
The photoinactivation of an RNA animal virus, vesicular stomatitis virus, with the aid of newly synthesized psoralen derivatives

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Received 7 January 1977

ABSTRACT

The newly synthesized psoralen derivatives, 4' hydroxymethyl 4,5',8 trimethylpsoralen, 4' methoxymethyl 4,5',8 trimethylpsoralen, and 4' aminomethyl 4,5',8 trimethylpsoralen hydrochloride photoreact with the single-stranded RNA animal virus, Vesicular Stomatitis virus, VSV. This virus is inactivated 10^3 times more effectively by photoreaction with these compounds than when photoreacted with 4,5',8 trimethylpsoralen. Under these conditions the RNA virus remains more than 10^3 times less sensitive to inactivation by these new photoreagents than were two double-stranded DNA viruses, Herpes Simplex type 2 (HSV-2) and Vaccinia. Preliminary evidence for the generality of this result is discussed.

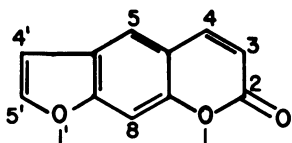
INTRODUCTION

In 1965, Musajo et al. (1) reported the photoinactivation of three DNA viruses [Pseudorabies virus (PrV), Infectious Canine Hepatitis virus (ICNV), Fowl Pox virus (FPV)] by photochemical reaction with psoralen (I). The RNA viruses [New Castle Disease virus (NDV), Foot and Mouth Disease virus (FMDV), Teschen Disease virus (TDV)] were also tested under the same conditions and found to be unaffected by photoreaction with psoralen.

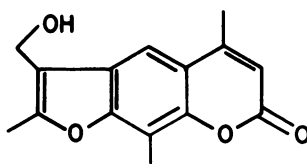
Isaacs, Shen, Hearst and Rapoport (2) have described the synthesis and properties of a new class of psoralen derivatives which are unusually effective in cross-linking double-stranded RNA. These compounds, 4' hydroxymethyl, 4, 5', 8 trimethylpsoralen (II), 4' methoxymethyl, 4, 5', 8 trimethylpsoralen (III), and 4' aminomethyl, 4,5',8 trimethylpsoralen (IV) all have the unique properties of strong binding to nucleic acids, high solubility in water, and photoreactivity with the nucleic acids so that one drug addition plus irradiation results in the near saturation of the nucleic acid with

psoralen photoadducts. Because these properties are directly related to the efficacy of these compounds as probes to *in vivo* secondary structures of nucleic acids, as aids to the inactivation of viruses for the purposes of vaccine production and also as chemotherapeutic reagents in the control of tumors, we have initiated experiments which measure their ability to inactivate many biological systems.

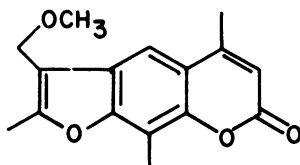
In this brief report, we demonstrate that at high doses (30 $\mu\text{g/ml}$) all three drugs are nearly one thousand times more effective than the common commercially available psoralen—4,5',8 trimethylpsoralen (V)—in the inactivation of the RNA animal virus, Vesicular Stomatitis virus, VSV. At lower doses, 1 to 3 $\mu\text{g/ml}$, the absolute superiority of 4' aminomethyl 4,5',8 trimethylpsoralen (IV) has been demonstrated. This superiority results from the stronger binding of IV to nucleic acids relative to the other derivatives.



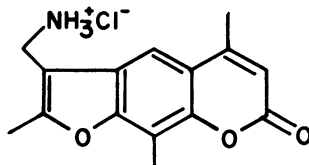
I. Psoralen



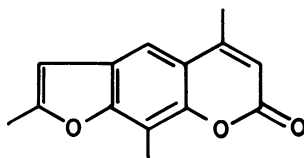
II. 4' Hydroxymethyl
4,5',8 trimethylpsoralen



III. 4' Methoxymethyl
4,5',8 trimethyl-
psoralen



IV. 4' Aminomethyl
4,5',8 trimethylpsoralen
hydrochloride



V. 4,5',8 Trimethylpsoralen

MATERIALS AND METHODS

Virus Strains and Titer Procedures. 0.5 ml samples of phosphate buffered saline (PBS-8g NaCl, 0.2 g KCl, 0.2 g KH_2PO_4 , 0.15 g Na_2HPO_4 in 1 liter) containing 5×10^4 virus plaque-forming units (PFU) were added to 3.5 cm diameter plastic petri dishes and irradiated through the covers.

At various time intervals, 50 μl aliquots were sampled from the dishes and the number of PFU were determined. Dilutions of the aliquot were plated on monolayers of primary chicken fibroblasts grown in plastic Sterilin trays (Triple Vent 305V). After virus adsorption, the cultures were overlaid with nutrient medium containing 20% calf serum and 3% methylcellulose. The vital stain, neutral red, was added after 2 to 4 days incubation at 35 C, and the plaques counted.

The single-stranded RNA virus, Vesicular Stomatitis virus (VSV) Indiana strain, was received from Dr. J. Zavada (Bratislava). The double-stranded DNA viruses used for comparison were Herpes Simplex virus type 2 (HSV2) received from Dr. J. Melnick (Baylor) and Vaccinia virus received from Dr. B. Moss (Bethesda).

Light Source and Irradiation. A Type A405-TLGW/05 long wavelength ultraviolet lamp manufactured by P. W. Allen Co., London was used for all irradiations. The lamp was placed above the virus samples in direct contact with the covers of 3.5 cm diameter plastic petri dishes at room temperature. Figure 1 shows the spectral distribution of the output of this lamp. The total intensity delivered to the sample under these conditions was 1.3×10^{15} photons/sec cm^2 or 0.7 mW/ cm^2 in the petri dish by actinometry (3).

Psoralen Derivatives. The synthesis of the three psoralen derivatives II, III, and IV has been described by Isaacs et al. (2). The derivatives were stored in ethanol stock solutions at concentrations from 0.3 to 1.0 mg/ml. The ethanol stock of appropriate volume was added to a 3.5 cm diameter petri dish and the ethanol partially air evaporated prior to the addition of the 50 μl of virus stock in PBS. The evaporation of the ethanol was designed to keep the ethanol concentration in the virus solution below 2%. The 4,5',8 trimethylpsoralen (v) was

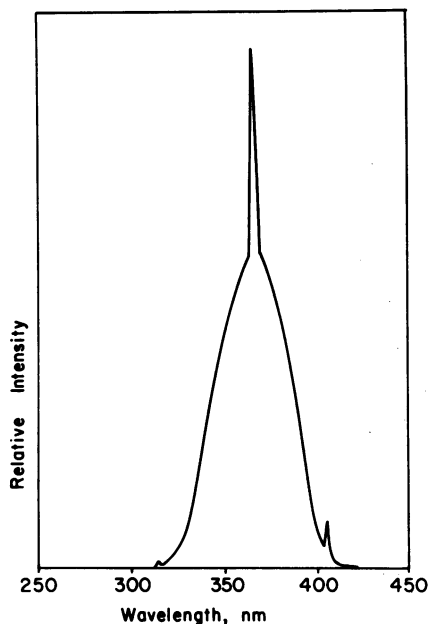


Figure 1. The relative spectral output of the long wavelength ultraviolet light source used in these inactivation experiments. The source is a Type A 405-TLW/05 manufactured by P. W. Allen Co. London.

purchased from the Paul B. Elder, Co., P. O. Box 31, Bryan, Ohio 43506.

RESULTS

At high concentrations (20-30 $\mu\text{g/ml}$), Figure 2 shows that all three derivatives; 4' hydroxymethyl 4,5',8 trimethylpsoralen (II), 4' methoxymethyl 4,5',8 trimethylpsoralen (III) and 4' aminomethyl 4,5',8 trimethylpsoralen (IV) are equivalent with respect to light dose response and are 10^3 times more effective than 4,5',8 trimethylpsoralen (V) in inactivating this virus after 30 minutes of irradiation. The equivalence of these three drugs at these high concentrations is essentially predictable on the basis of their dissociation constants from nucleic acids and their high solubilities (2). We view the virus binding capacity of non-covalently bound intercalated drugs to be saturated in these experiments and therefore all three drugs appear equivalent. The trioxsalen (V) is only soluble to the extent of 0.6 $\mu\text{g/ml}$ and therefore increas-

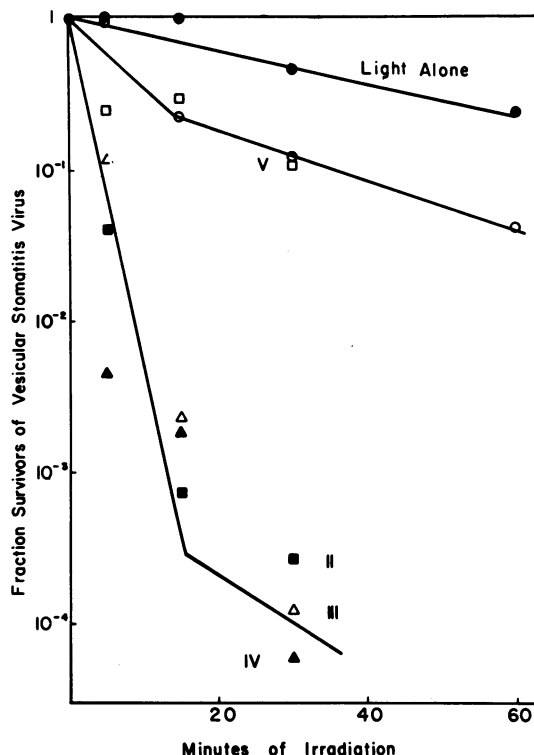


Figure 2. Survival curves at high concentrations of psoralen derivatives. The survival of plaque forming units of Vesicular Stomatitis virus (VSV) after different doses of long wavelength UV irradiation. ● - light alone, ○ - trioxsalen (V) at 10 µg/ml, □ - trioxsalen (V) at 30 µg/ml, ■ - 4' hydroxymethyl trioxsalen (II) at 30 µg/ml, △ - 4' methoxymethyl trioxsalen (III) at 30 µg/ml, ▲ - 4' aminomethyl trioxsalen (IV) at 20 µg/ml.

ing its concentration from 10 µg/ml to 30 µg/ml has no effect. We believe the non-linearity of the response to light is caused by the photodestruction of free derivative (2).

At low concentrations, Figure 3, the far stronger binding of the 4' aminomethyl trioxsalen (IV) to nucleic acid is clearly demonstrated, for only with this derivative is there less than a statistically significant amount of survival observed after 30 minutes of irradiation. The 4' hydroxymethyl trioxsalen (II) and the 4' methoxymethyl trioxsalen (III) both show a reduction of inactivation approximately proportional to the reduction in their concentrations when compared with the results presented in Figure 2.

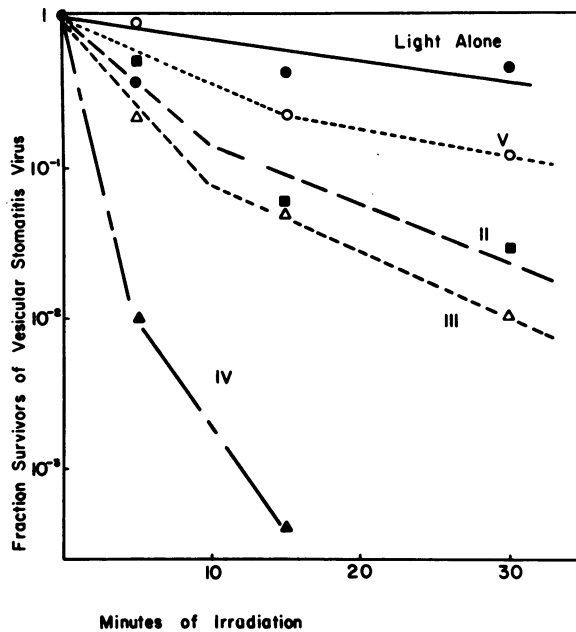


Figure 3. Survival curves at low concentrations of psoralen derivatives. The survival of plaque forming units of Vesicular Stomatitis virus (VSV) after different doses of long wavelength UV irradiation. ● - light alone, ○ - trioxsalen (V) at 10 $\mu\text{g/ml}$, ■ - 4' hydroxymethyl trioxsalen (II) at 1 $\mu\text{g/ml}$, Δ - 4' methoxymethyl trioxsalen (III) at 3 $\mu\text{g/ml}$, \blacktriangle - 4' aminomethyl trioxsalen (IV) at 2 $\mu\text{g/ml}$.

The slope of the response curves observed with the 4' aminomethyl trioxsalen (IV) derivative is light limited and because of the strong binding of this derivative to nucleic acid, the inactivation is independent of drug concentration at both concentrations used here (2 $\mu\text{g/ml}$ 59 20 $\mu\text{g/ml}$). Both of these concentrations are high enough to saturate the binding sites of the RNA in the virus (2). The slope of the response curve for 4' aminomethyl trioxsalen is 5 times greater than for trioxsalen.

Figure 4 provides a comparison between the ability of 4' aminomethyl trioxsalen (IV) in inactivating the RNA virus, Vesicular Stomatitis virus (VSV), and its ability in inactivating two double-stranded DNA viruses, Herpes Simplex virus type 2 (HSV 2) and Vaccinia virus. These experiments were all irradiated for a constant time of 15 minutes but at different concentrations of drug.

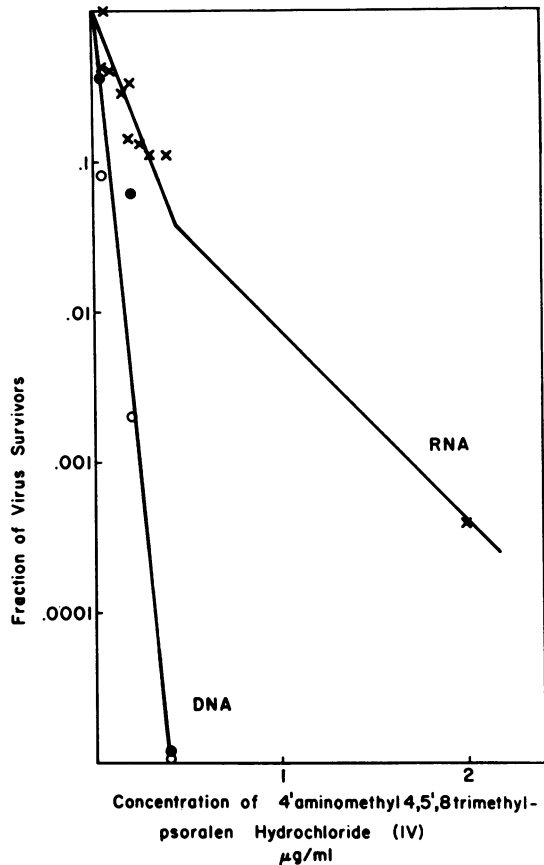


Figure 4. The effect of different concentrations of 4' aminomethyl 4,5',8 trimethylpsoralen upon the photoinactivation of the single-stranded RNA virus, Vesicular Stomatitis virus (VSV)-(X) and the two double-stranded DNA viruses, Herpes Simplex type 2 (HSV-2)-(O) and Vaccinia-(●). All samples were irradiated for 15 minutes and received an estimated dose of 0.63 joules/cm^2 of radiant energy.

DISCUSSION

The successful inactivation of a single-stranded RNA virus by psoralen photochemistry is likely to result, at least in part, from the cross-linkage of regions of double helix duplex which is part of the secondary structure in the intact virion. The cross-linkage of RNA helices by photoreaction with the psoralens was first observed by Isaacs et al. (2) and has subsequently been confirmed using the rRNA of *D. Melanogaster* (4) and intact Rous Sarcoma virus (RSV) particles (5) by our co-workers. A surprising feature of all the psoralen deriva-

tives that we have investigated is the ease with which they penetrate both cells and virus particles. This is a property rather different from that of proflavin which has been used in the photodynamic inactivation of animal viruses (6).

The inactivation of two other RNA viruses has been observed and although the results are too preliminary to present here the greater photochemical activities of the three new derivatives used in this study have been confirmed in these two additional cases as well. We have successfully inactivated Fowl Plague virus (7) and Western Equine Encephalitis virus (WEE) (7,8) by these same photoreactions.

Figure 4 shows that after 15 minute irradiation at the described conditions the DNA virus titers (Herpes Simplex virus type 2 (HSV-2) and Vaccinia virus) are reduced more than 10^3 times more by photochemical reaction with 4' aminomethyl 4,5',8 trimethylpsoralen (IV) than is the Vesicular Stomatitis virus (VSV) titer. The slope of the inactivation curve for the DNA viruses (Figure 4) is 5 times larger than for the RNA virus. The greater sensitivity of the DNA viruses under these conditions to photoreaction with 4' aminomethyl 4,5',8 trimethylpsoralen could be the consequence of a smaller number of psoralen binding sites per base pair in the RNA virus relative to the DNA virus because of the smaller amount of double-stranded secondary structure in the RNA virus. Additional explanations include a larger and more complex genome in the DNA viruses than the RNA virus, a difference in the photoreactivity of the psoralens with DNA and RNA, or any combination of all of these.

Finally, it should be emphasized that the commercial compound, trioxsalen (V), is nearly as effective in the photochemical inactivation of the two DNA viruses studied here as are the newly synthesized derivatives. The new derivatives, however, do provide a distinct advantage with the RNA viruses.

ACKNOWLEDGMENTS

We are very grateful to L. Tack and J. Stiénor for their excellent technical assistance with the virus inactivation studies. The synthesis of the psoralen derivatives was performed by S. Isaacs whom we thank. These experiments were

supported in part by the Fonds de la Recherche Scientifique Medicale in Belgium and by the American Cancer Society Grant #NP 185 in the United States.

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*In memory of Jerome Vinograd, the stoic - my educator and friend

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