Vibrio harveyi bioluminescence plays a role in stimulation of DNA repair

Agata Czyz, Borys Wróbel and Grzegorz Węgrzyn

Although the genetics and biochemistry of bacterial luminescence have been investigated extensively, the biological role of this phenomenon remains unclear. Here it is shown that luxA, luxB and luxD mutants (unable to emit light) of the marine bacterium Vibrio harveyi are significantly more sensitive to UV irradiation when cultivated in the dark after irradiation than when cultivated under a white fluorescent lamp. This difference was much less pronounced in the wild-type (luminescent) V. harveyi strain. Survival of UV-irradiated Escherichia coli wild-type cells depended on subsequent cultivation conditions (in the dark or in the presence of external light). However, after UV irradiation, the percentage of surviving E. coli cells that bear V. harveyi genes responsible for luminescence was significantly higher than that of non-luminescent E. coli, irrespective of the subsequent cultivation conditions. Moreover, it is demonstrated that luminescence of V. harveyi can be stimulated by UV irradiation even in diluted cultures, under conditions when light emission by these bacteria is normally impaired due to quorum sensing regulation. It is proposed that luminescent bacteria have an internal source of light which could be used in DNA repair by a photoreactivation process. Therefore, production of internal light ensuring effective DNA repair seems to be at least one of the biological functions of bacterial luminescence.

Keywords: bioluminescence, Vibrio harveyi lux genes, DNA repair, photoreactivation, SOS response

INTRODUCTION

The process of bioluminescence is relatively common in both prokaryotic cells and complex eukaryotic organisms. A biological role for light emission in animals (e.g. firefly) may be ascribed to sending signals from one individual to another. However, the role of luminescence in bacteria remains unclear.

There are several known bacterial species that are able to emit light (Meighen, 1994). In fact, light-emitting bacteria are the most abundant and widespread of the luminescent organisms found in marine, freshwater and terrestrial habitats. The process of luminescence is found in symbiotic, saprophytic, parasitic, as well as in free-living bacteria (for a review see Meighen, 1994). The ecological benefit for a fish or squid living in symbiotic association with luminescent bacteria has been established (Morin et al., 1975; Nealson & Hastings, 1979). The host organism can use light emitted by bacteria for attraction of prey, escape from predators or intraspecies communication (Morin et al., 1975; Bassler & Silverman, 1995). However, it is not understood what specific benefit free-living or symbiotic bacteria derive from producing light (compare Bassler & Silverman, 1995). On the other hand, it seems obvious that luminescence must have a positive selective value since as much as several per cent of the bacterial cell energy is consumed by this process (Makemson, 1986; Bassler & Silverman, 1995). Some speculations on the potential biochemical role of bacterial luminescence were reported (e.g. that the light-emitting system could function as an alternative pathway for electron flow) (Nealson & Hastings, 1979), but they have never been verified experimentally.

In the course of our studies on the free-living bioluminescent marine bacterium Vibrio harveyi, we have isolated many random mutants. Among these, several...
mutants were very sensitive to UV irradiation. Surprisingly, we found that most of these mutants had also lost the ability to emit light. Further studies, described in this article, led us to propose that production of internal light ensuring effective DNA repair, most probably by photoreactivation, may be at least one of the biological functions of bacterial luminescence.

METHODS

Bacterial strains, plasmids and phage. Bacterial strains are listed in Table 1. Plasmids containing the V. harveyi luxCDABE operon and the luxR gene, cloned in pLAFR2 and pACYC184 vectors, respectively, have already been described (Martin et al., 1989; Showalter et al., 1990). Plasmid pSUPTn5pMCS (MacKenzie et al., 1995) and bacteriophage P1CMcr100 (Rosner, 1972) were used in transposon mutagenesis experiments.

Culture media and growth conditions. The Luria–Bertani (LB) and BOSS media have already been described by Sambrook et al. (1989) and Klein et al. (1998), respectively. The minimal medium 3, described previously by Węgrzyń and Taylor (1992), was used, but in the case of V. harveyi cultivation, the concentration of NaCl was 3%. In all experiments, V. harveyi and Escherichia coli strains were cultivated at 30 and 37 °C, respectively.

Transposon mutagenesis. Transposon mutagenesis of V. harveyi was performed using a combination of previously described modified transposon mutagenesis (MacKenzie et al., 1995) and P1 transduction (Rosner, 1972) procedures.

UV sensitivity assays. V. harveyi strains were cultivated in BOSS medium, centrifuged and resuspended in 3% NaCl. Following UV irradiation of 1 × 10⁹ cells, bacteria were incubated in BOSS medium in the dark or under a white fluorescent lamp for 2 h, and then titrated on BOSS plates (the plates were incubated overnight in the dark). An analogous procedure was employed for E. coli strains, but LB medium was used instead of BOSS, and 0.9% NaCl was used instead of 3% NaCl. In the agar plate test, bacteria were streaked across the plate, and sectors of the plate were irradiated with different UV doses. The plate was incubated overnight in the dark, and growth inhibition was estimated.

Measurement of bacterial luminescence. V. harveyi strains were grown to high cell density (1 × 10⁸ cells ml⁻¹) in minimal medium 3 containing 3% NaCl. Then, the cultures were diluted 10000-fold in fresh minimal medium 3 and cultivated for 5 h in order to minimize luminescence. Bacteria were irradiated with different doses of UV and incubation was continued for the indicated time periods. Luminescence was monitored at each stage of cultivation in a scintillation counter using chemiluminescence mode as described previously (Bassler et al., 1994). The relative light units were calculated as counts min⁻¹ ml⁻¹ per cell. Luminescence of E. coli strains was measured by the method described above, but appropriate medium was used and the cultures were not diluted.

RESULTS AND DISCUSSION

Using a combination of P1 transduction and modified transposon mutagenesis procedures, we isolated 81 random V. harveyi mutants. Among them, six mutants were very sensitive to UV irradiation (in the agar plate test of UV sensitivity, growth of the wild-type V. harveyi strain was still observed at a UV dose of 15 J m⁻², whereas complete inhibition of growth of these six mutants was observed upon UV irradiation at a dose as low as 7 J m⁻²). Surprisingly, we found that five of these six mutants had also lost the ability to emit light.

One possible interpretation of the unexpectedly large proportion of non-luminescent mutants among UV-sensitive bacteria was that V. harveyi cells unable to emit light may be defective in the repair of DNA lesions caused by UV light. To test this hypothesis, we investigated the survival of UV-irradiated V. harveyi cells which were subsequently grown in the dark or in the presence of external light (under a white fluorescent lamp). This type of experiment has previously been used to investigate the efficiency of photoreactivation, a process of DNA repair by photolyase (Kato et al., 1997). We found that UV-mediated killing of wild-type (lumi-

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<td><strong>Strain</strong></td>
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Biological role of bacterial luminescence

UV sensitivity of wild-type *V. harveyi* BB7 strain (a) and its *luxA* (strain BB151) (b) and *luxB* (strain BB152) (c) derivatives. Bacteria were cultivated in BOSS medium to mid-exponential phase (OD575 0.3), centrifuged and resuspended in 3% NaCl. Following UV irradiation, bacteria were incubated in BOSS medium in the dark (squares) or under a white fluorescent lamp (circles) for 2 h, and then titrated on BOSS plates. The values presented are mean values from three experiments. In all cases, the standard deviation was below 10%.

UV sensitivity of wild-type *E. coli* MG1655 bearing pLAFR2 and pACYC184 vectors (a) and of the same strain bearing plasmids that contain the *V. harveyi luxCDABE* operon and *luxR* gene, cloned in pLAFR2 and pACYC184 vectors, respectively (b). Bacteria were cultivated in LB medium to mid-exponential phase (OD575 0.3), centrifuged and resuspended in 0.9% NaCl. Following UV irradiation, bacteria were incubated in LB medium in the dark (squares) or under a white fluorescent lamp (circles) for 2 h, and then titrated on LB plates. The values presented are mean values from three experiments. In all cases, the standard deviation was below 10%.

UV sensitivity of wild-type *V. harveyi* cells was somewhat more effective when bacteria were cultivated in the dark following irradiation compared to cultivation in the presence of external light (Fig. 1a). However, significantly less survival of cells was observed when UV-irradiated *luxA* or *luxB* (non-luminescent) mutants were cultivated in the dark (Fig. 1b, c). The *luxA* and *luxB* genes encode the two subunits of luciferase. To test whether the observed defects in DNA repair were caused by the loss of luminescence or the loss of the luciferase enzyme itself, we investigated the UV-sensitivity of the *luxD* mutant. The *luxD* gene encodes the acetyltransferase enzyme producing fatty acids for the luminescence reaction, thus *luxD* mutants are non-luminescent even in the presence of wild-type luciferase. We found that the *luxD* mutant was significantly more sensitive to UV irradiation when subsequent cultivation was performed in the dark than in the presence of external light, similarly to *luxA* and *luxB* mutants (results not shown). These results led us to propose that luminescence may serve as an internal source of light which is used in a photoreactivation-type reaction when bacteria grow in the dark.

If the above proposed hypothesis were true, one might expect that when bacteria naturally devoid of a light-emitting system are transformed with plasmids bearing the *V. harveyi* genes responsible for luminescence, they should become more resistant to UV irradiation during subsequent cultivation in the absence of external light. To test this, we introduced plasmids bearing the *V. harveyi luxCDABE* operon and the *luxR* gene (cloned in pLAFR2 and pACYC184 vectors, respectively) into *E. coli* cells. It was reported previously that these plasmids make *E. coli* cells luminescent (Showalter et al., 1990). We found that the survival of UV-irradiated *E. coli* wild-type cells bearing the plasmid vectors was lower when bacteria were subsequently cultivated in the dark relative to bacteria exposed to external light (Fig. 2a). However, the survival of UV-irradiated *E. coli* luminescent cells was not dependent on the conditions of subsequent cultivation, in the dark or in the presence of external light (Fig. 2b). Moreover, luminescent *E. coli* cells were significantly less sensitive to UV irradiation relative to their non-luminescent counterparts (Fig. 2a, b). These results support the hypothesis that luminescence may be...
an internal source of light used in DNA repair by photoreactivation.

When studied in *E. coli* cells, repression of the *lux* operon from another luminescent bacterium, *Photobacterium fischeri* (previously classified as *Vibrio fischeri*), by the LexA protein was reported (Ulitzur, 1989; Shadel et al., 1990). LexA is a negative regulator of the SOS regulon, and RecA protein activation and cleavage of the LexA repressor upon DNA damage result in SOS response induction (Little & Mount, 1982). The $\sigma^{32}$ factor is responsible for stimulation of transcription of the heat-shock genes (Grossman et al., 1984), and it was reported that $\sigma^{32}$-dependent production of GroEL and GroES heat-shock proteins is involved in stimulation of luminescence (Ulitzur & Kuhn, 1988; Adar et al., 1992; Dolan & Greenberg, 1992). Therefore, the above-mentioned results described by Ulitzur & Kuhn (1988), Ulitzur (1989), Shadel et al. (1990), Adar et al. (1992) and Dolan & Greenberg (1992) may indicate that bacterial luminescence can be enhanced under stress conditions which might potentially cause DNA damage. This would be compatible with our proposal that luminescence may enhance the efficiency of DNA repair and allow for the survival of light-emitting bacteria irrespective of the presence of an external source of light.

There is one more question concerning this hypothesis about a biological role for bacterial luminescence. It is well established that luminescent bacteria (including *V. harveyi*) emit light efficiently only when they are at high cell density. This regulation is known as quorum sensing (for a review see Swift et al., 1998). If *V. harveyi* were able to emit light only at high cell density irrespective of other environmental conditions, our hypothesis would seem rather unlikely as mechanisms ensuring efficient DNA repair should also operate at low cell density. To test if UV irradiation can induce luminescence of *V. harveyi* cells growing at low density, we have diluted a high-density bacterial culture 10000-fold, continued cultivation until the luminescence was negligible, and then irradiated the bacteria with UV light. Bioluminescence was monitored at each stage of the experiment in a scintillation counter using chemiluminescence mode. We found that while light emission by *V. harveyi* cells growing at low density was very low relative to high cell density conditions or immediately after dilution, UV irradiation of cells at low density caused transient but efficient induction of light emission (Fig. 3). A dose-response correlation between UV irradiation and luminescence was observed (Fig. 4). Most probably, stimulation of luminescence was caused by inactivation of the LexA repressor and subsequent induction of the SOS response. To test this hypothesis, we investigated the efficiency of luminescence of *E. coli lexA* and *lexA3* (unable to induce the SOS response due to the presence of the uncleavable form of LexA) cells bearing the *V. harveyi lux* genes on plasmids. UV irradiation of *lexA* cells caused a transient but about a 100-fold increase in luminescence, whereas these conditions had little effect on light emission by the *lexA3* mutant (Fig. 5). Some dose-response correlation between UV irradiation and stimulation of luminescence of *lexA* cells was observed (Fig. 6). These results support the proposal that *V. harveyi* genes responsible for the bioluminescence phenotype are under negative control of the SOS response regulator, and that effective luminescence of *V. harveyi* is possible at low cell density under conditions causing DNA damage.

In summary, we propose that at least one of the biological roles of bacterial luminescence is the pro-
the cells by causing putative energetic or biochemical changes. Another possible interpretation of our results is that mutations may result in general lower viability of the cells by causing putative energetic or biochemical changes. Another possible interpretation of our results is that mutations may result in general lower viability of the cells by causing putative energetic or biochemical changes.

![Fig. 5. Luminescence of E. coli AB1157 bearing plasmids that contain the V. harveyi luxCDABE operon and luxR gene, cloned in pLAFR2 and pACYC184 vectors, respectively (circles), and the lexA3 derivative of AB1157 (strain DM49) bearing the same plasmids (squares) at different times after UV irradiation (30 J m\(^{-2}\)). The luminescence was monitored in a scintillation counter using chemiluminescence mode and calculated as described in the legend to Fig. 3. The values obtained for non-irradiated cultures (time 0) were considered to be 1, and other presented values reflect this value. The presented values are the mean from two experiments. In all cases, the standard deviation was below 10%. The survival of the cells at the UV dose used in these experiments was about 40%.]

![Fig. 6. Dose-response correlation between UV irradiation and stimulation of luminescence of E. coli AB1157 bearing plasmids that contain the V. harveyi luxCDABE operon and luxR gene. The experiments were performed as described in the legend to Fig. 5 but different UV doses were used. The results of measurement of luminescence 15 min after irradiation are presented.]

Production of internal light that allows for efficient DNA repair in a photoreactivation-like reaction. Another possible interpretation of our results is that mutations in the lux genes may result in general lower viability of the cells by causing putative energetic or biochemical problems. This interpretation is unlikely as more efficient killing of non-luminescent V. harveyi was observed only during cultivation of UV-irradiated luxA, luxB and luxD mutants in the dark, and not in the presence of external light. V. harveyi emits light with a maximum intensity at 480–490 nm (for a review see Meighen, 1994), the wavelength potentially appropriate for photolyase activation as photoreactivation in E. coli is effective in the 300–500 nm range (Sancar et al., 1987). The luminescent bacterium V. harveyi is generally more sensitive to UV irradiation than E. coli (compare Figs 1a and 2a), indicating that perhaps other DNA repair systems are less effective in V. harveyi. Therefore, it is tempting to speculate that this bacterium (and possibly other luminescent bacteria) had to develop a mechanism ensuring that at least one DNA repair system (i.e. photoreactivation) is efficient irrespective of the external conditions. Since we demonstrated that introduction of genes responsible for bioluminescence into cells that naturally do not produce light (E. coli) caused increased resistance of the host to UV irradiation, it seems likely that stimulation of photoreactivation may be a general role for bioluminescence. In our experiments on UV irradiation of V. harveyi, we used UV doses of several J m\(^{-2}\). Under natural conditions, the Earth’s surface is exposed to UV rates of irradiation ranging from very low values (near zero) up to 0.2 J m\(^{-2}\) s\(^{-1}\), depending on weather, season, ozone content in atmosphere and other conditions (Wallace, 1977; Salby, 1996). UV rays are not reflected very much from the water surface, so they mostly penetrate it and may go relatively deep into the water (Gill, 1982). Therefore, in natural environments, free-living marine bacteria (like V. harveyi) may experience UV doses equal to those used in this work within relatively short periods, at least temporarily.

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REFERENCES


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