



Ultraviolet-induced DNA Damage and its Subsequent Repair in Field-collected *Aiptasia pallida* as Monitored by Single-cell Gel Electrophoresis

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ABSTRACT

Ultraviolet radiation (UVR) is a common occurring genotoxin in tropical marine environments. While shallow-water organisms have a variety of defenses against UVR, DNA damage may still occur. I documented the extent of DNA damage and subsequent repair response in the sea anemone *Aiptasia pallida* under field conditions.

Samples of *A. pallida* were collected from Walsingham Pond, Bermuda on June 19th 2007. Subsequently, an experiment was carried out to determine the efficiency of repair from DNA damage incurred from exposure to a natural levels of Ultraviolet Radiation (UVR).

It was found that field anemones produce relatively large quantities of Mycosporine-like amino acids (MAAs) and efficiently repaired DNA damage incurred from a reduced level natural UVR.

Results presented here suggest that the ability of *A. pallida* to repair DNA damage and / or protect themselves from the detrimental effects of UVR may be an important factor for their survival. These findings provide insight into how other tropical marine cnidarians survive high levels of UVR exposure.

RESULTS

Anemones processed using the comet assay immediately prior to 12 hr exposure to natural levels of UVR and PAR had higher DNA strand-breaks (SB) compared to controls (Fig. 1) as shown by a 127% increase in Tail Moment (TM). After 2 hr repair, TM increased 100% from the 0 hr repair group; indicating a peak in SB's observed. At 4 and 6 hr repair, TM decreased 30% and 35% respectively from the 2 hr repair group. DNA SB decreased 48% between the 6 hr and 8hr repair time, allowing TM to reduce to levels of pre-exposure (Fig.1).

Spectrophotometric scans of methanolic extracts of field-collected anemones (Figure 2) showed relatively high mean absorbance / μg protein in the range at which MAAs absorb UVR (302 nm – 360 nm). These scans indicated the presence of MAAs in field-collected organisms which was later confirmed by LC/MS. Field anemones were found to contain large quantities of mycosporine-2-glycine and mycosporine-glycine.

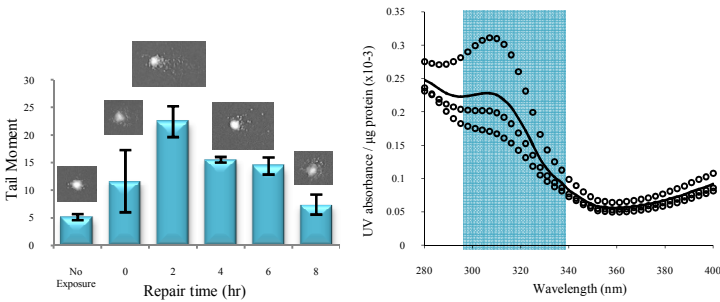
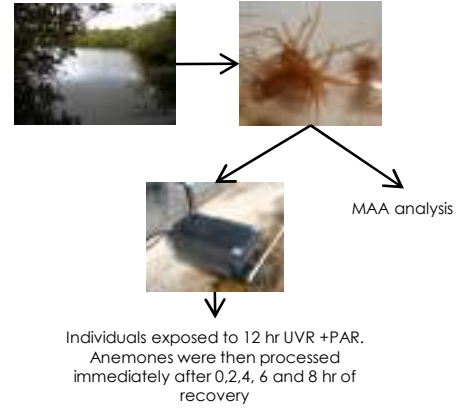


Figure 1. Genotoxic damage and repair evaluated in isolated nuclei in the field-collected symbiotic anemone *A. pallida* using the comet assay. Anemones were exposed to 12 hr UV followed by repair periods varying from 0-8 hr in the dark. No exposure (n=3) represents the control group – no UV exposure. Bars represent means \pm SEM (n=4 anemones with 2 replicate gels per individual). An asterisk indicates this repair time is significantly different from controls (No Exposure) at a =0.05.

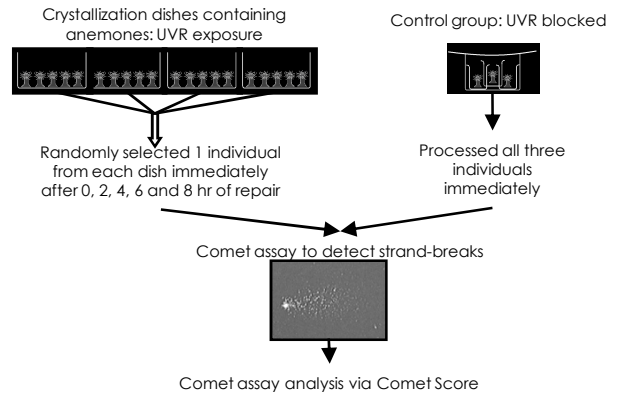
Figure 2. Spectrophotometric Scans of 100% methanolic extracts from field-collected *A. pallida* (n=3). Shaded area represents the range at which MAAs absorb UV light. The solid black line represents the mean absorbance, dotted lines represent the scans of three individual anemones.

MATERIALS AND METHODS

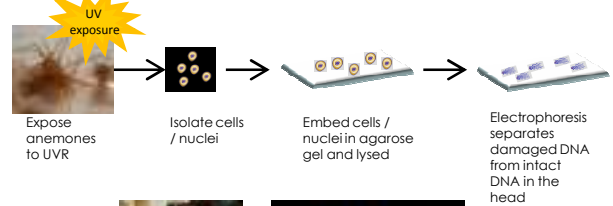
Experimental Design



Repair in Field Anemones



The Comet Assay



- Strand breaks detected:
- Alkali labile sites (AP sites)
 - SSBs formed during excision repair
 - DSBs formed from 2 SSBs close together

CONCLUSIONS

The Comet Assay is an effective tool to document the time course of DNA repair in *A. pallida*. This study is the first of its kind that documents a complete time course of repair in cnidarians as a result of UVR damage.

Total DNA SB formed reached an asymptote indicating a maximal amount of UVR damage had been reached. It is possible that DNA SB as measured by the comet assay reached an asymptote, for several reasons. Firstly, it is possible that at high UVR doses all potential CPDs and 6-4 photoproducts have formed. Secondly, it is possible that such high doses of UVR-induced programmed cell death or apoptosis. Lesser et al., (2003) found a positive correlation between CPD formation and the expression of p53 and p21 in the embryos of the green sea urchin when exposed to UVR.

Considerable lag times exist during exposure and repair, caused presumably by breakages in the DNA as a result of repair mechanisms excising photoproducts (NER). This study shows that field anemones can efficiently repair DNA damage induced from natural levels of exposure to UVR. Their ability may be in-part due to the relatively large quantities of MAAs found in field organisms.