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Free Amino Acid Pool of a Sea Anemone: Exposure and Recovery After an Oil Spill

Margaret R. Kasschau and Cynthia L. Howard

Division of Biological Sciences, University of Houston-Clear Lake, Houston, TX 77058

Numerous studies have been performed to determine the physiological effects of oil spills and water contamination on marine animals. Much has been learned about the physiological effects of oil pollution even though the majority of these studies have been performed under constant laboratory conditions rather than field situations. In addition, recovery from oil pollution is only observed occasionally. Chronic exposure of oysters to oil has been reflected by reduced food intake or utilization (Maloney and Noyes 1982). The shrimp, Palaemon adspersus, showed arrhythmic ventilatory behavior during a two week exposure to 100 ppb of the water soluble fraction (WSF) of North Sea crude oil (Baden and Hagerman 1981). However, following a five-week recovery the shrimp returned to their normal ventilatory pattern. These shrimp were also less able to maintain hyperosmolality during exposure to WSF (Baden 1982), but after three weeks of recovery P. adspersus regained their hyperosmolality.

Field studies on the physiological effects of oil contamination, usually from spills, are more rare. Farrington et al. (1982) followed the removal of hydrocarbons from tissues of Mytilis edulis after several different oil spills, but did not look for changes in any physiological parameters during recovery. Macoma inquinata artifically exposed to oil contaminated sediments under field conditions for 38 days showed significant decreases in several tissue free amino acids (FAA's) with glycine showing the greatest drop in concentration (Roesijadi and Anderson 1979). The authors did not study recovery.

A number of laboratory studies on marine invertebrates have shown changes in FAA pools in response to various pollutants. Jeffries (1972) observed changes in the taurine to glycine ratio in Mercenaria mercenaria, while Roesijadi (1979) found a significant decrease in glycine concentration after long term exposure of a clam to chlorine but no change in taurine concentration. We have shown significant increases in free glutamate levels after sea anemones were exposed to heavy metals and organic amines for 1 and 7 days (Kasschau et al. 1980). A study by Aarset and Zachariassen (1982) showed that following a 7-day exposure of M. edulis to oil-polluted sea water there were no changes in body fluid concentrations of inorganic ions and FAA's or muscle levels of FAA's. In none of these studies was recovery measured.

During a nineteen-month field study to determine the effects of natural

environmental parameters on the FAA pools of the Gulf Coast sea anemone, Bunodosoma cavernata (Howard and Kasschau 1980), an oil tanker collision occurred about 8 miles off Galveston Island. More than 95,000 barrels of Nigerian crude oil spilled, 85% of which burned (Houston Chronicle, Nov. 1-20, 1979). The initial spill from the tanker Burma Agate occurred on November 1, 1979 with large leakages continuing for several weeks. The oil reached shore in different areas at different times. There was no visible sign of oil on our first collection date 13 days after the spill, but 11 days later the anemones were covered with an oil sheen. As a result of this natural exposure to the oil, we decided to monitor the sea anemones for changes in the FAA pool during the oil exposure and recovery period.

MATERIAL AND METHODS

Sea anemones from the Gulf of Mexico were collected at 7 to 15 day intervals from the 53rd Street jetty in Galveston, Texas starting in early November, 1979 and continuing through mid-January, 1980. Four or more animals were collected from the same site on each collection day and each anemone was immediately frozen after scraping off any clinging shells or stones. Anemones were lyophilized and dry weights were individually determined. Samples were prepared for amino acid analysis as previously reported (Kasschau et al. 1980) and amino acids were quantified on an Aminco Aminalyzer. Significant differences between means was determined by t-tests for independent means.

RESULTS AND DISCUSSION

The free amino acid pool of B. cavernata is known to change with varying salinities in laboratory experiments (Benson-Rodenbough and Ellington 1982; Kasschau et al. 1983) and under field conditions (Howard and Kasschau 1980). In addition, the levels of some FAA's have been found to fluctuate inversely with temperature and change with season (Howard et al. 1984). During the peak reproductive period, from April through September, male B. cavernata accumulate excessive amounts of free glycine in their gonads (Kasschau and McCommas 1982). In this study the spill occurred in early November and during the next two months there was very little change in either salinity (25-28 o/oo) or water temperature (9-12^{o}C) (see Fig. 1).

Statistical comparisons were made between mean FAA concentrations from oil stressed anemones and those animals collected at other times during the nineteen month study when salinity and temperature conditions were similiar to those during this collection period. The FAAs of the anemones in the sample just prior to when the oil came ashore at the collection site (11/13/79) were not significantly different from the composite (Table 1). At the time the oil sheen was observed (11/24/79) there was a significant drop in alanine, β-alanine, and leucine levels and a statistically nonsignificant increase in taurine concentration (Fig. 1, Table 1). The loss of FAA's during oil exposure was not surprising since Powell et al. (1982) observed a similar response of the FAA pool in oysters exposed to drilling effluents for 5 days. Roesijadi and Anderson (1979) also found significantly lower concentrations of several amino acids in the clam M. inquinata after a 38 day exposure to oil contaminated sediments. However, while B. cavernata shows no significant change in free glycine, M. inquinata showed its greatest FAA loss in glycine concentration. Roesijadi and Anderson's (1979) experiments

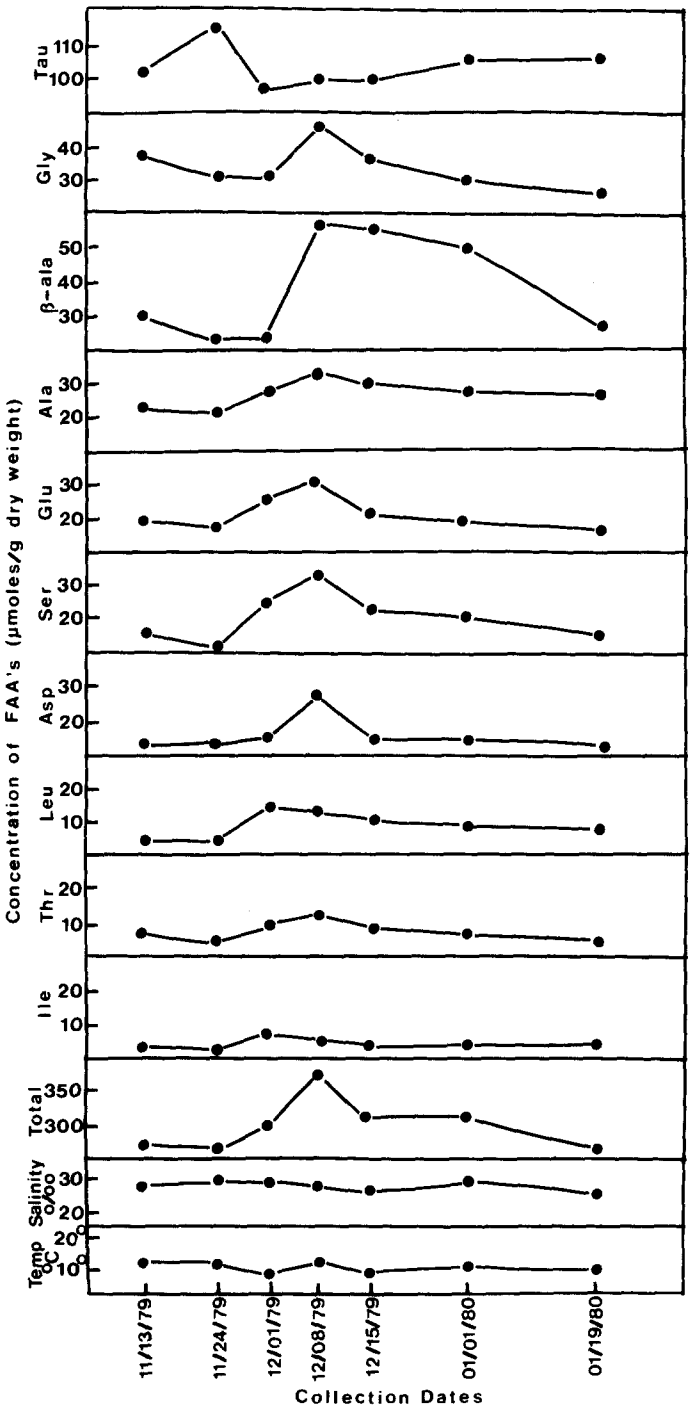

Fig 1. Mean concentrations of FAA's and salinity and temperature conditions during the Burma Agate oil spill. Oil sheen was observed on 11/24/79.

Table 1. Free amino acid pools[†] from Bunodosoma cavernata in normal conditions and after exposure to an oil sheen

FAA	Composite of 9-12°C 25-29 o/oo	Collection Dates 11/13/79	11/24/79	12/1/79	12/8/79	12/15/79	1/1/80	1/19/80
Tau	98.2 (12.0)	103.2 (13.4)	114.7 (5.3)	96.0 (14.2)	100.7 (27.8)	101.2 (27.9)	107.7 (20.2)	90.1 (22.0)
Gly	32.0 (6.2)	38.2 (15.0)	31.4 (6.3)	31.2 (6.6)	45.7 (8.7)	36.4 (14.6)	30.2 (4.6)	27.4 (11.7)
β-ala	29.7 (7.1)	30.4 (3.6)	++22.9 (3.1)	24.0 (3.1)	*56.9 (11.4)	*55.6 (13.1)	*50.1 (9.1)	26.2 (5.1)
Ala	30.4 (3.0)	23.4 (2.0)	+21.6 (2.7)	27.2 (2.6)	31.6 (2.8)	30.6 (6.0)	27.0 (1.4)	27.1 (8.8)
Glu	15.7 (2.7)	19.7 (2.4)	17.6 (2.1)	**25.4 (4.2)	*28.9 (3.4)	21.4 (2.8)	19.6 (2.3)	15.5 (4.2)
Ser	12.1 (1.1)	16.4 (3.2)	11.1 (2.3)	*25.1 (4.1)	*32.9 (2.1)	**21.2 (7.2)	*21.8 (3.1)	15.9 (3.7)
Asp	14.1 (2.1)	12.9 (2.2)	12.6 (1.7)	15.7 (2.7)	*28.2 (3.3)	14.7 (2.6)	15.4 (1.3)	12.5 (2.3)
Leu	7.7 (1.3)	4.7 (0.8)	+3.9 (0.5)	*13.9 (0.8)	12.3 (2.8)	10.7 (1.9)	9.1 (2.4)	8.4 (3.3)
Thr	4.3 (0.7)	6.5 (1.5)	4.7 (0.8)	**9.3 (1.8)	*10.7 (0.7)	*8.0 (1.9)	**7.3 (1.7)	4.3 (0.8)
Ile	2.4 (0.3)	2.1 (0.5)	1.9 (0.3)	*7.1 (0.4)	*6.4 (2.2)	**4.2 (1.2)	**4.1 (1.1)	3.0 (1.0)
Total	254.6 (21.3)	270.8 (13.6)	260.2 (14.0)	298.0 (31.6)	366.8 (55.4)	306.7 (62.4)	313.0 (35.0)	258.8 (32.8)

[†] FAA are expressed as mean ± (SEM) for 4 or more samples in μmoles/g dry weight. Increase in concentration over composite: *$p < 0.05$, **$p < 0.1$; Decrease in concentration below composite: +$p < 0.05$, ++$p < 0.1$

were done in June which is often a time of peak reproductivity. Since a number of invertebrates are known to accumulate glycine during reproduction (Barnes 1963; Cook et al. 1972; Ferguson 1975; Kasschau and McCommas 1982), we speculate that the apparent loss of glycine may be due to slower gonadogenesis in the oil treated animals and thus actually less accumulation, rather than loss, of glycine.

The following week (12/1/79) the oil sheen was gone but the FAA pool continued to reflect the effects of oil exposure. At this time significant increases, expressed as a doubling in concentration, were observed in leucine, isoleucine, threonine, serine, and glutamate levels (Table 1). One week later (12/8/79) aspartate and β-alanine levels doubled over control values and the total pool increased 69 μmoles/g dry weight (from 298.0 to 366.8 μmoles/g dry weight) (Fig. 1, Table 1). All free amino acids quantified (except taurine) showed some increase in concentration one or two weeks following oil exposure. Three weeks after the observed oil sheen (12/15/79) all of the FAA concentrations dropped except β-alanine which remained above 50 μmoles g dry weight for two more weeks before returning to its normal concentration of 26-29 μmoles/g dry weight (at 25-28 o/oo) (Fig. 1, Table 1).

The increase in so many free amino acids following oil exposure suggests that proteolysis has occurred, releasing amino acids from proteins. There is evidence that lysosomes may be destablized in animals found in polluted waters (Moore et al. 1982), and thus hydrolytic enzymes could be released, leading to protein breakdown. In addition, we observed a doubling in the concentration of the non-proteinaceous amino acid, β-alanine (from 29 to 57 μmoles/g dry weight). In contrast to our controlled laboratory studies on osmoregulation (Kasschau et al. 1983), this increase was not salinity related. Increased levels of cellular and urine free β-alanine have often been suggested as indicators of stressful conditions. Shick (1976) found that during starvation Aurelia aurita doubled its level of β-alanine and showed increases in taurine and glycine. After extensive space flight (60 to 84 days) astronauts exhibited a 4-fold increase in urinary β-alanine, a larger percent increase than for any other amino acid (Leach et al., 1979). Neurological symptoms have also been linked to high urinary β-alanine levels (Vaughan et al. 1979). During the entire 2 months of oil exposure and recovery there were no statistically significant changes for either taurine or glycine concentrations, due in part to large standard deviations within samples. Jeffries' (1972) hypothesis of changes in taurine to glycine ratios as well as Roesijadi's (1979) suggestion of a drop in glycine concentration as indicators of pollution is inappropriate for the sea anemone, Bunodosoma cavernata.

Thus it appears that the sea anemone, Bunodosoma cavernata can tolerate an oil sheen, at least during cold weather and normal salinity conditions, without any dramatic and immediate adverse effects. The major changes in the FAA pool take about two weeks to appear, and show up as increases in a number of FAA's. B. cavernata apparently returns to normal about 7 weeks following exposure.

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