relatively high frequency in Mexican Indians. Therefore, our findings indicate that albumin variants occur with greater frequency than previous evidence had indicated.

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Antibodies to Photoproducts of Deoxyribonucleic Acids Irradiated with Ultraviolet Light

Abstract. *A rabbit immunized with complexes of methylated bovine serum albumin and ultraviolet-irradiated DNA from calf thymus produced antibodies directed toward the photoproducts in the DNA. Serologic activity appeared after irradiation of DNA at 270 mμ and decreased upon irradiation at 235 mμ. The antigenic determinants of the ultraviolet-treated DNA appear to be photoproducts associated primarily with thymine, as measured by direct dependence of serologic activity on the adenine-thymine content of the DNA, and by inhibition of the serologic reaction by the irradiated di-, tri-, and tetra-(thymidine-5'-phosphate) nucleotides.*

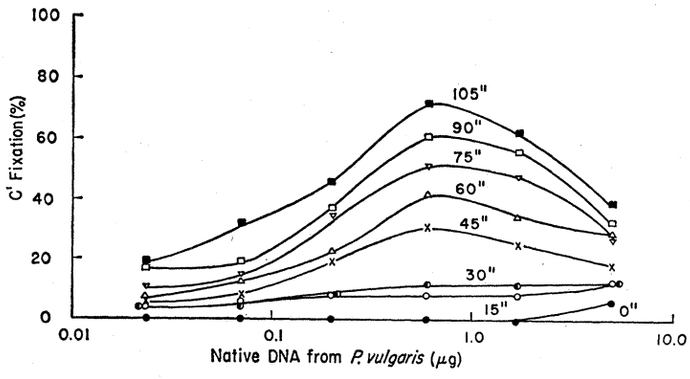
Deoxyribonucleic acid (DNA) can be rendered immunogenic by formation of complexes with methylated bovine serum albumin (MBSA) (*1*). Rabbits, immunized with DNA that has been irradiated in the presence of methylene blue, oxygen, and tris buffer and complexed with MBSA, produce antibodies not only to the normal bases, but also to a specific product of photooxidation (*2*).

The characterization of the specific reaction of photoproduct with antibody to photoproduct was possible since the immune response to the photooxidation product was so strong that the activity of the antibodies to normal constituents of DNA could be eliminated by dilution of the antiserum. We now describe the preparation of antibodies directed toward lesions in DNA induced by ultraviolet light. Evidence is presented that the antigenic determinants, resulting from ultraviolet irradiation, are composed of thymine photoproducts.

For preparation of the immunogen, calf thymus DNA (Calbiochem; used without further purification) was denatured at a concentration of 125 μg/ml in a mixture of 0.15*M* NaCl and 0.015*M* sodium citrate, *p*H 7.4, for 10 minutes at 100°C, and quickly chilled. The denatured DNA was irradiated with 1×10^5 erg/mm^2 of monochromatic light at 270 mμ. A single rabbit was injected with MBSA complexes of the irradiated denatured DNA by the method of Plescia *et al.* (*1*). Native DNA from *Proteus vulgaris* was exposed to various dosages of ultraviolet light from a germicidal lamp and assayed by complement (C′) fixation (*3*) at a 1/2000 dilution of the antiserum (Fig. 1). No serologic activity was observed with either native or denatured unirradiated DNA at this dilution of antiserum, although the antibodies to the normal DNA bases could be measured with five to ten times more antiserum (depending on the DNA used). Serologic activity appeared after 15 to 30 seconds of irradiation at a distance of 5 cm and continued to increase with time of irradiation. There is neither an increase nor decrease of serologic activity after 105 seconds of exposure. After varying doses of ultraviolet irradiation, denatured DNA from *P. vulgaris* showed a somewhat faster increase in complement-fixing activity. The photoproducts, therefore, are formed in both double- and single-strand DNA although the rate of their formation appears to be influenced by the conformation of the DNA.

There is some controversy concerning the exact chemical nature of the photoproduct or products responsible for the biological effects of ultraviolet irradiation. Much evidence exists that thymine and cytosine may be sites of damage in the irradiated DNA (*4*). Since thymine dimers are formed more readily than cytosine dimers, and are more stable under physiological conditions than are cytosine hydrates, it was anticipated that antibodies would be directed primarily to the thymine photoproducts. Thus, the serologic activity of DNA exposed to ultraviolet irradiation would reflect the thymine content of the DNA, much as the ultraviolet sensitivity of bacteria reflects the thymine content of their DNA (*5*). When DNA's that varied in A+T (*6*) content from 62 to 28 percent were exposed to 1×10^5 erg/mm^2 of monochromatic light (270 mμ), the resulting serologic activity was highest with *P. vulgaris* DNA (62 percent of A+T) and lowest with *Micrococcus lysodeikticus* DNA (28 percent of A+T). DNA's with intermediate A+T contents (*7*) were intermediate with respect to serologic activities. Thus it appears that thymine photoproducts are part of the antigenic determinants of the irradiated DNA.

The dimerization of pyrimidines by ultraviolet irradiation is a reversible reaction, and the equilibrium between dimer formation and dimer splitting depends, in part, on the wavelength of irradiation (*4, 8*), monomerization being favored at short wavelengths, while dimerization is favored at longer wavelengths. Thus irradiation of DNA at 270 mμ should lead to increased serologic activity, while exposure of the



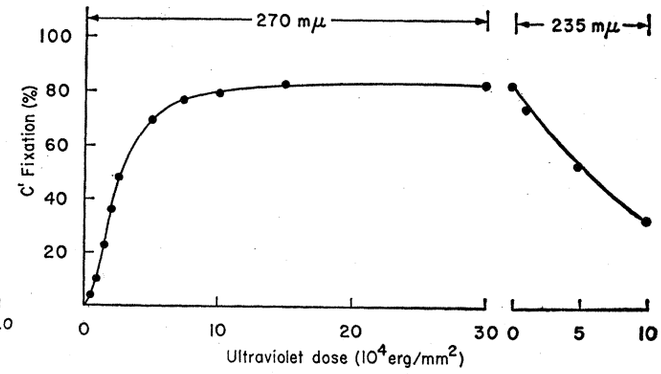


Fig. 1 (left). Fixation of C′ by increments of native *P. vulgaris* DNA exposed to varying doses (seconds) of ultraviolet light from a germicidal lamp at a distance of 5 cm. Antiserum diluted 1/2000. Fig. 2 (right). Maximum C′ fixation of native *P. vulgaris* DNA exposed to varying doses of monochromatic ultraviolet light at 270 mμ and then 235 mμ. Antiserum diluted 1/2000.

serologically active DNA to irradiation at 235 mμ should lead to a loss of activity. This anticipated increase in serologic activity after irradiation at 270 mμ, and the subsequent decrease in activity following irradiation at 235 mμ, were in fact observed (Fig. 2).

If the antibodies are directed toward thymine photoproducts, irradiated thymine oligonucleotides might be expected to inhibit the reaction of irradiated DNA with its specific antiserum. When di-, tri-, and tetra-(thymidine-5′-phosphate) nucleotides (*7*) were irradiated with monochromatic light (270 mμ), they became potent inhibitors of the reaction (Table 1). Of these, the irradiated tri- and tetra-nucleotides were the most effective inhibitors. The 40-fold more effective inhibition with $(Tp)_4$ and $(Tp)_3$ (*6*) over that observed with $(Tp)_2$ cannot be explained at present but two obvious possibilities are either that the antigenic determinant includes a $\overline{TTT}$ sequence or that the rate of formation of the effective photoproduct is slower in $(Tp)_2$ than in $(Tp)_3$ and $(Tp)_4$. The irradiated tri- and tetra-cytosine oligodeoxynucleotides (*7*) are 100 to 1000 times less effective than the thymine photoproducts of equivalent chain length and the substitution of a thymine for a cytosine in the tetra-nucleotide C_3Tp_5 gives products which are about eightfold more effective than the tetra-nucleotide C_4p_5. Thus the antigenic determinants of the irradiated DNA appear to be thymine-associated photoproducts. The antibody also appears capable of cross-reacting, to a small extent, with a photoproduct consisting of one thymine and one or more cytosine residues and, to a lesser extent, with photoproducts involving cytosine oligonucleotides.

Table 1. Inhibition of reaction of irradiated DNA with its specific antiserum by ultraviolet-irradiated oligodeoxypyrimidines (ODP). The test system contained 0.1 μg of *P. vulgaris* DNA irradiated at 270 mμ, 5×10^5 erg/mm², and rabbit antiserum to irradiated DNA from calf thymus. The antiserum was diluted 1 : 2000.

Inhibitor	ODP required for 50% inhibition*		Hypochromicity after irradiation (%)
	Exp. 1 (mμ mole)	Exp. 2 (mμ mole)	
$(Tp)_2$	0.085	0.13	29
$(Tp)_3$	.002	.005	33
$(Tp)_4$	.003	.006	29
C_3Tp_5	.11	.23	24
C_4p_5	.70	2.0	25
C_3p_4	.57	1.3	24

* Unirradiated oligodeoxypyrimidines were not inhibitory.

When thymidylyl-(5′-3′)-thymidine (TpT) is subjected to ultraviolet irradiation several photoproducts are formed (*9*). Two of the characterized photoproducts, $\overline{TpT}^1$ and $\overline{TpT}^2$, yield cyclobutane-type thymine dimers after acid hydrolysis. Since the chromatographic mobility and acid stability of thymine dimers obtained from acid hydrolysates of irradiated DNA and $\overline{TpT}^1$ are the same, $\overline{TpT}^1$ may be the thymine photoproduct in DNA (*10*). This hypothesis becomes amenable to a direct test with each isolated photoproduct as an inhibitor of the reaction between irradiated DNA and antibody to irradiated DNA.

Since ulraviolet-irradiated DNA becomes refractory to enzymatic digestion, it is chemically hydrolyzed in order to isolate specific photoproducts. Such treatment results in loss or alteration (or both) of some photoproducts (*4*). Antibodies directed toward a specific photoproduct can be used to detect the presence of such a photoproduct in intact DNA. It should be possible to produce antibodies to DNA irradiated under a variety of conditions and to assess the relevance of the numerous photoproducts obtained in model systems to the photochemical events which can occur in the biological macromolecule.

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6. A, adenine; T, thymine; $(Tp)_2$, di-(thymidine-5′-phosphate); $(Tp)_3$, tri-(thymidine-5′-phosphate); $(Tp)_4$, tetra-(thymidine-5′-phosphate); C_3p_4, tri-(cytidine-5′-phosphate)-3′-phosphate; C_4p_5, tetra-(cytidine-5′-phosphate)-3′-phosphate; C_3Tp_5, a tetranucleotide containing three cytosine residues and one thymine residue. The base sequence is not known. The bar above, as $\overline{TT}$, indicates dimerization.
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