THYMINE DIMER FORMATION IN DNA BETWEEN 25°C AND 100°C*

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Various workers have shown that pyrimidine dimer formation in a polynucleotide is dependent upon the state of the polymer. Dellweg and Wacker (1962) showed that in aqueous solution more dimers were formed in heat-denaturated DNA than in native DNA; denaturation with glycol, however, was not as effective in increasing the dimer yield. Setlow also found a higher rate of thymine dimer formation in single-strand poly dT than in the ordered complex poly dA·dT. Another example of the influence of conformation on dimer formation can be found in poly U, where a striking enhancement of dimer yield occurs upon going from the double-stranded helix poly (A+U) to single-stranded poly U (Pearson and Johns, 1966). Hence, it appears that dimer formation is favored in the single-strand conformation as opposed to the rigid double-strand helix, in which the bases have much less motional freedom (McDonald et al., 1964).

It was proposed by Sinanoglu and Abdulnur (1964) that base stacking favors thymine dimer formation in DNA and that base stacking is most highly favored in aqueous single strand DNA. As pointed out by Michelson and Monny (1966), photochemical studies are particularly suitable for the investigation of single-strand base stacking in polynucleotides because of the changes with time in the local ordering due to stacking and unstacking of the bases. To investigate the influence of structure on thymine dimer formation in DNA, we irradiated both native and denatured DNA with and without glycol present at temperatures above and below the melting point of the double-stranded form.

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METHODS AND MATERIALS

The DNA (Escherichia coli, 15 T^-) labeled with tritiated thymidine was obtained from W. L. Carrier. Denatured DNA was obtained by heating DNA in water at 98°C for 20 minutes and then rapidly cooling it to 0°C. Samples were placed into quartz tubes (3 mm I.D.) which were continuously rotated during the course of the irradiation in order to provide uniform illumination of the sample. Samples were irradiated with a low pressure mercury lamp that provided 254 nm excitation. Measurements of the incident intensity were made with a thermistor bolometer at the site of the sample. Temperatures between 25°C and 100°C were obtained by blowing prewarmed gaseous nitrogen over the sample, which was located in a vacuum-jacketed cylindrical quartz tube. The temperature at the bottom of the sample tube was measured with a thermocouple.

After irradiation, the samples were hydrolyzed in 98% formic acid at 175°C for 30 minutes. The hydrolysates were then chromatographed on paper (Whatman No. 1) with a solvent mixture of n-butanol/acetic acid/water (80/12/30) (Smith, 1963). The distribution of radioactivity along the chromatogram was measured and used to calculate the percentage of thymine photoproduct. The identification of the thymine-thymine dimer ($\overset{\frown}{TT}$) was based on its known chromatographic mobility in this solvent.

RESULTS AND DISCUSSION

The yield of ($\overset{\frown}{TT}$), as a function of temperature, is shown in Fig. 1 for native and denatured DNA dissolved in either water or a 50% solution of ethylene glycol (EG:W). A pH of 8 was maintained with 0.01 M Tris buffer.

Native DNA in water irradiated below the melting temperature, T = 79°C, showed little variation in the yield of thymine dimer with temperature. Upon passing through the melting temperature, there was a sharp decrease in the dimer yield. When DNA was first thermally denatured and then irradiated as a function of temperature, the yield of dimer decreased in a linear way as the temperature increased, and the sharp transition found in native DNA was not observed. Above the melting temperature the yield of dimer was, as expected, independent of whether or not the starting material was denatured.

Similar results were obtained for native DNA in EG:W, except that the drop in dimer yield occurred about 30°C lower than it did in water. The breadth of the

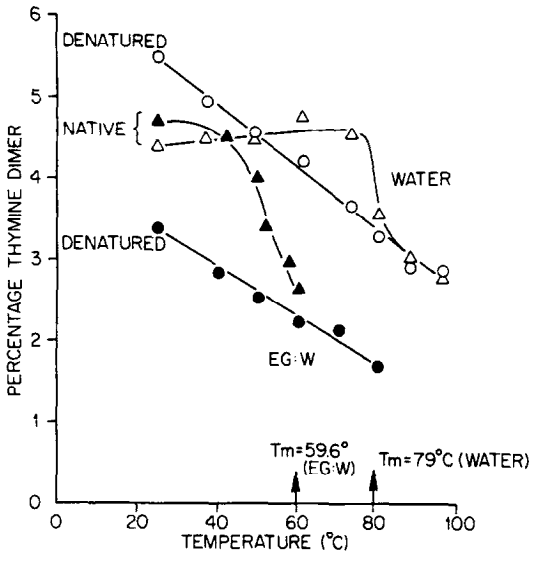

Fig. 1. Temperature dependence of thymine dimer formation in E. coli DNA at a concentration of 2.5 μ gm/ml. Samples labeled "native" were never exposed to temperatures greater than the irradiation temperature. The incident dose at 254 nm was 1×10^4 ergs/mm^2 where the variation of thymine dimer with dose was linear up to 10^4 ergs/mm^2 at both 25°C and 76°C. The melting temperature, T_m, was 20°C lower in EG:W than in water, as determined by separate absorbance measurements at 260 nm.

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transition appeared broader in EG:W than in water. Dimer formation in denatured DNA in EG:W approximately paralleled the corresponding curve for denatured DNA in water but was displaced by 55°C to lower temperatures.

If we assume that changes in the ability to form thymine dimer are solely dependent upon changes in the structure of the DNA, then the relatively constant yield of thymine dimer between 25°C and 70°C for native DNA in water is consistent with the maintenance of the double-stranded helix over this temperature range. At temperatures sufficient to melt the polymer and disrupt the pair-wise interactions between the bases, the dimer yield decreased and approached that of the denatured DNA. The sharpness of the transition indicates that the change in yield follows the cooperative structural change of the DNA. In either solvent the sharp change in dimer yield occurs at a temperature slightly less than the melting temperature (T_m). This result is consistent with the assumption that the A-T rich regions tend to melt first (McDonald et al., 1964).

The linear decrease with temperature of thymine dimer formation in denatured DNA appears to follow the non-cooperative structural change due to unstacking of the bases that occurs in DNA as the temperature is raised (Luzzati et al., 1964). It appears from Fig. 1 that further heating above 96°C in either solvent would lead to a further decrease in thymine dimer yield, and that even at this high temperature there is some kind of organization of the DNA which can be destroyed upon further heating. Recent NMR work (MacDonald, et al., 1967) indicates that at 90°C there still exists vertical interaction between the adjacent bases in DNA.

The idea that base stacking favors dimer formation receives support from the fact that more dimers are formed in denatured DNA in water than in the presence of glycol, which is a denaturing agent and disrupts base stacking (Hanlon, 1966). Absorbance measurements from 25°C to 80°C of heat-denatured DNA in EG:W showed a 4% absorbance increase in the DNA, while heat-denatured DNA in water showed a 12% increase. Hence, denatured DNA has a greater amount of single strand ordering in water than in EG:W, and this ordering is destroyed either by heating or by addition of glycol.

REFERENCES

Dellweg, V. H., and Wacker, A. Z., Naturforsch., 17b, 827 (1962).
Hanlon, S., Biochem. Biophys. Res. Commun., 23, 861 (1966).
Luzzati, V., Mathis, A., Masson, F., and Witz, J., J. Mol. Biol., 10, 28 (1964).
McDonald, C. C., Phillips, W. D., and Lazar, J., J. Am. Chem. Soc., 89, 4166 (1967).
McDonald, C. C., Phillips, W. D., and Penman, S., Science, 144, 1234 (1964).
Michelson, A. M., and Monny, C., Proc. Natl. Acad. Sci. U. S., 56, 1528 (1966).
Pearson, M., and Johns, H. E., J. Mol. Biol., 20, 215 (1966).
Setlow, R. B., private communication.
Sinanoglu, O., and Abdulnur, S., Photochem. Photobiol., 3, 333 (1964).
Smith, K. C., Photochem. Photobiol., 2, 503 (1963).