Using Uric Acid for Singlet Oxygen Detection in a Laser Virus Inactivation Experiment

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Abstract: We demonstrate the generation of singlet oxygen in a laser virus inactivation experiment using a low power diode light at 405 nm by detecting photobleaching of the absorption peak of uric acid at 294 nm.
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1. Introduction

Uric acid is a well-known singlet oxygen quencher. The reaction of the excited oxygen with the uric acid molecule produces photobleaching of its light absorption at 294 nm. The method has been applied widely to estimate the photosensitizing properties of different compounds [1-3]. Krasnowsky et al. have proposed to use uric acid for the detection of singlet oxygen in an aqueous environment [4]. We have recently reported that a continuous wave 500 mW laser at 405 nm can effectively inactivate viruses within tissue culture media without the use of additional photosensitizers [5]. We attributed the inactivation to the generation of singlet oxygen in the sample. In this work, we use uric acid to confirm the production of singlet oxygen upon illumination of a virus sample by blue light of relatively low power. We added low concentrations of uric acid to the virus sample to get a high enough absorption at 294 nm. Experiments show strong photobleaching of this peak when illuminating the sample for over two hours with blue light confirming the presence of singlet oxygen during the irradiation procedure. The results demonstrate the photochemical character of the laser inactivation of the virus.

2. Method

The experimental set-up used for the sample's irradiation and details of the method of preparation and analysis of the virus samples have been described elsewhere [5]. We use a 500-mW (405±5) nm diode laser to irradiate the virus samples. We prepare working stocks of murine norovirus (MNV). MNV stock titers ranged from 0.5×10^5 to 1×10^7 pfu/ml. We use 1-cm path-length spectroscopic quartz cell to contain 2 to 3 ml of the sample. We focus the laser light using a 15-cm lens. Using magnetic stirrer, we make sure that the light affects all sample's volume. We use 99% purity uric acid (Alfa Aesar) to demonstrate the generation of singlet oxygen during the irradiation procedure. We measure the absorption spectra of the virus and tissue culture media samples with uric acid before and after the irradiation procedure using a Thermo Scientific UV-VI spectrophotometer model Evolution 201. We monitor the UV peak of uric acid at 294 nm.

3. Results and Analysis

Figure 1a shows the fraction of survival in a logarithmical scale of the MNV virus grown in tissue culture media for different irradiation times. We did not add chemicals or photosensitizers to the sample. After irradiating for over four hours using the 405 nm laser, we reduce the fraction of virus population by four orders of magnitude. To show the photochemical character of the laser virus inactivation process, we measure the UV spectra of the media sample without and with minute amounts of uric acid. The solid black line in figure 1b shows the UV spectra of the media without uric acid. The spectra show the two-band structure characteristic of organic molecules (proteins, amino acids) [6]. The uric acid exhibits a similar structure slightly shifted toward larger wavelengths with a maximal peak at 294 nm. We increase the concentration of uric acid up to 1.2 mM, so the 294-nm peak is seen clearly (red line in figure 1b). Irradiation for over two hours reduces this peak substantially (blue line in figure 1b). As pointed up by previous works [1-3], the effect is evidence that the irradiation is producing singlet oxygen. Culture media is a complex sample containing amino acids, glucose, inorganic salts, vitamins, and a variety of proteins. Of all these compounds, vitamins are the primary suspects for the photosensitizing mechanism. We have performed similar irradiation experiments in samples containing only proteins (bovine serum albumin) showing no effect on the uric

acid. More studies are undergoing to find the compound of the tissue culture responsible for the generation of singlet oxygen in the irradiation experiment.

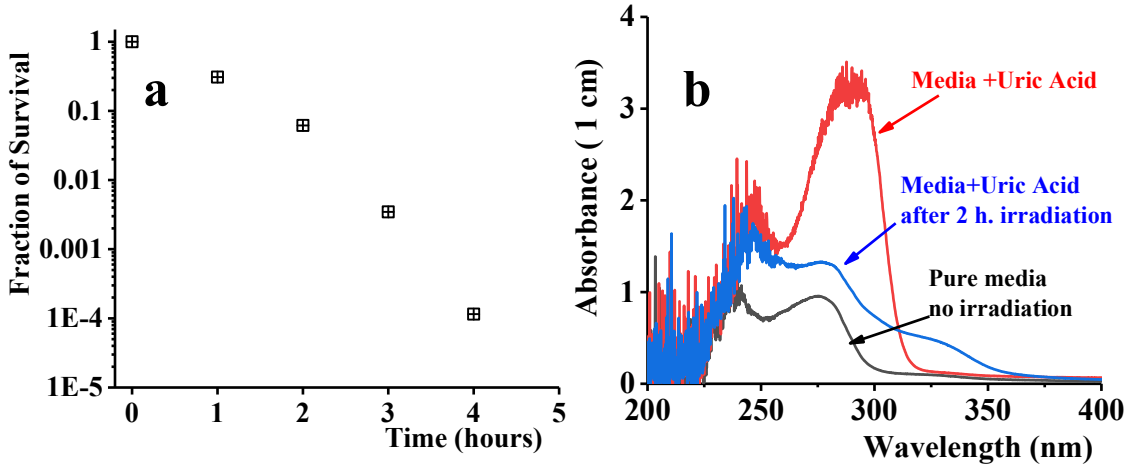

Figure 1. a) The fraction of survival of MNV irradiated using 500 mW of 405 nm light from a diode laser. b) UV spectra of the tissue culture media without uric acid before irradiation (solid black line), the spectrum of the sample after adding uric acid at a 1.2 mM concentration (solid red line) and the spectrum of the same sample after two hours irradiation with the blue light (solid blue line).

4. Conclusions

We have completed irradiation experiments of MNV virus sample using a blue light of relatively low power. The method shows an effective inactivation of the viruses even without the use of additional photosensitizers. A study of the UV spectra of a sample containing uric acid confirms the generation of singlet oxygen upon the irradiation procedure. The results demonstrate a photosensitizing activity of the tissue culture media, which includes the virus.

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6. References

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