Photochemical Destruction of the Virucidal Activities of Retinoids and Unsaturated Fatty Acids

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Photochemical damage either to retinoids by near-ultraviolet radiation or to unsaturated fatty acids by near-ultraviolet radiation in the presence of a hydrophobic photosensitizer destroys the virucidal activities of the compounds, as determined by studies on the enveloped bacteriophage φ6.

We have reported that some unsaturated fatty acids (UFAs) (7, 8) and retinoids (1, 5) have potent virucidal effects against lipid-containing viruses, particularly herpesviruses. Kohn et al. (3) recently extended some of these studies and showed that arboviruses, myxoviruses, and paramyxoviruses are readily inactivated by UFAs, whereas we found that even some non-lipid-containing viruses are sensitive to several retinoids (1, 5). The virucidal activities observed for the lipid-containing viruses seem to be due to a perturbative interaction of the fatty acid or vitamin molecules with the viral lipid-protein envelope resulting in release of protein from the envelope at low concentration (4) and envelope delipidation at high concentration (3) of the virucidal agent.

Retinoids and UFAs are both sensitive to light. Relevant to any possible future medical uses of these classes of molecules as topical virucidal agents is the question of the effect of light on the virucidal activities of these compounds. I report here that photodynamic damage to oleic and linoleic acids and photochemical damage to retinal totally destroy their virucidal activities.

The optical absorption spectrum of retinal has a broad maximum centered at 380 nm. To irradiate retinal, Westinghouse BLB bulbs having a broad emission spectrum centered at 355 nm (near ultraviolet) were used. By visual inspection, the yellow color of retinal solutions in TB buffer (9) disappeared within 2 min of exposure to four BLB bulbs at a distance of 6.5 cm (9). To quantitate the observation of this bleaching of retinal, I measured the optical density of the retinal solution at 400 nm (Table 1).

The virucidal activity of irradiated retinal was determined against the enveloped bacteriophage φ6 (10), which has been repeatedly shown to be very similar to enveloped mammalian viruses in its sensitivity to lipophilic virucidal agents (4-8). The data reported in Table 1 show that retinal bleaching results in a loss of the virucidal activity of the vitamin.

Studies similar to those reported here for retinal have been performed for several other retinoids with similar results being obtained; i.e., retinoid bleaching in all cases led to loss of virucidal activity against φ6 and several other bacteriophages (data not shown).

UFAs are not nearly as sensitive to light as is retinal. The sensitivity of UFAs is greatly increased, however, in the presence of a hydrophobic photosensitizer. I therefore investigated the effects of exposure of UFAs to near-ultraviolet radiation in the presence of acridine (9) on their virucidal properties. Figure 1 shows that, when exposed to near-ultraviolet radiation in the presence of acridine, both oleic and linoleic acids lost virucidal activity. The polysaturated (linoleic

<table>
<thead>
<tr>
<th>Near-UV dose (s)</th>
<th>Retinal absorbance (400 nm)</th>
<th>Virucidal activity (% φ6 inactivation)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>0.57</td>
<td>&gt;99</td>
</tr>
<tr>
<td>15</td>
<td>0.45</td>
<td>43</td>
</tr>
<tr>
<td>30</td>
<td>0.38</td>
<td>20</td>
</tr>
<tr>
<td>45</td>
<td>0.31</td>
<td>8</td>
</tr>
<tr>
<td>300</td>
<td>0.06</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

* Irradiation was carried out at a distance of 6.5 cm from four vertically mounted Westinghouse BLB bulbs. UV, Ultraviolet.
* Retinal from a 20-mg/ml stock solution in ethanol was added to TB buffer to give 200 µg/ml. Optical density at 400 nm was measured before and after various intervals of exposure to near-ultraviolet radiation.
acid) was more sensitive to this photodynamic treatment than was the monounsaturated, as expected by the presumed singlet oxygen-mediated oxidation of the UFA at unsaturation sites.

Further studies (Table 2) showed that the presence of O$_2$ was required for this photodynamic damage to oleic and linoleic acids and that the sensitivity to damage did not depend significantly on pH (and hence on head-group charge state of the fatty acids).

I also carried out studies to determine whether damaged retinoids or UFAs, which have completely lost virucidal activity, could interfere with the virucidal activity of undamaged agents of the same type. These studies, which involved assaying the virucidal properties of mixtures of irradiated and unirradiated molecules, showed no evidence for any interfering effect of the damaged agents up to a concentration five times greater than that of the undamaged agent.

From the results of the studies reported here, I concluded that photochemically damaged retinoids and photodynamically damaged UFAs have little if any virucidal activity compared with the undamaged molecules. The potent virucidal activity of retinal is readily lost upon exposure to near-ultraviolet radiation, which is prevalent in sunlight at the surface of the earth. This suggests that protection from high-intensity light might need to be a prime concern in any future use of retinoids and other unsaturated lipophilic molecules as virucidal agents.

The virucidal retinoids and UFAs have been shown to act as fusogenic agents in an erythrocyte assay system by inducing a conversion from a bilayer to a hexagonal phospholipid packing phase in at least a portion of the cell membrane (2). In our system, retinoids and UFAs bind to the bacteriophage φ6 lipid-protein envelope, resulting in the release of envelope proteins (4), perhaps because of a similar conversion to a hexagonal lipid phase in the envelope. Photochemically or photodynamically damaged retinoids or UFAs probably are similar to saturated fatty acids in their inability to significantly disrupt phospholipid bilayers.

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**LITERATURE CITED**


