Synergism of UV Radiation and Heat for Cell Inactivation

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1. Introduction

Organisms including human beings are constantly exposed to UV radiation. The potential hazards of UV radiation have risen due to a depletion of the protective ozone layer in the stratosphere and the formation of ozone holes. Moreover, the effect of UV radiation may greatly increase due to synergistic interaction of UV radiation with other environmental factors. Fluence rate is known to constitute a very important parameter in photobiology. While it is generally accepted that lowering the UV radiation fluence rate results in a decrease of the cell killing or mutagenesis efficiency per unit dose [1], the matter is still unclear with regards to the synergistic interaction of UV radiation and another environmental agent. It is of great interest to investigate whether or not the synergistic interaction can take place at low intensities of such environmental factors. Heat is known to be an important modifier of UV radiation sensitivity [2,3]. Exposure of skin to UV radiation is often encountered at hot ambient temperatures. Therefore, the elucidation of new fundamental aspects of the simultaneous action of UV radiation and heat is an actual task. Thus, the purpose of the present work was to establish whether the UV fluence rate alters the synergistic interaction between heat and UV radiation for cell inactivation.

2. Materials and Methods

Wild-type diploid yeasts of *Saccharomyces cerevisiae* strain T1 were used in this study. The cells were grown before irradiation up to a stationary stage of growth for 3-5 days at 30°C on a complete growth medium. In order to determine the cell survival after the treatments with UV and hyperthermia a complete nutrient medium was used.

Cell suspension (1.5 mL, 10^6 cells/mL) in a quartz vessel was irradiated with a germicidal lamp that predominantly emitted UV radiation of a wavelength of 254 nm at fluence rates of 0.033**,** 0.15, 0.25 and 1.5 W/m^2 at different temperatures (47.5-60.0°C). Variation of the intensity was achieved by means of a calibrated metal wire net. The UV radiation source was calibrated by potassium ferrioxalate actinometry [1], and the fluence rates were checked with a germicidal meter (General Electric Co.).

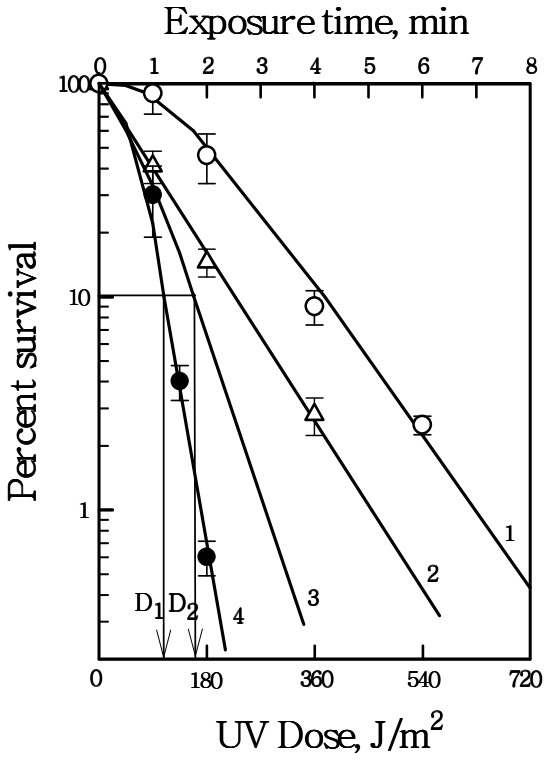
The following heating method was used: 0.1 mL of cell suspension at room temperature (about 1.5 x 10^6 cells) was placed into 1.4 mL of sterile water preheated to a required temperature in a water bath. Thus, the final volume and the concentration of the treated cells were 1.5 mL and 10^6 cells/mL, respectively. For a simultaneous thermal and UV radiation treatment, the time interval between the introduction of the cells into the preheated water and the beginning of the exposure was about 0.1 min. At the end of the treatment, the samples were rapidly cooled to room temperature. To avoid photoreactivation, the UV exposure, dilution and other procedures were performed under a red ambient light, while the post-irradiation incubation was carried out under dark conditions.

3. Results and Discussions

Figure 1 exhibits the basic experimental data obtained in this study for cell inactivation. Here, the survival curves were obtained for *S. cerevisiae* diploid yeast cells after UV irradiation alone (at room temperature, 1.5 W/m^2, curve 1), heat treatment alone (57.5°C, curve 2), the theoretical survival curve expected for an independent action of these agents (curve 3) and the experimental survival curve obtained after a simultaneous action of both factors (curve 4).

To quantify the synergistic effect, we also used the synergistic enhancement ratio (k). For cell inactivation, this coefficient was estimated as the ratio of the expected UV radiation dose (assuming an independent effect of UV radiation and hyperthermia) to that obtained from the experimental survival curve after a simultaneous action of both agents at a fixed level of survival, i.e. for a 10% survival, $k = D_2 / D_1$. From the experimental data, the synergistic enhancement ratio as a function of the exposure temperature and fluence rate could be determined. These data were calculated on the basis of a whole set of dose-effect curves It is worth noting that presented here are the ultimate findings based on the most complete set of our experimental data on this subject, part of which was published before [3,4].

Figure 1. Survival curves of yeast cells: curve 1 – UV irradiation (1.5 W/m^2) alone (room temperature); curve 2 – heat treatment alone (57.5°C); curve 3 – calculated



curve for independent action of UV radiation and high temperature; curves 4 – experimental curve obtained after simultaneous action of both agents.

It is evident that, for a given intensity, there is a specific temperature maximizing the synergistic effect. From these findings, it can be concluded that the temperature at which the UV radiation is delivered should be reduced to obtain the highest synergistic effect with a decrease in the fluence rate. It means also that the temperature range, synergistically increasing the inactivation effect of UV radiation, should be shifted to lower temperatures with a decrease in the fluence rate.

For a long duration of interaction, which is important for problems of UV radiation protection and health effects, one would expect a synergistic interaction of this factor with ambient temperatures. To this end, the biological meaning and significance of the results obtained in this study are apparent. Despite the fact that all of the yeast cells were treated with temperatures far beyond the physiological range, the data may, in principle, serve as an indicator that low UV radiation intensities existing in the biosphere may synergistically interact with environmental heat or physiological temperatures of homoiothermal animals and humans and thereby increase their inactivating consequences.

4. Conclusions

For cell inactivation, there was a specific temperature maximizing the synergistic effect for any constant fluence rate and the temperature range, synergistically increasing the inactivation effect of UV radiation, should be shifted to lower temperatures with a decrease in the fluence rate. To interpret the results observed, a simple mathematical model of the synergistic interaction was applied. The model suggests that the synergistic interaction of UV radiation and heat is expected to result from additional effective damage arising from the interaction of some sublesions induced by both agents. It is supposed that the synergistic interaction of these factors might take place at small intensities of UV radiation and temperatures existing in the biosphere.

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