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MOLECULAR STUDIES ON THE EFFECT OF GERMANIUM OXIDE ON SPONGES, CORALS AND ASCIDIANS

Mohammed Shokry Ahmed Ammar and Mohammed Abdel-Fattah Hamed
National Institute of Oceanography and Fisheries, Suez, P.O. Box 182, Egypt.
e-mail: maamoaz@yahoo.com

ABSTRACT: The stress response, at the molecular level, of the soft corals *Dendronephthya klunzingeri* and *Heteroxenia* sp., hard corals *Acropora hyacinthus* and *A. valenciennesi,* an ascidian *Symplegma* sp. and sponges *Latruncula cortica* and *Callyspongia crassa* to germanium oxide (GeO_2) was evaluated. Evaluation was carried out using bioindicators, such as the level of expression of each of the heat shock proteins (HSPs) and the silicatein enzyme in response to the compound. However, the expression was measured by SDS Polyacrylamide Gel Electrophoresis (SDS PAGE) and Western blotting. The harmful concentration of GeO_2 that produced noticeable molecular changes in the studied samples during the first 6-24 hours was 6 µg/ml. The two studied soft corals as well as the ascidian responded to the harmful concentration of germanium oxide by expressing the heat-shock protein 90 (hsp90), while the two hard corals responded by expressing hsp70, *C. crassa* by decreasing the level of silicatein enzyme and sponge *L. cortica* produced no change by any of the used biomarkers. The soft coral *Heteroxenia* sp. was found to be sensitive to mechanical stress during the experiment and it was more sensitive to 6 µg/ml of GeO_2 than the other soft coral *D. klunzingeri.* The two studied hard corals were sensitive to mechanical stress during the experiment, but *A. hyacinthus* showed higher sensitivity than *A. valenciennesi.* However, these 2 corals displayed reverse response to GeO_2. Primitive evidences were found in the SDS PAGE to distinguish the tissue of the soft coral from that of the hard coral on the molecular level; the soft coral showed two prominent protein bands (45 and 50 kDa) while the two prominent protein bands for hard corals were 31 and 116 kDa.

KEY WORDS: Germanium oxide, sponges, corals, ascidians.

INTRODUCTION

Stress response and acquired stress tolerance are commonly seen in all organisms from bacteria to higher vertebrates and considered to be essential defence mechanisms of the cell against environmental stress (Yonehara *et al.*, 1996). Besides, to the best of our knowledge, there have been no natural field studies in which germanium levels were monitored using bioindicators from corals, ascidians or sponges. However, monitoring the effect of metals using biomarkers have been studied in some other invertebrates. Mueller *et al.* 1998 used the marine sponge *Suberites dumuncula* in a natural field study to assess the genotoxic risk of cadmium exposure for sponges as a model for invertebrates, and as an early stress marker of expression of heat-shock protein 70. The most frequently discussed protection systems against exposure to toxic heavy metals present in higher invertebrates are (1) metal binding proteins (e.g. metallothioneins) and (2) heat-shock proteins (Bauman *et al.*, 1993). Cadmium has been reported to cause a series of toxic effects, DNA single-strand breaks (Hassoun and Stohs, 1996). The expression of

metallothioneins has been found to be induced by several heavy metals, e.g. Cd, Zn, and Hg (reviewed in Hamer, 1986). The purpose of the present study is to follow the stress response, on the molecular level, of selected species of corals, ascidians and sponges to treatment with GeO_2. Wiens *et al.*, 2003 found that okadaic acid acts as a potential defence molecule for the sponge *Suberites domuncula* against some endotoxins.

MATERIALS AND METHODS

Tissues of the soft corals *Dendronephthya klunzingeri* and *Heteroxenia* sp., hard corals *Acropora hyacinthus* and *A. valenciennes*, the ascidian *Symplegma* sp. and sponges *Latruncula cortica* and *Callyspongia crassa* were exposed to 1, 3 and 6 µg/ml of GeO_2, for 24 hours. Samples were taken after different intervals and analysed for the expression of heat shock protein 70 (hsp70) and heat shock protein 90 (hsp90). Silicatein enzyme was analysed for expression only in the two sponges. The tissues of the hard corals, were extracted and analysed for hsp70 and hsp90 by the method described in Ammar and Mueller 2001, however, for the soft coral *Dendronephthya klunzingeri* (a zoxanthellae-free coral) the tissue was analysed by the method described by Wiens *et al.*, 2000, while the tissues of both the soft coral *Heteroxenia* sp. and the ascidian *Symplegma* sp. (freed of zooxanthellae) as described in Ammar and Mueller, 2001. These were then analysed for expression of hsp70 and hsp90. Samples of sponges were analysed for hsp70 and hsp90. Similarly, silicatein was monitored in the same steps except for using anti-silicatein as the first antibody. In each of the studied samples, a separate gel was prepared beside the gel used for the Western blot.

RESULTS

The present study showed that exposure in the corals, ascidians and sponges to 1 and 3 µg/ml of GeO_2 produced no changes in molecular parameters under investigation. However, exposure to 6 µg/ml of GeO_2 induced marked molecular changes. Levels of expression of heat shock protein in each of soft corals, hard corals, ascidians as well as silicatein in sponges are indicated in Table 1 and figs. 1B, 2B, 3B, 4B, respectively. No heat shock protein 70 (hsp70) was recorded in the two studied species of soft corals while the heat shock protein 90 (hsp90) was expressed in the control sample (0 time) of *Heteroxenia* sp. (0.5 fold) and that expression increased to 4.5 fold after 24 hours. On the other hand, the control sample of the other soft coral *Dendronephthya klunzingeri* expressed no hsp90 and a little expression was noticed (0.5 fold) in the 24-hour sample. Contrary to soft corals, the two studied species of hard corals expressed no hsp90 while the hsp70 was expressed by each of the control and treated samples of both species. However, the control sample of the hard coral *Acropora valenciennesi* expressed a low value of hsp70 (0.3 fold) that increased to 3 fold after 24 hours. On the other hand, the control sample of *Acropora hyacinthus* had a higher expression of hsp70 than that of the control of *A. valenciennesi*. Furthermore, *Acropora hyacinthus*, after 24 hours, produced a level of expression of hsp70 lower than that of *Acropora valenciennesi*. The only studied species of ascidians *Symplegma* sp. was similar to soft corals in expressing hsp90 and not expressing hsp70. No increase in the expression of hsp90 by the used ascidian was recorded after 6 hours, however, the expression increased considerably after 24 hours (5

fold). The two studied species of sponges *Latruncula cortica* and *Callyspongia crassa*, did not express either hsp70 or hsp90, while enzyme silicatein was expressed only by the second one having a level of 3 fold by the control. This decreased 2 folds after 24 hours.

Table 1. Levels of expression of hsp70 and hsp90 (in folds) in corals, ascidians and sponges as well as the levels of expression of silicatein in sponges after exposure to ermanium oxide (6 µg/ml).

Corals	Hsp70				Hsp90				Silicatein	
	0t	6h	18h	24h	0t	6h	18h	24h	0t	24h
Soft corals										
Dendronephthya klunzingeri	0	-	-	0	0	-	-	0.5	-	-
Heteroxenia sp.	0	-	-	0	0.5	-	-	4.5	-	-
Hard corals										
Acropora valenciennesi	0.3	0.3	1	3	0	0	0	0	-	-
Acropora hyacinthus	0.5	-	-	1	0	-	-	0	-	-
Ascidians										
Symplegma sp.	0	0	-	0	0.5	0.5	-	5	-	-
Sponges										
Latruncula cortica	0	0	0	0	0	0	0	0	0	0
Callyspongia crassa	0	0	0	0	0	0	0	0	3	2

Hsp70 = heat shock protein 70, hsp90 = heat shock protein 90, 0t = 0 time, 6h = 6 hours, 8h = 18 hours, 24h = 24 hours, the dash means no treatment was carried out at that interval.

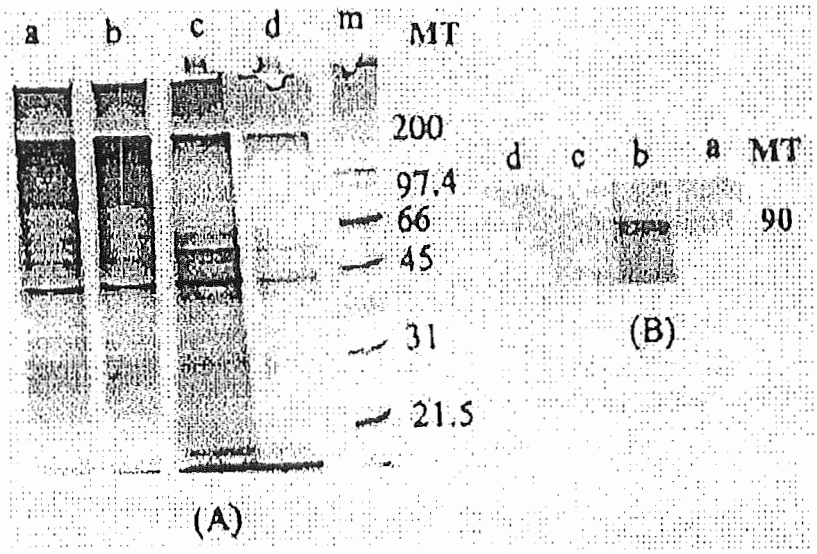

Fig. 1. (A), SDS PAGE for soft corals treated with 6 µg/ml of GeO_2. MT=molecular weight, m=marker, a=*Heteroxenia* after 1 day of treatment, b=*Heteroxenia* control, c=*Dendronephthya klunzingeri* 1 day, d=*D. klunzingeri* control; (B), Western blotting for the expression of hsp90 from the samples of (A). MT=molecular weight, a=*Heteroxenia* control, b=*Heteroxenia* 1 day, c=*D. klunzingeri* 1 day, d=*D. klunzingeri* control.

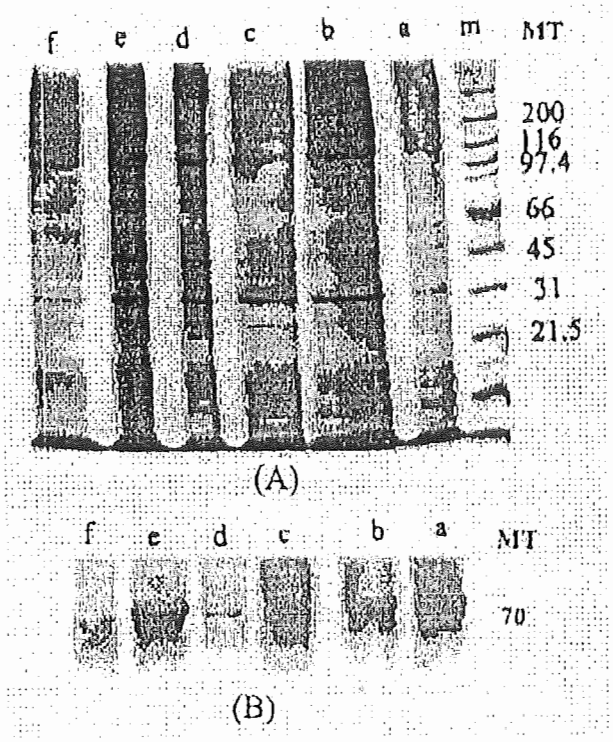

Fig. 2. (A), SDS PAGE for hard corals treated with 6 µg/ml of GeO_2. MT=molecular weight, m=marker, a=*Acropora hyacinthus* control, b=*A. hyacinthus* 1 day, c=*A. valenciennesi* 6 hours, d=*A. valenciennesi* 18 hours, e=*A. valenciennesi* 24 hours, f=*A. valenciennesi* control; (B), Western blotting for the expression of hsp70 from the samples of (A). MT=molecular weight.

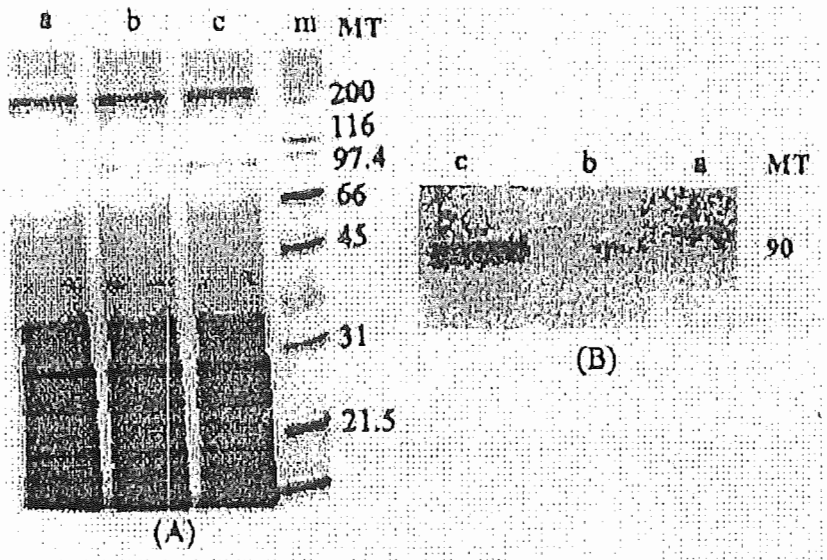

Fig. 3. (A), SDS PAGE for the ascidian *Symplegma* sp. MT=molecular weight, m=marker, a=control sample, b=6 hours sample, c=1 day sample; (B), Western blotting for the expression of hsp90 from the samples of (A). MT=molecular weight, a=0 time sample, b=6 hours sample, c=1 day sample.

SDS PAGE for each of soft and hard corals is shown in figs. 1A, 2A, respectively. There are several bands in the two studied soft corals starting from lower than 21.5 kilodaltons to 116 kilodaltons. However, two prominent bands at 45 and 50 kilodalton are found in both species. The 90 kilodalton band (hsp90 band) is most prominent in *Heteroxenia* sp. after 1 day of treatment. Similarly, the two studied hard corals expressed several protein bands in the SDS PAGE, ranging from 14.5 to more than 200 kilodalton. Two prominent bands at 31 and 116 kilo Dalton were found in each of the two studied species. SDS PAGE for each of the ascidian species and sponges is shown in figs. 3A, 4A, respectively. A lot of protein bands ranging from lower to higher molecular weights are found in the ascidian *Symplegma* sp., the most prominent one is found at around 29 kilodalton. Besides, the 90 kDa band (hsp90 band) is most expressed in the one day chart, matching the result of the western blotting. The results of the SDS PAGE of sponges is matching that of the western blotting for the same species where the silicatein band (35 kDa band) was expressed only by the *Callyspongia crassa*, however, that band was lower in the one day sample compared to the control.

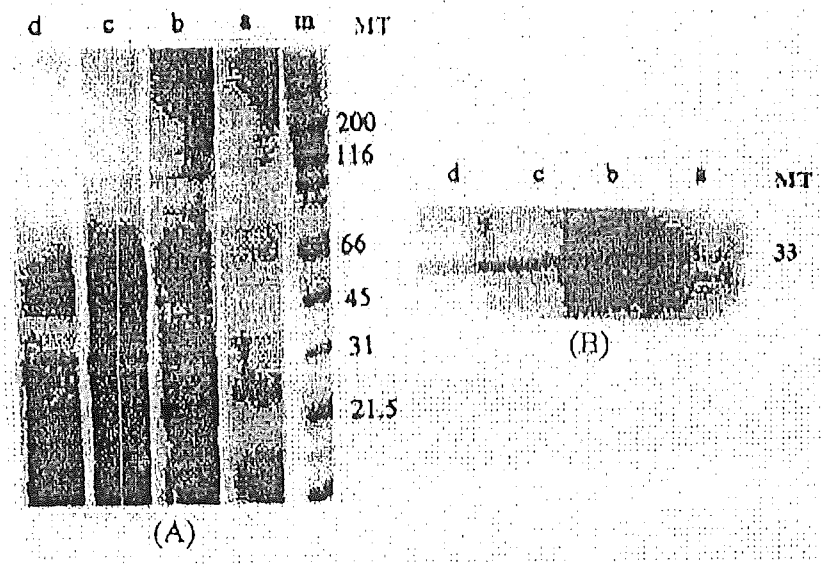

Fig. 4. (A), SDS PAGE for two species of sponges. MT=molecular weight, m=marker, a=*Latruncula cortica* 1 day, b=*L. cortica* control, c=*Callyspongia crassa* control, d=*C. crassa* 1 day; (B), Western blotting for the expression of silicatein enzyme from the samples of (A). MT=molecular weight.

DISCUSSION

The absence of molecular response in corals, ascidians and sponges after exposure to 1 and 3 µg/ml of GeO_2 is a clear evidence that this concentration limit of GeO_2 is not harmful to the studied samples for up to 24 hours of exposure. The study proved that the two studied soft corals *Dendronephthya klunzingeri* and *Heteroxenia* sp. as well as the ascidian *Symplegma* sp. respond to higher concentration of GeO_2 by expressing hsp90 while the two studied hard corals *Acropora hyacinthus* and *A. valenciennesi* respond by expressing hsp70. However, the two studied sponge species *Latruncula cortica* and *Callyspongia crassa* respond to such concentration of GeO_2 by decreasing the expression

of silicatein enzyme. The lack of expression of either hsp70 or hsp90 by the two sponge species disagree with the statement of Yonehara *et al.*, 1996 that the 90-kDa stress protein, hsp90, is a major cytosolic protein ubiquitously distributed in all species and also disagree with the results of Koziol *et al.*, 1997 in which they cloned the hsp70 genes from the marine sponges *Sycon raphanus* and *Rhabdocalyptus dawsoni*. The expression of hsp90 by the two studied soft corals and hsp70 by the two hard corals can not be stated as a general behavior in all soft and hard corals since hsp90 has been expressed in both soft (Wiens *et al.*, 2000) and hard corals (Ammar and Mueller, 2001; Ammar, 2001). Furthermore, hard corals have expressed hsp70 Sharp *et al.*, 1997 the small heat shock/alphacrystallin protein (Branton *et al.*, 1999) or alteration in the DNA strand scission factor (Ammar, 2001) in response to stress. The reason for these variations of molecular expression in different groups may be related to the different stresses, habitats or individual fitness. However, further experiments should be undertaken to confirm this conclusion. The expression of hsps, which are induced under a variety of stressful conditions, has been shown to be a reliable marker for environmental stress (reviewed in Mueller *et al.*, 2000). These hsps are subdivided into five families; hsp90, hsp70, hsp58-60, hsp20-30 and 8 Kda protein (ubiquitin) (Lindquist and Craig, 1988).

With regard to the two soft corals, the presence of a little bit expression of hsp90 in the control *Heteroxenia* sp. (0.5 fold) may be due to the fact that the control *Heteroxenia* is sensitive to the physical stress during the experiment, furthermore, the sharp increase in the hsp90 expression in the same species after 1 day (4.5 fold) compared to the 0.5 fold for *Dendronephthya klunzingeri* is a clear evidence that treatment with 6 μg/ml of GeO_2 for one day is more harmful for *Heteroxenia* sp. than for *D. klunzingeri*. Regarding the two hard corals, each of the two studied species are sensitive to physical stress during the experiment, but such sensitivity is higher in case of *Acropora hyacinthus* than that shown by *A. valenciennesi* as it is indicated by the level of expression of hsp70. Contrary to this result, *A. valenciennesi* was shown to be more sensitive to the high concentration of GeO_2 as indicated by its higher expression of hsp70 than that shown by *A. hyacinthus* after 24 hours. Similarly, the ascidian *Symplegma* sp. is sensitive to the physical stress. In addition, the harmful effect of GeO_2 on that ascidian, after 24 hours, was higher than its effect on any of the two soft corals. The ascidian *Symplegma* sp. could be compared only with the two studied soft corals since they all responded to the harmful effect of GeO_2 by expressing hsp90. The lack of response of *Latruncula cortica* may be due to either the non-harmful effect of such concentration (6 μg/ml of GeO_2) or to the fact that the healthy status of the animal might have been initially critical, so that the enzymes have been degraded even before the treatment. However, this requires confirmation with more experiments in future. The non-expression of hsps and the decrease in silicatein expression by *Callyspongia crassa* support the result of Le Pennec *et al.*, 2003 that the unfavourable environmental conditions can be monitored with the use of suitable biomarkers, and also support the result of Ammar *et al.*, 2000 who used the variations in molecular reponse to select the sites for rehabilitation of coral reefs by asexual recruits.

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