with poly C gives a very stable 1:1 complex, no indication of complex formation with a stoichiometry of 2G:1C, or 1G:2C, is observed. However, degraded poly G readily forms a complex 2G:1C. Poly G and poly BrC also form a 1:1 complex which is even more stable than that between poly G and poly C.

The authors thank Mlle Christiane Monny for technical assistance.

* Part V: Massoulié, J., and A. M. Michelson, *Compt. Rend.*, **259**, 2923 (1964).
[1] Inman, R. B., *J. Mol. Biol.*, **9**, 624 (1964).
[2] Radding, C. M., J. Josse, and A. Kornberg, *J. Biol. Chem.*, **237**, 2869 (1962).
[3] Inman, R. B., and R. L. Baldwin, *J. Mol. Biol.*, **8**, 452 (1964).
[4] Schildkraut, C. L., J. Marmur, J. R. Fresco, and P. Doty, *J. Biol. Chem.* **236**, PC 2 (1961).
[5] Lipsett, M. N., *J. Biol. Chem.*. **239**, 1256 (1964).
[6] Sigler, P. B., D. R. Davies, and H. T. Miles, *J. Mol. Biol.*, **5**, 709 (1962).
[7] Inman, R. B., *J. Mol. Biol.*, **9**, 624 (1964).
[8] Massoulié, J., and A. M. Michelson, unpublished work.
[9] Lipsett, M. N., *Biochem. Biophys. Res. Commun.*, **11**, 224 (1963).
[10] Fresco, J. R., in *Informational Macromolecules*, ed. H. J. Vogel, V. Bryson, and J. O. Lampen (New York: Academic Press, 1963), p. 121.
[11] Fresco, J. R., J. Massoulié, and R. D. Blake, quoted in ref. 10, p. 129.
[12] Thang, M. N., M. Graffe, and M. Grunberg-Manago, *Biochim. Biophys. Acta*, in press.
[13] Fresco, J. R., and D. F. Su, *J. Biol. Chem.*, **237**, PC 3305 (1962).
[14] Hirschbein, L., and J. R. Fresco, *Bull. Soc. Chim. Biol.*, Reunion, Feb. 1964.
[15] Michelson, A. M., *Nature*, **182**, 1502 (1958).
[16] Schramm, G., *Acides Ribonucléiques et Polyphosphates* (Paris: CNRS, 1962), p. 25.
[17] Michelson, A. M., *The Chemistry of Nucleosides and Nucleotides* (London: Academic Press, 1963).
[18] Michelson, A. M., and F. Pochon, in preparation.
[19] Fresco, J. R., and J. Massoulié, *J. Am. Chem. Soc.*, **85**, 1352 (1963).
[20] Grunberg-Manago, M., and A. M. Michelson, *Biochim. Biophys. Acta*, **80**, 431 (1964).

---

*THE CHEMICAL NATURE OF PHOTOREACTIVABLE LESIONS IN DNA**

BY JANE K. SETLOW, M. E. BOLING, AND F. J. BOLLUM

BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY, OAK RIDGE, TENNESSEE

Communicated by Alexander Hollaender, March 29, 1965

Ultraviolet-inactivated transforming DNA can be reactivated by photoreactivating enzyme from yeast in the presence of visible light.[1] The ability of ultraviolet-irradiated polydeoxynucleotides to compete with ultraviolet-inactivated transforming DNA for yeast photoreactivating enzyme provides a method of determining the effect of the enzyme plus photoreactivating light on compounds which themselves are lacking in biological activity. A synthetic polymer may be said to be photoreactivable if (1) it competes, and (2) its ability to compete is reduced by treating the irradiated polymer with enzyme plus light before addition of the transforming DNA, as shown by Rupert for poly dG:dC but not for poly d(AT):d(AT).[2]

This communication describes the photoreactivability of a wider range of simple polydeoxynucleotides of known composition and sequence, and includes experiments on competition by several DNA's of varying base composition. The homopolymer

complexes dA:dT, dI:dC, dG:dC, and dC are photoreactivable, whereas poly dA and poly dI are not. Pyrimidine dimers are formed after the irradiation of polymers that are competitors, and the removal of these dimers is correlated with reduced competition. From the experimental results we infer that pyrimidine dimers are photoreactivable lesions in DNA.

Methods.—Polymers: The general method for enzymatic synthesis of polydeoxynucleotides using terminal deoxynucleotidyl transferase and DNA polymerase has been described.[3] Purified DNA was obtained according to the method of Marmur.[4]

Poly dA:dT spectroscopy: A solution containing 20 μg/ml poly dA:dT in 0.06 *M* phosphate buffer, pH 7.0, was exposed to radiation from a Hilger high-power quartz-prism monochromator in quartz microcells (1-cm path length). Absorbancy changes were measured with a Beckman DU spectrophotometer.

Competition measurement: A mixture was prepared containing the following components: (1) 1 ml polymer at 1–2 μg/ml in 0.06 *M* phosphate buffer, pH 7.0, exposed to radiation from a germicidal lamp (3730 ergs/mm^2 incident dose) or from the Hilger monochromator (the same volume of phosphate buffer was used for a control experiment); (2) 0.15 ml *Hemophilus influenzae* transforming DNA at 1 μg/ml exposed to an incident dose of 2440 ergs/mm^2 from a germicidal lamp (survival of ability to transform to streptomycin resistance was 1%); (3) 0.25 ml of stock yeast enzyme[4a] (containing 20 mg protein/ml) diluted $\times$ 40 in phosphate buffer. For photoreactivation, 0.2-ml samples were distributed in white porcelain spot plates. The samples were exposed at 37°C for times up to 25 min to the illumination from blacklight bulbs, filtered to eliminate all wavelengths below 3250 Å. The incident intensity was about 7000 ergs/mm^2. Samples of 0.1 ml were withdrawn and assayed for ability to transform *H. influenzae* cells to streptomycin resistance.[5] Incubation of the mixture at 37°C in the dark for 25 min produced no observable change in the amount of transforming activity.

In some experiments synthetic polynucleotides were treated with photoreactivating enzyme plus light before addition of the transforming DNA. Samples of 0.2 ml of irradiated polymer at 10 μg/ml were mixed with 10 μl stock yeast enzyme in white porcelain spot plates. After blacklight illumination, 0.1 ml of the mix was diluted with 1.15 ml phosphate buffer, 0.15 ml transforming DNA was added, and 0.2-ml samples were placed in spot plates for photoreactivation as before. The yeast enzyme showed no evidence of inactivation for as long as 3 hr under the blacklight at 37°C.

Thymine dimer assay: Tritium-labeled thymidine triphosphate was used to make poly dA:dT (H^3) with a specific activity of about 3000 cpm/μg, determined by scintillation counting. Irradiated, photoreactivated samples were hydrolyzed with formic acid at 175°C and chromatographed in two dimensions on Whatman #1 paper as previously described.[6] Hydrolyzed calf thymus DNA was added to each sample for aid in locating the compounds on the paper. Paper strips were cut, eluted in water, placed in 15-ml dioxane-naphthalene fluid,[7] and counted in a scintillation spectrometer.

Results and Discussion.—Poly dA:dT: The homopolymer complex dA:dT, unlike the alternating polymer d(AT):d(AT), contains adjacent thymine residues. Hence, we expect poly dA:dT but not d(AT):d(AT) to contain thymine dimers after irradiation. If thymine dimers in DNA are one type of photoreactivable lesion, we would also expect the homopolymer, but not necessarily the alternating polymer, to be photoreactivable.

Poly dA:dT undergoes the spectroscopic changes associated with the formation of thymine dimers,[8] as seen in Figure 1. The absorbance decreases markedly after 2805 Å irradiation, and the effect of a large dose of 2805 Å is largely reversed by subsequent 2378 Å irradiation, a phenomenon similar to that found in oligo T[8] and in DNA.[9] Similar results were *not* obtained with the alternating polymer d(AT):d(AT).[8] Formation of thymine dimers in poly dA:dT may also be shown chemically (Fig. 2).

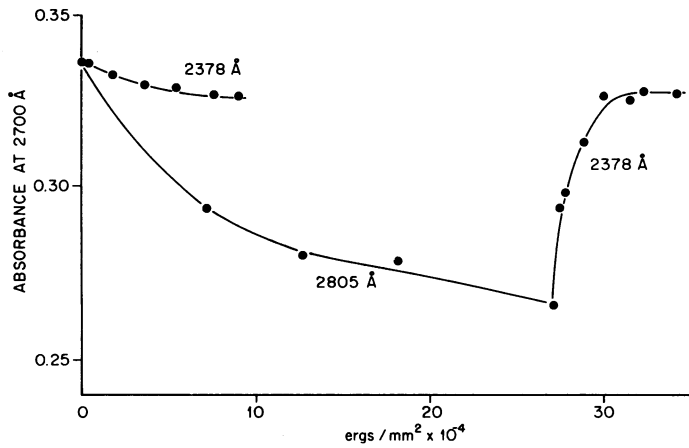

FIG. 1.—Optical density changes at 2700 A in poly dA:dT produced by 2378 Å irradiation alone and following 2805 Å irradiation.

Illumination of irradiated transforming DNA in the presence of photoreactivating enzyme results in a rapid increase in the number of transformations, but when irradiated poly dA:dT is also present, the number increases more slowly (Fig. 2). The increase is more rapid when the polymer has not been irradiated, or when the polymer has been treated with the enzyme plus light before addition of transforming DNA. It can be seen that longer periods of preillumination of the polymer before addition of the transforming DNA result in increasing rates of photoreactivation of transforming DNA. After 40 min of this treatment the competing ability is completely eliminated, as indicated by the fact that the treated polymer behaves like unirradiated polymer in the reaction. There is a slight depression of transforming

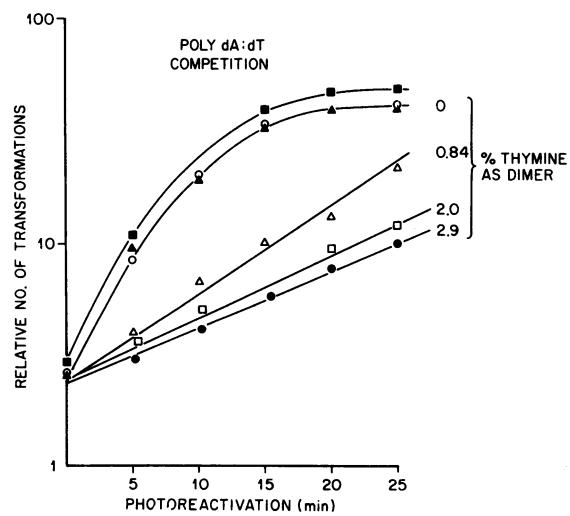

FIG. 2.—Illumination of the polymer in the presence of photoreactivating enzyme for ●, ○; □, 10; △, 20; ○, 40 min before addition of transforming DNA. ■, No poly dA:dT present; ▲, unirradiated poly dA:dT present with the enzyme and transforming DNA. Germicidal lamp irradiation at 2537 Å.

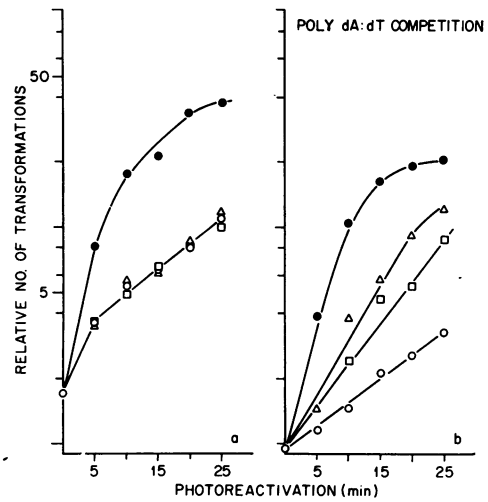

FIG. 3.—(*a*) One μg/ml poly dA:dT irradiated with ●, 0 dose; Δ, 3 × 10^4; □, 6 × 10^4; ○, 12 × 10^4 ergs/mm² at 2378 Å. (*b*) Two μg/ml poly dA:dT irradiated with ●, 0 dose; Δ, 1 × 10^3; □, 2 × 10^3; ○, 5 × 10^3 ergs/mm² at 2805 Å. The corresponding per cent thymine as dimer as Δ, 1.1; □, 1.95; ○, 5.

ability due to the presence of unirradiated or completely photoreactivated irradiated poly dA:dT, indicating competition for transformation by the synthetic material. The unirradiated polymer does not appear to compete for photoreactivating enzyme, since the curves obtained with and without unirradiated polymer present have the same shape.

At large doses of short-wavelength radiation, the number of thymine dimers in DNA and in synthetic polymers reaches a constant level (see also Fig. 1). Figure 3*a* shows that there is also a constant level of competition in poly dA:dT at such doses, whereas at lower doses or at long wavelengths an increase in competing ability is observed with increasing dose, as shown in Figure 3*b*.

Illumination with the photoreactivating enzyme eliminates both the competing ability and the thymine dimers in poly dA:dT, strengthening the earlier conclusion[10] that the thymine dimer produced from adjacent thymines in DNA is a photoreactivable lesion. Since neither the homopolymer dA nor the alternating copolymer d(AT):d(AT)[2] shows competing ability, we conclude that the ultraviolet-induced thymine dimers in poly dA:dT can account for all the competing effect in this synthetic polymer.

Poly dI:dC: In an earlier paper,[11] Setlow, Carrier, and Bollum demonstrate the presence of cytosine dimers in ultraviolet-irradiated poly dI:dC. They also describe several chemical transformations of cytosine dimers in this polymer. (1) Cytosine dimers can be eliminated from poly dI:dC by exposure to short-wavelength ultraviolet irradiation (2378 Å), as well as by exposure to yeast enzyme plus visible light. (The dimer elimination presumably results from a process of monomerization, that is, conversion to cytosine.) (2) Cytosine dimers are converted to uracil dimers by heat. (3) Uracil dimers are also monomerized by short-wavelength ultraviolet radiation as well as by yeast enzyme plus visible light. The *rates* of monomerization differ considerably for the two dimers. Cytosine dimers are mono-

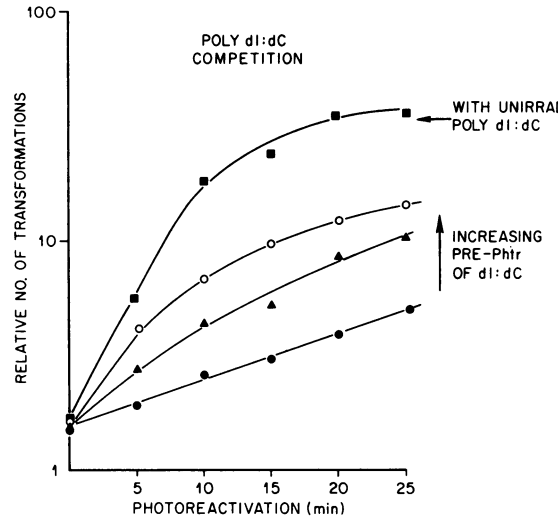

FIG. 4.—Illumination of the polymer in the presence of photoreactivating enzyme for ●, 0 min; ▲, 50 min; ○, 130 min before addition of the transforming DNA. Germicidal lamp irradiation at 2537 A.

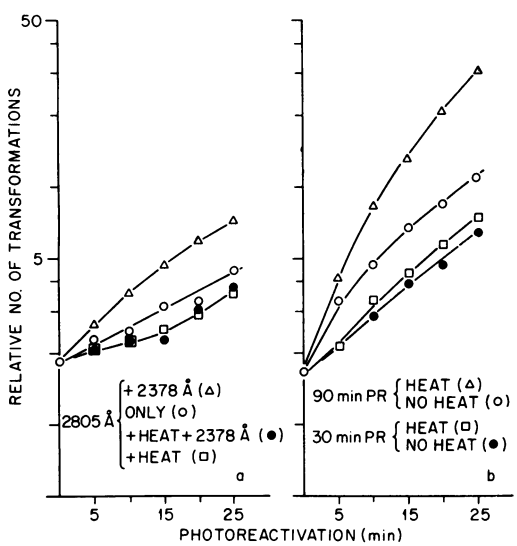

FIG. 5.—Effect of heat on elimination of competition by (*a*) short-wavelength reversal and (*b*) photoreactivation of poly dI:dC. Two μg/ml of polymer irradiated. (*a*) 2805 Å dose: 4×10^4 ergs/mm²; 2378 Å dose: 1×10^4 ergs/mm²; heat: 1 hr at 60°C. (*b*) Germicidal lamp irradiation of the polymer at 2537 Å. Heat before photoreactivation (PR): 1 hr at 60°C. Illumination of the poly dI:dC in the presence of photoreactivating enzyme for the times indicated before addition of transforming DNA.

merized faster than uracil dimers by short-wavelength irradiation, but more slowly by yeast enzyme plus visible light.[11] These facts form the basis for the competition experiments presented below.

Irradiated poly dI has no effect on the rate of photoreactivation, but poly dI:dC, like poly dG:dC,[2] competes for the photoreactivating enzyme (Fig. 4). The ability of this polymer to compete is eliminated relatively slowly; even after 130 min of preillumination the competing ability is not completely gone. The slow rate of photoreactivation of the polymer compared to that of poly dA:dT probably reflects a structural difference of the polymers rather than an intrinsic difference in rate of monomerization of the cytosine and thymine dimers. Poly dI:dC has a very low melting temperature (47°C in 0.15 *M* NaCl; ref. 12—see also ref. 13), whereas poly dA:dT behaves in this respect more like native DNA

(melting at 71°C in 0.15 *M* NaCl[12]). We have observed that denaturation of DNA decreases its ability to compete. Furthermore, heat denaturation after irradiation is known to decrease the rate of elimination of dimers in DNA by photoreactivating enzyme.[14] Hence, it is reasonable to suppose that structural differences between polymers might also affect the rate of elimination of dimers by photoreactivating enzyme.

If competition from irradiated poly dI:dC is primarily due to the presence of cytosine dimers, we would expect short-wavelength irradiation to decrease competing ability more slowly when the polymer has been heated (since uracil dimers are less susceptible to short-wavelength monomerization). Figure 5*a* shows that heat does indeed inhibit the reversal of competition by 2378 Å radiation following a large dose of 2805 Å radiation.

Heat alone seems to increase the competing ability of the 2805 Å-irradiated polymer, possibly because the photoreactivating enzyme binds more readily to the uracil dimer than to the cytosine dimer in the polynucleotide chain. Since uracil dimers in poly dI:dC are monomerized more rapidly than cytosine dimers by the photoreactivating enzyme,[11] preheating should increase the rate of elimination of competition. The number of transformants does rise more rapidly with time of illumination when the competing polymer has been heated before treatment with the enzyme (Fig. 5*b*).

We conclude that the dimers primarily responsible for the competing ability of the polymer are cytosine dimers before heating, and uracil dimers after heating.

Poly dG:dC and poly dC: We have repeated Rupert's experiment[2] showing competition by irradiated poly dG:dC. Irradiated poly dC also competes, but somewhat less, possibly due to a lesser degree of secondary structure in the single-chain polymer.

DNA competition: DNA irradiated with large doses of 2378 Å radiation shows increasing ability to compete, although the number of thymine dimers produced has reached a constant level.[15] Since the irradiation lasted 3 hr at room temperature in this experiment, some of the cytosine dimers might have been converted to uracil dimers during the irradiation. The resulting shift in equilibrium could cause the formation of more cytosine dimers. If this interpretation is correct, then irradiation at a much lower temperature should prevent the conversion of one form of dimer to the other, and there should be little or no increase in competition at the higher doses of 2378 Å radiation. Figure 6 shows the results of one such experiment, and indicates that competi-

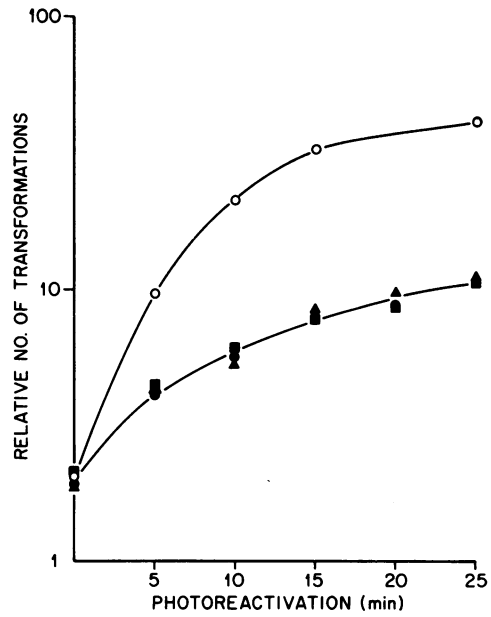

FIG. 6.—Competition by *Micrococcus lysodeikticus* DNA. 2378 Å irradiation of micrococcal DNA at 4°C with doses O, 0; ●, 3×10^4; ▲, 6×10^4; and ■, 12×10^4 ergs/mm².

tion reaches a constant level when the DNA is irradiated at 4°C. Likewise, poly dI:dC shows constant competition with these doses when irradiated at 4°C, but not at room temperature.

Since cytosine dimers are not formed in DNA as efficiently as thymine dimers,[16] it is of interest that T2 DNA competes more readily after irradiation than does similarly irradiated *Micrococcus radiodurans* DNA and *M. lysodeikticus* DNA, the last two having a higher G-C content.[17-19] Likewise calf thymus DNA, with a higher A-T content,[20] competes better than *M. lysodeikticus* DNA.

Summary.—The presence of thymine dimers in irradiated poly dA:dT and cytosine dimers in irradiated poly dI:dC, poly dG:dC, and poly dC is correlated with the ability of these substances to compete with transforming DNA for the photoreactivating enzyme. Heating polynucleotides that contain cytosine dimers converts the dimers to uracil dimers, and the resulting polymers also compete. All three types of dimers are monomerized by the photoreactivating enzyme, which also decreases the ability of the irradiated polymers to compete. Experiments on competing ability of irradiated DNA indicate that in the naturally occurring polynucleotides all these pyrimidine dimers also are substrates for the photoreactivating enzyme.

We thank Charles Steinberg for suggestions on the manuscript.

* Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

[1] Rupert, C. S., *J. Gen. Physiol.*, **43**, 573 (1960).

[2] Rupert, C. S., *J. Cellular Comp. Physiol.*, **58** (Supp. 1), 57 (1961).

[3] Bollum, F. J., E. Groeniger, and M. Yoneda, these PROCEEDINGS, **51**, 853 (1964).

[4] Marmur, J., *J. Mol. Biol.*, **3**, 208 (1961).

[4a] Kindly supplied by Dr. Amir Muhammed. The crude yeast extract had undergone ammonium sulfate and Sephadex purification.

[5] Goodgal, S. H., and R. M. Herriott, *J. Gen. Physiol.*, **44**, 1201 (1961).

[6] Setlow, R. B., P. A. Swenson, and W. L. Carrier, *Science*, **142**, 1464 (1963).

[7] Butler, F. E., *Anal. Chem.*, **33**, 409 (1961).

[8] Deering, R. A., and R. B. Setlow, *Biochim. Biophys. Acta*, **68**, 526 (1963).

[9] Setlow, R. B., and W. L. Carrier, *Photochem. Photobiol.*, **2**, 49 (1963).

[10] Setlow, J. K., and R. B. Setlow, *Nature*, **197**, 560 (1963).

[11] Setlow, R. B., W. L. Carrier, and F. J. Bollum, these PROCEEDINGS, **53**, 1111 (1965).

[12] Bollum, F. J., in *Abstracts*, Biophysical Society Meeting, San Francisco, February 1965.

[13] Inman, R. B., and R. L. Baldwin, *J. Mol. Biol.*, **8**, 452 (1964).

[14] Setlow, R. B., *J. Cellular Comp. Physiol.*, **64** (Suppl. 1), 51 (1964).

[15] Setlow, J. K., *Photochem. Photobiol.*, in press.

[16] Setlow, R. B., W. L. Carrier, and F. J. Bollum, in *Abstracts*, Biophysical Society Meeting, San Francisco, February 1965.

[17] Setlow, J. K., and D. E. Duggan, *Biochim. Biophys. Acta*, **87**, 664 (1964).

[18] Belozersky, A. N., and A. S. Spirin, in *Nucleic Acids*, ed. E. Chargaff and J. N. Davidson (New York: Academic Press, 1960), vol. 3, p. 147.

[19] Sinsheimer, R. L., in *Nucleic Acids*, ed. E. Chargaff and J. N. Davidson (New York: Academic Press, 1960), vol. 3, p. 187.

[20] Chargaff, E., in *Nucleic Acids*, ed. E. Chargaff and J. N. Davidson (New York: Academic Press, 1955), vol. 1, p. 307.