INACTIVATION OF TOBACCO MOSAIC VIRUS RIBONUCLEIC ACID BY NEAR- AND MIDDLE- ULTRAVIOLET LIGHT: SENSITIZATION BY SULFANILAMIDE AND CHLORTETRACYCLINE

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The inactivation of tobacco mosaic virus-ribonucleic acid infectivity by near (320-400 nm) or middle (290-320 nm) ultraviolet light was promoted by sulfanilamide and chlortetracycline, as well as acetone, hydroquinone, $AgNO_3$, and $HgCl_2$. Sulfanilamide was an especially effective sensitizing agent. Sulfanilamide and possibly chlortetracycline, like acetone, induced the formation of cyclobutadipyrimidines in the RNA. Damage to nucleic acids may result from clinical use of sulfanilamide and chlortetracycline and may partially explain the skin photosensitization associated with such use.

The presence of sensitizing agents can increase many-fold the rate at which near- and middle-wavelength ultraviolet light damages nucleic acids. Recent work has identified several agents and mechanisms by which DNA (1-9) and RNA (10-11) can be sensitized to photoreactions. Sensitization can occur *in vivo*, and sensitizers can greatly increase the damage induced in tissues by the ultraviolet components of sunlight (1).

I have recently observed that two compounds with pharmaceutical uses, sulfanilamide and chlortetracycline, sensitize TMV[1]-RNA to ultraviolet-light induced inactivation. The observation is particularly significant, since it suggests that RNA (and possibly DNA) damage may account in part for clinical evidence (12) that these compounds sensitize human skin to sunlight. This report describes the sensitization of TMV-RNA by sulfanilamide and chlortetracycline and compares this with sensitization by four other agents, acetone, hydroquinone, and Ag^+ and Hg^{++} ions.

[1]Abbreviation: TMV, tobacco mosaic virus.

MATERIALS AND METHODS

TMV-RNA assay. Procedures for the growth of TMV, the preparation of TMV-RNA, and the determination of TMV-RNA infectivity were followed as described by Murphy and Gordon (13). Nicotiana tabacum var Xanthi nc was used as host plant for local-lesion assays of infectivity. Inactivation of infectivity was measured by applying irradiated and control preparations of RNA to opposite halves of a leaf. Relative numbers of lesions on the different leaf-halves, corrected for differences in RNA concentration in the two preparations, indicated relative survival of the irradiated RNA. Statistical analysis of results from replicate leaves was performed according to a non-parametric method (14). Each value reported represents results from approximately 30 replicate leaves and 3-4 independent experiments. With this sample, a variation of ± 20% of the reported value gave a 95% confidence interval for the median of the population.

Irradiation. Samples of RNA (1-5 ml, 5 µg TMV-RNA per ml) were irradiated in 0.1 M potassium phosphate buffer, pH 7, at 0°C and with continuous stirring. The samples were placed 54 cm from two Westinghouse FS40 fluorescent sunlamps, filtered either with a 5 mil (0.13 mm) Kodacel (Eastman) plastic film or with a 10 mil (0.25 mm) Mylar Type A plastic film. The emission peak of the lamp is at 360 nm. Kodacel absorbs $\geq$ 90% of radiation below 293 nm; Mylar Type A absorbs > 90% below 321 nm. The total incident irradiance was 3.5 W/m^2 (Kodacel) or 3.1 W/m^2 (Mylar). Samples were irradiated in open 100 ml beakers, unless oxygen was to be excluded, in which case they were irradiated in quartz cuvettes sealed after the solutions were bubbled with N_2 gas.

Photoreactivation. All leaves were inoculated with TMV-RNA in a room illuminated only with F40R (red) fluorescent lamps, which do not photoreactivate ultraviolet-light-damaged TMV-RNA (13). To test for photoreactivation, inoculated plants were placed under three F40WW (warm white) fluorescent lamps (15 W/m^2 irradiance) for 10 min, then were placed in the dark overnight. The survival of the irradiated RNA, as measured on these illuminated plants (y_L), was compared with the survival of the same preparation of irradiated RNA, as measured on control plants kept in the dark after inoculation (y_D). The extent of photoreactivation ("photoreactivated sector", f_p) was calculated by the formula, $f_p = 1 - (\log y_L / \log y_D)$. This formula is strictly valid only when values for survival, with and without photoreactivation, are exponential functions of dose.

Zonal Centrifugation. Samples of irradiated RNA were layered on gradients (12.5-50%) of sucrose dissolved in 0.1 M potassium phosphate buffer, pH 7, and were centrifuged in an SW 41 rotor (Beckman) for 240 or 360 min at 40,000 rpm. After centrifugation, the distribution of RNA in the tubes was measured spectrophotometrically (A_{260}) using a flow-through cuvette.

RESULTS AND DISCUSSION

The radiation from the FS40 lamps, filtered through Kodacel plastic, inactivated the infectivity of TMV-RNA (Figure): 15 min of irradiation had approximately the same effect as 80 min of summer noontime sunlight in Davis (15). The logarithm of the surviving fraction of infectivity dropped more strongly as a function of dose for survival above 30% than for survival below

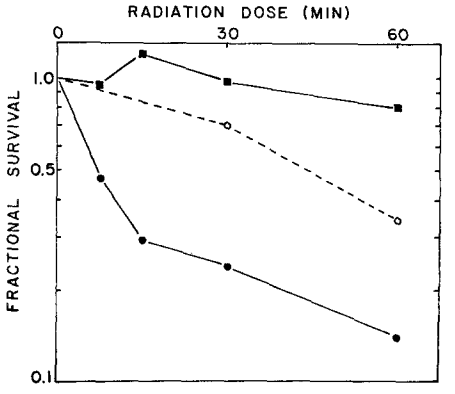

Figure. Inactivation of TMV-RNA infectivity by radiation from FS40 fluorescent sunlamps, filtered with Kodacel (●,○) or with Mylar Type A (■). Closed symbols: no photoreactivation. Open symbol: RNA photoreactivated after inoculation. No sensitizing agents were used in these experiments.

30%, suggesting that different fractions of RNA had different sensitivities to the radiation or that the sensitivity of RNA decreased during irradiation. Radiation from the same lamps, filtered through Mylar plastic, had little if any effect on the infectivity, demonstrating that most of the inactivating radiation passing through Kodacel was at the low end of the emission spectrum (290-320 nm).

The addition of sulfanilamide, chlortetracycline, and four other compounds increased the inactivation of TMV-RNA by a standard 10 min dose of radiation (Table, column A). Among these compounds, sulfanilamide, $AgNO_3$, and $HgCl_2$ were most effective: concentrations on the order of 10^{-3} M resulted in survivals after irradiation of approximately 1/10 that of the control irradiated without sensitizer. Acetone, which has been previously described as a sensitizer for both DNA (6, 8) and RNA (10, 11), was much less effective, even at a concentration as high as 3% (0.4 M). Hydroquinone and chlortetracycline were slightly more effective than acetone at molar concentrations approximately 100-fold lower.

When the radiation was filtered through Mylar plastic (Table, column B), the sensitizing effects of sulfanilamide and acetone were negligible. Sensi-

TABLE

Sensitized inactivation of TMV-RNA infectivity. Each value in columns A through D represents the fractional survival of infectivity after a 10 min treatment with FS40 radiation under the conditions described. Conditions: A, Kodacel filter, open beaker, no photoreactivation; B, Mylar Type A filter, open beaker, no photoreactivation; C, Kodacel filter, closed cuvettes flushed with N_2, no photoreactivation; D, Kodacel filter, open beaker, photoreactivating light given after inoculation.

Sensitizing compound	Concentration (M)	A Kodacel air no PR	B Mylar air no PR	C Kodacel N_2 no PR	D Kodacel air PR	f_p
sulfanilamide	0.0005	0.078		$\leq$ 0.02		
	0.0015	0.031	0.96		0.062	0.20
chlortetracycline	0.0017	0.32		0.19		
	0.005	0.19	0.24		0.32	0.30
acetone	0.13	0.30		0.076		
	0.40	0.23	0.90		0.33	0.25
hydroquinone	0.001	0.15		0.56		
	0.003	0.15	0.32		0.19	0.12
$AgNO_3$	0.0001	0.15		0.12		
	0.001	0.042	0.63		0.11	0.30
$HgCl_2$	0.0001	0.15		0.21		
	0.001	0.062	0.26		0.054	-0.05

tization by these compounds must depend on middle ultraviolet wavelengths (290-320 nm) emitted by the FS40 lamp. The sensitizing effects of $AgNO_3$ and $HgCl_2$ were also reduced in Mylar-filtered light. The effects of hydroquinone and chlortetracycline, however, were only slightly reduced by the use of the Mylar filter; sensitization by these compounds must involve the longer wavelengths emitted by the lamps.

The possibility that a fraction of the damage might occur through the induction of chain breaks was tested by zonal centrifugation of irradiated TMV-RNA. Samples of RNA were irradiated for 10 min in open beakers and in the presence of the higher concentration of each sensitizing agent indicated

in the Table. In each case, the RNA appeared primarily in a single peak. Irradiation (unsensitized) did not change the amount of RNA in the peak or the rate of sedimentation. In the presence of each sensitizer, the recovery of RNA in the main peak was 80-85% of that found with unsensitized, irradiated samples. In the presence of $AgNO_3$ and $HgCl_2$, the peak sedimented 1.7 and 2.5 times faster than normal, indicating the formation of Ag-RNA and Hg-RNA complexes; in all other cases, sensitizing agents did not affect the rate of sedimentation of the RNA. Sensitization apparently produced only a small degree of breakage of the RNA molecules.

To test for the presence of cyclobutadipyrimidines, I measured the photoreactivation of inactivated TMV-RNA; photoreactivation of UV-damaged RNA has been correlated with these photoproducts (11). I observed photoreactivation of TMV-RNA inactivated in the presence of each of the sensitizing agents tested, except $HgCl_2$ (Table, compare Columns A and D, see Column f_p). The photoreactivated sectors ranged from 0.12 (hydroquinone) to 0.30 ($AgNO_3$ and chlortetracycline). These sectors represent an estimate of the fraction of the damage attributable to cyclobutadipyrimidines, but the estimate is probably low, since it is likely that not all cyclobutadipyrimidines are photoreactivable. These sectors are comparable to those reported for TMV-RNA inactivated (unsensitized) at 254 nm (ref. 13, $f_p = 0.30$) and for TMV-RNA inactivated in the presence of acetone at 313 nm (ref. 11, $f_p = 0.21$). The data for survival of TMV-RNA irradiated (unsensitized) for 15 min or less with the FS40 lamps suggested an unusually large sector of 0.75 (Figure), though this sector decreased with longer irradiation times.

The induction of cyclobutadipyrimidines is thought to occur through triplet excitation. The exclusion of O_2, a triplet-quenching agent, has been shown to increase the rate of formation of cyclobutadipyrimidines in acetone-, acetophenone-, and benzophenone-sensitized DNA (8). If transfer of triplet energy from sensitizer to nucleic acid represents a mechanism of damage of TMV-RNA, then the exclusion of O_2 should lead to increased inactivation.

Such increased inactivation of TMV-RNA was found when acetone, sulfanilamide, and chlortetracycline were used as sensitizing agents, but was not observed with the other compounds (Table, compare Columns A and C). The exclusion of O_2 strongly inhibited sensitization by hydroquinone. A limiting role for oxygen in hydroquinone sensitization would explain why an increase in the concentration of hydroquinone from 0.001 to 0.003 M did not increase the rate of inactivation (Table, Column A).

The various compounds tested apparently act in diverse ways. Acetone probably transfers triplet energy to TMV-RNA, thus inducing the formation of cyclobutadipyrimidines, though several other reactions may also occur (11). Ag^{+} and Hg^{++} both form complexes with TMV-RNA. Ag^{+} seems to stimulate formation of cyclobutadipyrimidines in TMV-RNA, as it does in DNA (7). However, Hg^{++}-complexes with TMV-RNA act differently from those with DNA, since Hg^{++} protects DNA by stimulating dipyrimidine reversal (16). Hydroquinone apparently acts through some oxygen-dependent mechanism, perhaps by promoting formation of singlet oxygen. None of these compounds cause any appreciable chain breakage.

The effects of sulfanilamide are like those of acetone; thus sulfamilamide probably transfers triplet energy to RNA. The effects of chlortetracycline are in part similar to these, but the differences suggest the chlortetracycline action may be more complex. Further experiments are needed to determine the exact nature of the sensitized photoreactions and to identify the RNA bases involved. Since sulfanilamide and chlortetracycline are used clinically, it seems especially important to determine whether they affect DNA as they do RNA.

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