteration; the maximum rate depression reported is 80% for heavily glyoxylated DNA with a melting temperature 24°C lower than that of native DNA.[11]

MATERIALS AND METHODS

DNA

T7 wild-type phage was obtained from M. Chamberlain; *E. coli* strain B/r thy$^-$ was obtained from H. Nakayama. Unlabeled T7 DNA was prepared in a manner similar to that of England.[13] Tritium labeled DNA was prepared by growing *E. coli* B/r thy$^-$ in 0.2% glucose, 0.1% casamino acids (Difco) to an OD of 0.8 in a medium containing ^{3}H (methyl) thymine (1 μg/ml, 2 μm Ci/ml NEN) and infecting with T7 at a multiplicity of 0.1. Phage were prepared following Thomas and Abelson.[14] All phage were sedimented in CsCl step gradients and banded at equilibrium. The DNA was extracted with phenol, dialyzed against 0.06 *M* NaH_2PO_4, 0.06 *M* Na_2HPO_4, (0.12 *M* NaP), and stored over chloroform. Concentration was measured by determining A_{260}.

Formation of Dimers

DNA was irradiated at 7 or 70 μg DNA/ml for various lengths of time with a high-pressure mercury lamp (PEK 10010) through a 1.6-mm glass filter. The glass had a transmittance of less than 2% at 300 mμ and 47%

at 310 mμ. Silver nitrate was added to a Ag:DNA-phosphate ratio of greater than 1. Water-saturated nitrogen was bubbled through the DNA–silver solution prior to and during irradiation. An aliquot of the irradiated DNA was acid hydrolyzed and analyzed for photoproducts on Dowex formate columns and by paper chromatography.[15]

Hybridization

The irradiated DNA was dialyzed once against 0.02 *M* NaCN and then twice against 0.12 *M* NaP to remove Ag^+. Release of silver was complete as judged by disappearance of Ag^{110m} in control experiments. The DNA was then sonicated and its molecular weight determined by sedimentation through a 5–20% alkaline sucrose gradient using a method similar to that of Abelson and Thomas[16] (Spinco 50.1 rotor, 46,000 rpm, 6 hr, 20°C). Molecular weights were in the range of $3–5 \times 10^5$ daltons.

DNA samples were sonicated (Bronson LS-75 sonifier) and melted at 100°C for 10 min, then quick cooled in ice water. The solutions were transferred to shell vials mounted in a floating shell vial holder and placed in a constant temperature bath; 0.1-ml samples were taken at predetermined times and placed in 1 ml of ice-cold 0.12 *M* NaP. These were stored cold until analyzed for duplex by a hydroxylapatite-centrifuge method similar to that of Brenner et al.[17] The rate constant K was determined and corrected for length and concentration effects.[2]

Melting Temperature

Sonicated DNA was dialyzed against 0.01 *M* NaCl, 0.001 *M* Na_2H_2EDTA, 0.003 *M* NaP pH = 7, and placed in quartz cuvettes. Temperature was raised using a temperature-controlled cuvette holder connected to a Haake circulating water bath. Absorbance at 260 nm was measured with a Zeiss PMQ II spectrophotometer. Temperature regulation was accurate to 0.2°C (R. Baldwin, personal communication) and DNA standards were melted at the same time. Change in melting temperature ΔT_m was taken to be the temperature difference between the midpoints of the hyperchromicity curve of the sample and that of the standard.

RESULTS AND DISCUSSION

The specificity of the silver-sensitization technique for dimer formation was examined. Acid stable nondimer photoproducts contributed less than 0.1% of total label as determined by column chromatography.[15] The contribution of cytosine dimers (CT) was less than 1% of the total label, under conditions in which 30% of the label was identified as dimer associated. Strand breakage was observed but only with unsonicated DNA and times of irradiation longer than those used in this study. Sonication after irradiation eliminated strand breakages as a factor in the actual renaturation. No evidence for an increased extent of strand breakage was observed in dimerized DNA at higher temperatures.

Figure 1 is a plot of total dimers (mostly thymine dimers) versus ΔT_m. Regression of the points leads to the empirical formula

$$\Delta T_m = K_{[\widehat{TT}]}/T$$

where $K = 0.6°C$ per dimer per 100 base pairs (°C/%). Such a figure is lower than the value of $K = 1.2°C/\%$ obtained by Hutton and Wetmur[11] for glyoxylated DNA and 1.6°C/% obtained by Ullman and McCarthy for deaminated DNA.[18] A single dimer is thus approximately half as destabilizing as these other alterations.

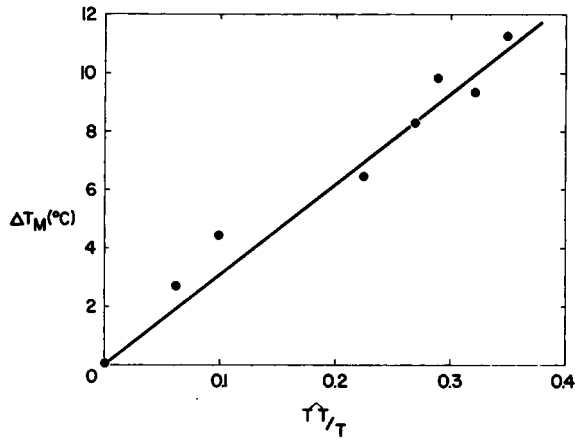

Fig. 1. Effect of thymine dimers on melting temperature. Irradiated DNA hydrolyzed by heating at 121°C for 3 hr in equal volume of $HClO_4$. Solution then neutralized with KOH and solid $KClO_4$ removed. Sample applied to column of AG 1-X8 resin (formate form, BioRad), which had been pre-equilibrated with 0.02 *M* NH_4OH. Dimers eluted with 0.02 *M* NH_4OH, 0.02 *M* HCOOH pH = 8, monomers with 0.02 *M* HCOOH. Plotted are dimer counts per total counts. ΔT_m determined as described in "Methods."

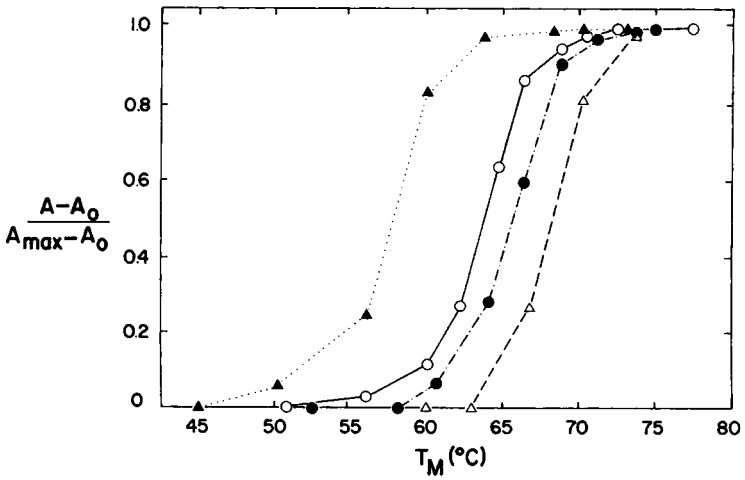

Fig. 2. Melting profiles of irradiated DNA. Dimer concentration 29.0% (▲), 10% (○), 6.8% (●), or 0% (△).

The width of the melting curves indicates a sharp transition (Figure 2). This contrasts with the broad melt of DNA irradiated at $\lambda = 254$ mμ but is consistent with results obtained by irradiating at $\lambda = 310$ mμ in the presence of acetophenone.[19] It is probable that the former results are due to a variety of photoproducts other than dimers, which have greater destabilizing effects than dimers. These photoproducts are absent in the DNA used in the present work.

Renaturation Rate

Sonicated T7 DNA was reannealed as described in "Methods" and the C_0t curves plotted in Figure 3. The minimum rate observed at 65°C is 62% of that for unaltered DNA. This change, with DNA containing 29% of its thymine label as dimers and having a ΔT_m of 10.2°C indicates that in a stability region comparable to reannealed eukaryotic DNA, the rate constant is not altered by even a factor of 2. The extent of dimerization is not extensive enough to test the hypothesis of Hutton and Wetmur[11] concerning the precise form of the dependence of rate constant on ΔT_m but is consistent with their data in the range explored in this study.

In order to see how the rate constant varies as the conditions of hybridization were made more stringent, the incubation temperature was raised. Results are plotted in Figure 4. The dimer-dependent decrease in renaturation rate becomes more pronounced as temperature rises.

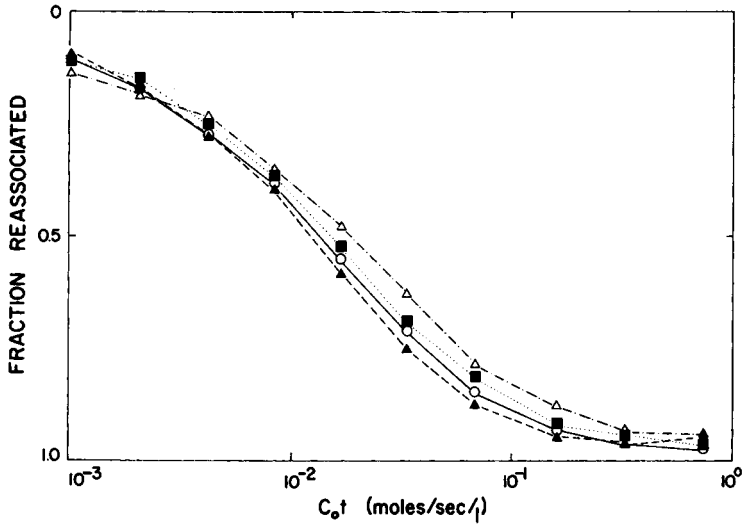

Fig. 3. Reassociation of irradiated DNA. Samples obtained as described in text were added to 0.5 g hydroxylapatite (DNA grade, BioRad) suspended in 10 ml of 0.12 *M* NaP; after equilibration at 65°C samples centrifuged in Sorvall desk-top centrifuge. Single-stranded DNA in supernatant precipitated in 5% cold Cl_3CCOOH. Pellet resuspended in 0.4 *M* NaP, centrifuged, and supernatant double-stranded DNA precipitated. Samples filtered through Millipore filters, and counted in toluene-PPO-POPOP. Plotted is ratio SS/(SS + DS). Dimer content 29.0% (△), 22.6% (■), 10.0% (○) and 0% (▲).

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