

Interactions Between Light and Gas Vacuoles in *Halobacterium salinarium* Strain 5: Effect of Ultraviolet Light

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The potential light shielding by intracellular gas vacuoles in *Halobacterium salinarium* strain 5 was examined by looking at the ultraviolet light inactivation curves of both wild-type cells and mutants which are defective in the production of gas vacuoles. Whereas strains defective in gas vacuole production were slightly more sensitive to ultraviolet inactivation, no significant differences in ultraviolet sensitivity were seen, indicating that these subcellular inclusion bodies are not effective as light-shielding organelles. In addition, it was shown that ultraviolet light acts as a plasmid-curing agent in *Halobacterium*.

Gas vacuoles are subcellular inclusions found in a variety of aquatic microorganisms. These bodies consist of aggregates of individual gas vesicles, cylindrical structures which are filled with gas (17). Because of the large difference between the refractive index of cell cytoplasm and the contents of the vesicles (gas), the inclusions intensively scatter light. Gas vacuoles can be collapsed by the application of a critical pressure, which in vivo is a function of the character of the individual gas vesicles as well as of the cell turgor pressure (15). Light scattering drops dramatically after pressurization of a gas vacuolate culture, and the comparison of control and pressurized cultures has been one of the major ways by which the function of gas vacuoles has been analyzed. Colonies of many gas vacuolate microorganisms have a distinct creamy color, and by examining colony color it is relatively easy to identify isolates which do not produce gas vacuoles, as well as those in which there may be alterations in the timing of synthesis or the amount of gas vacuoles (gas vacuole defective, gv^{def}) (12, 16; R. D. Simon, *J. Gen. Microbiol.*, in press).

Gas vacuoles provide a way for aquatic organisms to change their position in the water column (1, 16, 17). Thus, photosynthetic microorganisms can regulate the amount of light they receive, and obligate aerobic heterotrophs such as *Halobacterium* potentially have greater access to oxygen because they can float to the surface. In addition, there is a controversy over whether the light-scattering properties of gas vacuoles may also serve to protect individual cells from the potentially destructive effects of high light intensities. Based upon studies of the spectroscopic character of gas vacuolate micro-

organisms, it has been suggested that the vacuoles act as light-shielding bodies (9, 14). However, such a function would most obviously depend upon the position of the inclusion body in the cell relative to the light-sensitive site. More recent studies suggest that the shielding reported may be an artifact of the techniques used to measure the absorption spectra (8, 11, 17).

The only direct test of the light-shielding hypothesis of gas vacuole function has been reported by Shear and Walsby (11), who studied a photosynthetic, filamentous cyanobacterium, *Anabaena flos-aquae*. They suggest that if gas vacuoles act as light shields, then at limiting light intensities photosynthetic rates should rise after pressure-induced gas vacuole collapse. However, at best there is only a 4% increase in the photosynthetic rate. Light scatter increases exponentially as the wavelength of light decreases, and Shear and Walsby (11) also examined the effect of brief ultraviolet (UV) light exposure on the growth of *A. flos-aquae*. They irradiated dilute suspensions of cyanobacteria to avoid significant shielding of one cell by others in the suspension; no differences in growth inhibition were noted in irradiated cells with or without intact gas vacuoles, and they suggest that the scattering of UV light by gas vacuoles is not enough to protect individual cells from light-induced damage. These experiments are somewhat difficult to further analyze because it is not possible to characterize the effects of UV light on individual cells. In addition, no allowance was made for possible photoreactivation repair of UV-induced damage, repair which itself is light dependent.

It is likely that gas vacuoles have evolved to play different functions in different ecological

niches. Thus, the potential effects of light shielding might best be studied in an organism normally associated with an environment receiving extremely high levels of light irradiation. The halobacteria are found in such an environment: both saline lakes and salterns which contain the elevated salt concentrations required for *Halobacterium* growth are usually located in areas of high solar irradiance. The gas vacuolate forms of halobacteria would float to the surface under these conditions, and it might be expected that gas vacuoles serve as light shields in this situation. It has previously been shown that *Halobacterium* are relatively resistant to killing by sunlight (2; unpublished data) and that the colored carotenoids present in these organisms protect against photooxidative death induced by the addition of exogenous photosensitizers (3).

In this study, I tested the light-shielding hypothesis by repeating the UV-inactivation experiments of Shear and Walsby (11) with *Halobacterium salinarium* strain 5. Experiments were carried out under conditions where only single cells were irradiated and in the absence of photoreactivation repair of DNA damage. Because spontaneous gas vacuole mutants of *H. salinarium* strain 5 are available, it was also possible to compare UV sensitivities among strains producing greatly decreased amounts of gas vacuoles. The major conclusion of this report is that in *Halobacterium*, as in the cyanobacteria, gas vacuole collapse does not increase the sensitivity to UV light, and thus, gas vacuoles do not act as light shields. In addition, strains which were genetically impaired in gas vacuole production were slightly more sensitive to UV light. However, this may only reflect a small change associated with this mutation.

The origin and character of the strains used in the experiments reported here are given in Table 1. The mutant strains used in these studies produced small amounts of gas vacuoles only after several weeks of growth in culture. There-

fore, they were classified as gv^{def} , rather than nonproducers of gas vacuoles (Simon, in press). However, the response of gv^{def} strains was analyzed at times before the formation of any gas vacuoles, as indicated by the absence of a difference in turbidity after pressurization of the experimental material. The major carotenoid present in *Halobacterium* is bacteriorubrin (6). A strain lacking colored carotenoids and gas vacuoles was included to determine whether the absence of this primary pigment would affect UV sensitivity.

The response of *Halobacterium* to UV light was measured by plating known numbers of bacteria on medium solidified with 1.8% agar (7) and directly irradiating the plates with a germicidal lamp. Irradiating cells in this manner avoids any population effects such as shielding of cells by other cells. Previous studies show that *Halobacterium* has 100% plating efficiency under the conditions used here. UV lamp output was calibrated with a thermopile, and the time of irradiation was varied to provide a kill curve of at least four logs. Irradiation was carried out under dim yellow light, and, after irradiation, plates were immediately wrapped in aluminum foil to prevent photoreactivation repair of the damaged DNA (5). Plates were incubated at 42°C for 1 week; colonies were then counted, and the percent survival rates were calculated. To determine whether the physical presence of intact gas vacuoles affected UV sensitivity, some samples of *Halobacterium* were treated in a pressure bomb at 370-kN/m² nitrogen gas pressure for 3 min immediately before plating and UV treatment. Walsby (15) has provided experimental evidence to show that 99% of the gas vacuoles in *H. salinarium* strain 5 would be collapsed by this treatment.

The UV-inactivation curve for wild-type *Halobacterium* with intact gas vacuoles is shown in Fig. 1, and the UV doses required for 37 and 1% survival rates are given in the inset of the same

TABLE 1. Characteristics of isolates of *H. salinarium* strain 5

Strain isolated	Characteristics					Source
	Gas vacuoles ^a	Colored carotenoids ^b	Plasmid composition ^c			
			pRDS101	pRDS102	pRDS103	
Wild type	gv^+	+	+	+	+	H. Larsen
7705	gv^{def}	+	+	-	+	Spontaneous, from wild type (12)
7734	gv^{def}	-	+	-	+	UV-induced mutation

^a Strains are characterized as producing assembled gas vacuoles (gv^+) or producing gas vacuoles but in amounts less than the wild-type strain or after a time delay (gv^{def}).

^b Presence (+) or absence (-) of the major colored carotenoid.

^c Wild-type strains contained three species of plasmid DNA: pRDS101, pRDS102, and pRDS103 with molecular weights of 26×10^6 , 44×10^6 , and 86×10^6 , respectively. Plasmid composition of the various strains was determined as previously described (12).

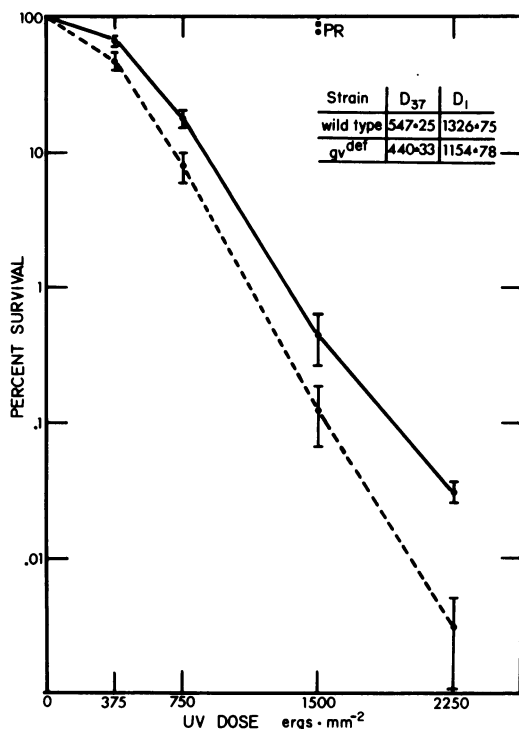


Fig. 1. Effect of UV light on the survival of wild-type *H. salinarium* strain 5 and gv^{def} isolates. Cells were grown as previously described (12), and survival rates after UV irradiation were determined as described in the text. The curves presented are the average of five independent experiments, and the standard deviation of the mean is indicated by the bars through each point. D_{37} and D_1 , Doses of UV light necessary to give 37 and 1% survival, respectively. The inserted box gives the D_{37} and $D_1 \pm$ the standard deviation, which were determined from the survival curves for wild-type and gv^{def} strains. Survival was measured under conditions which would preclude photoreactivation repair (PR) of UV-induced damage. However, to test the photoreactivation of wild-type and gv^{def} strains, samples given a UV dose of $1,500 \text{ ergs mm}^{-2}$ were exposed to 1 h of white light. The survival in these samples is given (PR) for wild-type (—) and gv^{def} (-----) strains.

figure. In a parallel series of measurements, no differences were noted in the rates of inactivation of *Halobacterium* with gas vacuoles collapsed immediately before irradiation. The shape of the inactivation curves and the doses necessary to produce 37 and 1% survival rates were statistically identical. Thus, the presence of intact gas vacuoles does not protect *Halobacterium* from the destructive effects of UV light.

The effect of photoreactivation repair of DNA damage was measured by illuminating UV-irradiated plates for 1 h in cool white fluorescent light at an intensity of $10^{-4} \text{ ergs cm}^{-2} \text{ s}^{-1}$. Plates were then wrapped in aluminum foil and treated

as un-photoreactivated sets. Samples of *Halobacterium* which were given doses of UV light which would normally result in 0.1 to 0.5% survival rates ($1500 \text{ ergs mm}^{-2}$) could be photoreactivated to between 75 and 90% colony survival rates. Although the detailed kinetics of photoreactivation were not studied, there were no major differences between wild-type strains with or without intact gas vacuoles or between wild-type and gv^{def} isolates.

Strains which were gv^{def} (e.g., 7705) were slightly more sensitive to UV inactivation than wild-type strains (Fig. 1). The differences are statistically significant, and the dosages necessary for equivalent killing are approximately 20% higher in the wild-type isolate. Strain 7734, an isolate without carotenoids, shows the same inactivation curves as gv^{def} strains containing colored carotenoids. Thus, the presence of these pigments does not protect the cells in any way from UV-induced damage. The gv^{def} strains used here all lack the plasmid pRDS102 (12; Simon, in press). Although it is known that repair systems for UV-induced damage can be specified by plasmid-localized genes (10), the increase in UV sensitivity is small and not the order-of-magnitude difference often associated with the loss of a dark repair system (13). Thus, pRDS102 does not encode a UV repair system. This is supported by the findings of Grey and Fitt (4), who show that there may be no dark repair in *Halobacterium*. The slightly increased UV sensitivity of gv^{def} strains could result from small changes in the UV-sensitive target, e.g., variations in cell size or morphology, changes in nucleoid structure or DNA content per cell, etc. (13). Thus, although it is not possible to simply explain the origin of the altered UV sensitivity, the data do suggest that there are probably differences between strains with and without pRDS102 other than the presence of wild-type gas vacuoles.

Strains of *Halobacterium* which lose the plasmid pRDS102 are gas vacuole defectives. It becomes easy to identify isolates cured of pRDS102 because the colony color is different from that of the wild-type strain (12). Studies show that although plasmid pRDS102 is lost under normal growth conditions at a very high frequency (10^{-2}) (12), many compounds which normally act as plasmid-curing agents in bacteria are without affect on *Halobacterium* (12, 18) and do not increase this frequency. However, Willets (19) has shown that mutagenic agents, including UV light, are able to induce the curing of the F *lac*⁺ plasmid in *Escherichia coli*. Whereas several chemical mutagens will not act as plasmid-curing agents in *Halobacterium* (12; Simon, unpublished data), data in Fig. 2 show that doses of UV light will increase the frequency

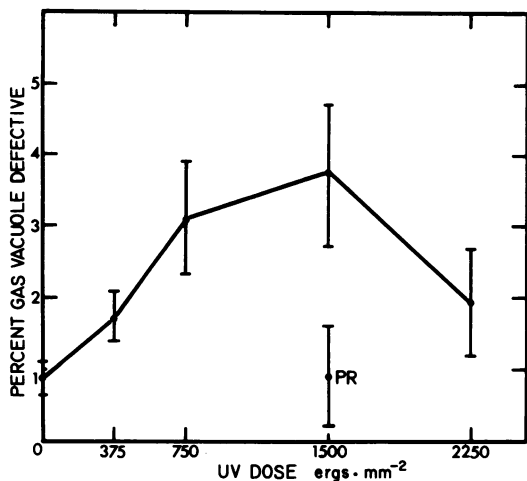


FIG. 2. Effect of UV light on the frequency of *gv*^{def} isolates induced in wild-type *H. salinarium* strain 5. Wild-type *Halobacterium* was treated with various doses of UV light as described in the text. The morphology of colonies formed from cells surviving the treatment was examined. All colonies which were redder than the creamy-pink color of the untreated wild-type colonies were scored as *gv*^{def}. The results given are the average of five experiments, and the standard deviation of the mean is indicated by the bars drawn through each point. The experiments were normally carried out under conditions which preclude photoreactivation repair (PR) of UV-induced damage. However, the effect of allowing PR is shown for samples irradiated with UV at 1,500 ergs mm⁻².

of *gv*^{def} progeny in the survivors. At a dose of 1,500 ergs mm⁻², which gives only about a 0.5% survival rate, almost 4% of the survivors are *gv*^{def}. Analysis of the plasmid composition of selected UV-induced *gv*^{def} strains indicated the absence of pRDS102 (not shown). The plasmid loss is associated with the UV-induced mutagenic effect, as shown by the fact that conditions which allow for photoreactivation decrease the percentage of *gv*^{def} progeny to the level normally seen in untreated cultures (Fig. 2).

Gas vacuolate halobacteria often are found in areas receiving high rates of solar influx for long periods of time. In addition, the presence of gas vacuoles would tend to accumulate cells at the water surface where the potential for light-induced damage was the greatest. Data provided here and elsewhere (11) indicate that the light-scattering properties of gas vacuoles do not provide the individual cell with protection from light-induced damage. If such protection occurs, it most likely does so as a population phenomenon where the upper cells in a layer are them-

selves sacrificed, but provide some protection for some cells lying below them.

I thank M. Shilo for providing facilities with which to prepare the manuscript and Rachel Yehezkel for secretarial help.

This work was supported in part by grant PCM 77-202322 from the National Science Foundation.

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