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Tolerance, Bioaccumulation and Biotransformation of Arsenic in Freshwater Prawn (*Macrobrachium rosenbergii*)

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Tolerance bioaccumulation and biotransformation of arsenic compounds by a freshwater prawn (*Macrobrachium rosenbergii*) were investigated. *M. rosenbergii* was exposed to 10, 20, 30 and 35 μg As cm^{-3} of disodium arsenate [abbreviated as As(V)], 25, 50, 100 and 120 μg As cm^{-3} of methylarsonic acid (MMAA), or 100, 200, 300 and 350 μg As cm^{-3} of dimethylarsinic acid (DMAA). Tolerances (50% lethal concentration: LC_{50}) of the prawn against As(V), MMAA, and DMAA were 30, 100, and 300 μg As cm^{-3}, respectively. The prawn accumulated arsenic compounds directly from aqueous phase and biotransformed them in part. Both methylation and demethylation of the arsenicals were observed *in vivo*. Highly methylated and less toxic arsenicals were less accumulated in *M. rosenbergii*.

Keywords: arsenic; prawn; tolerance; bioaccumulation; biotransformation; methylation; demethylation; freshwater

INTRODUCTION

There seems to be a significant difference in the level and chemical forms of arsenic between terrestrial and marine organisms. Terrestrial organisms rarely contain more than 1 μg As g^{-1} (dry weight), whereas marine organisms contain from several to more than 100 μg As g^{-1}.[1] The source of arsenic in the marine ecosystem is, of course, inorganic arsenate [abbreviated as As(V)] and arsenite [abbreviated as As(III)] dissolved in seawater and the concentration is generally constant at a few parts per million in most areas of the sea. On the other hand, arsenic concentrations in unpolluted rivers, ponds and lakes are of the parts per billion (10^{-9} ppb) order and less, and are greatly affected by the circumstances. It has been found that a large part of the arsenic in marine organisms is in organic forms, e.g. dimethylarsenic compounds such as arsenosugars in algae, and trimethylarsenic compounds such as arsenobetaine and arsenocholine in fish, mollusks, and crustaceans.[2-6]

Chemical forms of methylated arsenic compounds in marine organisms have been reported by many researchers. Arsenobetaine was first reported as a major constituent of total arsenic in the tail muscle of the western rock lobster by Edmonds,[7] who found arsenobetaine in many marine organisms.[3-5, 8, 9]

Much less is known about arsenic compounds in freshwater organisms. A variety of aquatic plants was collected from 22 pools resulting from tin-mining activities in Kuala Lumpur, Malaysia. The concentration of arsenic in these plants partially reflected the concentrations of arsenic in pool waters, which ranged from 0.002 to 0.25 μg As g^{-1}.[10] Cotton plants (*Gossypium hirsutum* L, 20 days old) were grown in a solution culture containing arsenic trioxide (0–8 mg As dm^{-3}), cacodylic acid (0–40 mg As dm^{-3}), monosodium methylarsonate, and disodium methylarsonate.[11] Arsenite was readily taken up by the roots but was not translocated to the shoots. In the case of cacodylic acid, arsenic was found not only in the roots but also in the leaves and reproductive organs.

Five bacteria (*Proteus* sp., *Escherichia coli*, *Flavobacterium* sp., *Corynebacterium* sp., and *Pseudomonas* sp.) capable of biotransformation of sodium arsenate were grown in the presence of the arsenate to determine arsenic uptake and distribution in the microbial cells.[12] Species grown

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in the presence of arsenate showed an average accumulation of 25 $\mu g\ g^{-1}$ and 3.0 $\mu g\ g^{-1}$ total arsenic following 48 h exposure to 100 $mg\ dm^{-3}$ and 10 $mg\ dm^{-3}$ arsenic respectively.

Maeda *et al.* collected algae from sites which had once been polluted with arsenic.[13, 14] The algae were screened for arsenic tolerance; some mixed algal systems grew well in 50–2000 μg As(V) g^{-1} media. The alga *Chlorella vulgaris* was isolated and found to survive in the medium containing 10 000 μg As(V) g^{-1}. Growth of *C. vulgaris* increased with increase in arsenate concentration up to 2000 μg As(V) g^{-1}.

Fowler reviewed the toxicity of arsenic trioxide to blue tropical fish and found mean LC_{50} values at 24 h to be 24 $mg\ dm^{-3}$, and at 48 h to be 18 $mg\ dm^{-3}$.[15]

We reported the toxicity of As(V), methylarsonic acid (MMAA), dimethylarsinic acid (DMAA) and arsenobetaine (AB) to shrimp (*Neocaridina denticulata*) in a previous paper.[16] No shrimp could survive in media containing arsenic higher than 2 μg As cm^{-3} for As(V), 12 μg As cm^{-3} for MMAA, 50 μg As cm^{-3} for DMAA, and 200 μg As cm^{-3} for AB.

This paper presents the tolerance, accumulation and biotransformation of aqueous As(V), MMAA and DMAA by freshwater prawn (*Macrobrachium rosenbergii*).

MATERIALS AND METHODS

Culture of prawn

The omnivorous prawn (*Macrobrachium rosenbergii*) was fed under the following general conditions.

Juvenile prawns (about 2–3 months old, about 5 cm in length) were obtained from Usui Freshwater-Fish Farming Center. The prawns were fed with a commercial basic diet ('prawn food', Higashimaru Foods Co., Ltd.) in aerated 50-fold diluted 'modified Detmer medium' [KNO_3 1.0 g, $CaCl_2$ 0.1 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, NaCl 0.1 g, K_2HPO_4 0.25 g, $FeSO_4 \cdot 7H_2O$ 0.02 g, H_3BO_3 2.86 mg, $MnCl_2 \cdot 4H_2O$ 1.81 mg, $ZnSO_4 \cdot 7H_2O$ 0.22 mg, $CuSO_4 \cdot 5H_2O$ 0.08 mg, Na_2MoO_4 0.021 mg, pure water 1 dm^3, pH 8]. Before exposure to arsenicals, the prawns were fed for seven days with the arsenic-free diet, of which the composition is shown in Tables 1–3.

Table 1 Composition of basic diet

Ingredient	Concentration (g/100 g diet)
Casein (from milk)	42.8
α-Starch	5.0
Glucose	7.0
Sucrose	13.0
D(+)-Glucosmine–HCl	0.8
Sodium citrate	0.3
Sodium succinate	0.3
Vitamin mix[a]	5.0
Mineral mix[b]	5.0
Soybean oil	7.0
Soybean lecithin	2.0
Cholesterol	1.0
Cellulose	5.5
Trimethylamine–HCl	0.3
Gluten	5.0
Total	100.0

[a] See Table 2. [b] See Table 3.

Determination of total and methylated arsenic compounds

Each prawn was harvested by a net, washed with pure water and dried at 60 °C to a constant weight.

The method for the determination of total arsenic in the organisms was as follows. The dried organisms (10–20 mg) were mineralized in the presence of magnesium nitrate (50 wt%). The ash

Table 2 Composition of vitamin mixture

Vitamin	Concentration (mg/100 g dry diet)
p-Aminobenzoic acid	15.08
(+)-Biotin	0.63
Inositol	632.00
Niacin	63.20
Calcium panthothenate	94.80
Pyridoxine–HCl	18.96
Riboflavin	12.64
Thiamine–HCl	6.32
Menadione	6.34
β-Carotene	15.17
DL-α-Tocopherol	31.60
Calciferol	1.90
Cyanocobalamin	0.13
Sodium L-ascorbate	3160.00
Folic acid	1.26
Choline chloride	948.00
Total	5008.73

Table 3 Composition of mineral mixture

Mineral	Concentration (mg/100 g diet)
K_2HPO_4	1169.0
$Ca_3(PO_4)_2$	1591.0
$MgSO_4 \cdot 7H_2O$	1778.5
$NaH_2PO_4 \cdot 2H_2O$	461.5
Total	5000.0

was dissolved with 10 mol dm^{-3} hydrochloric acid (10 cm^3) and with 40% potassium iodide aqueous solution (1 cm^3). The solution was extracted with chloroform (5 cm^3). The chloroform phase was back-extracted with 0.02% magnesium nitrate aqueous solution (2 cm^3), and the aqueous phase was analyzed by graphite-furnace atomic absorption spectrometry. Disodium arsenate [$Na_2HAsO_4 \cdot 7H_2O$: As(V)] was used as an authentic sample for total and non-methylated arsenic compounds.

The methods for the determination of methylated arsenic compounds were as follows. The dried organism (*ca* 10 mg) was digested with 2 mol dm^{-3} NaOH (5 cm^3) at 90–95 °C for 3 h using an aluminium heating block (hot-base digestion). Methylated arsenic compounds in the digest were reduced with 20% $NaBH_4$ in 0.1 mol cm^{-3} NaOH to the corresponding arsine compounds. The arsines generated were at once frozen in a liquid-nitrogen U-trap. Upon warming the U-trap, the arsines were borne out of it successively, passed through a quartz tube atomizer and determined chromatographically using an atomic absorption spectrometer on the basis of the difference in the boiling points of the arsines [b.p.: AsH_3 −55 °C, CH_3AsH_2 2 °C, $(CH_3)_2AsH$ 35.6 °C (747 mmHg), $(CH_3)_3As$ 52 °C (736 mmHg)].

MMAA, DMAA and AB were used as authentic samples for monomethylarsenic (MMA), dimethylarsenic (DMA) and trimethylarsenic (TMA) compounds, respectively. These three authentic methylated compounds (MMAA, DMAA and AB) were degraded into monomethyl-, dimethyl- and trimethyl-arsine oxides on hot-base digestion, and then hydride-generated by borohydride to monomethyl-, dimethyl- and trimethylarsines, respectively. Non-methylated arsenic (abbreviated as IA) concentration was calculated by the following equation:

$$\mathrm{IA} = \text{total As} - (\mathrm{MMA} + \mathrm{DMA} + \mathrm{TMA}).$$

The concentrations of all arsenic compounds are expressed in μg As g^{-1} units.

The absolute detection limits for total and methylated arsenic in a single injection were 0.5 ng and 5 ng, respectively. The coefficients of variations for the total and methylated arsenic were below 5% and 10%, respectively.

RESULTS AND DISCUSSION

Tolerance of prawn (*M. rosenbergii*) against arsenicals

Each of eight prawn samples was fed for seven days with the arsenic-free diet (Tables 1–3) in 50-fold diluted modified Detmer media (2 dm^3) containing As(V) (0, 10, 20, 30 and 35 μg As cm^{-3}), MMAA (0, 25, 50, 100 and 120 μg As cm^{-3}), and DMAA (0, 100, 200, 300 and 350 μg As cm^{-3}). The experiments were carried out in triplicate. Experimental results are summarized in Table 4.

Almost all prawns survived in media containing arsenic at 0 and 10 μg As cm^{-3} for As(V), 0 and 25 μg As cm^{-3} for MMAA, and 0 and 100 μg As cm^{-3} for DMAA. No prawn could survive in media containing arsenic higher than 35 μg As cm^{-3} for As(V), 120 μg As cm^{-3} for

Table 4 The number of prawns surviving in water containing various concentrations of three arsenicals

Arsenical	Concentration in water (μg As cm^{-3})	Number of prawns surviving[a]
As(V)	0	7.7 ± 0.6
	10	7.7 ± 0.6
	20	6.0 ± 1.0
	30	3.0 ± 1.0
	35	0.0 ± 0.0
MMAA	0	8.0 ± 0.0
	25	7.7 ± 0.6
	50	6.3 ± 0.6
	100	3.7 ± 1.5
	120	0.0 ± 0.0
DMAA	0	8.0 ± 0.0
	100	8.0 ± 0.0
	200	7.3 ± 0.6
	300	4.3 ± 0.6
	350	0.3 ± 0.6

[a] Average of data from three independent series of experiments ±SD.

Table 5 Arsenic accumulation and transformation by prawn from water containing As(V)

As concn in water (μg As cm^{-3})	As, μg As g^{-1} dry wt (%)[a] Total	IA	MMA	DMA	TMA
10	93.8±7.5	86.3±4.0 (91.3±3.3)	—	1.0±0.4 (1.2±0.4)	6.5±3.4 (7.5±3.2)
20	141.3±24.9	134.1±25.7 (94.6±2.5)	—	0.9±0.6 (0.7±0.4)	6.3±3.1 (4.7±2.9)
30	118.2±14.9	111.4±18.3 (93.9±4.1)	—	0.8±0.3 (0.7±0.3)	6.0±3.6 (5.4±3.9)

Abbreviations: IA, non-methylated arsenic compounds; MMA, monomethylarsenic compounds; DMA, dimethylarsenic compounds; TMA, trimethylarsenic compounds; —, not detected.

[a] Average of data from three independent series of experiments ±SD.

Table 6 Arsenic accumulation and transformation by prawn from water containing MMAA

As concn in water (μg As cm^{-3})	As, μg As g^{-1} dry wt (%)[a] Total	IA	MMA	DMA	TMA
25	103.1±7.1	61.7±7.9 (59.8±8.5)	39.8±9.9 (38.6±7.7)	1.6±0.9 (1.6±0.8)	—
50	103.2±13.5	52.9±14.0 (51.3±6.7)	48.9±2.4 (47.4±7.8)	1.4±0.7 (1.3±0.6)	—
100	98.2±20.3	32.4±12.1 (33.0±6.7)	65.8±9.0 (67.0±6.7)	—	—

Abbreviations: IA, non-methylated arsenic compounds; MMA, monomethylarsenic compounds; DMA, dimethylarsenic compounds; TMA, trimethylarsenic compounds. —, not detected.

[a] Average of data from three independent series of experiments ±SD.

Table 7 Arsenic accumulation and transformation by prawn from water containing DMAA

As concn in water (μg As cm^{-3})	As, μg As g^{-1} dry wt (%)[a] Total	IA	MMA	DMA	TMA
100	22.4±3.3	5.1±1.3 (22.8±6.0)	—	17.3±3.1 (77.2±6.0)	—
200	20.5±2.0	4.8±2.0 (23.4±8.3)	—	15.7±0.9 (75.6±8.3)	—
300	35.1±6.0	1.2±1.1 (3.3±2.8)	8.6±5.3 (24.4±14.1)	25.4±5.2 (72.3±16.9)	—

Abbreviations: IA, non-methylated arsenic compounds; MMA, monomethylarsenic compounds; DMA, dimethylarsenic compounds; TMA, trimethylarsenic compounds, —, not detected.

[a] Average of data from three independent series of experiments ±SD.

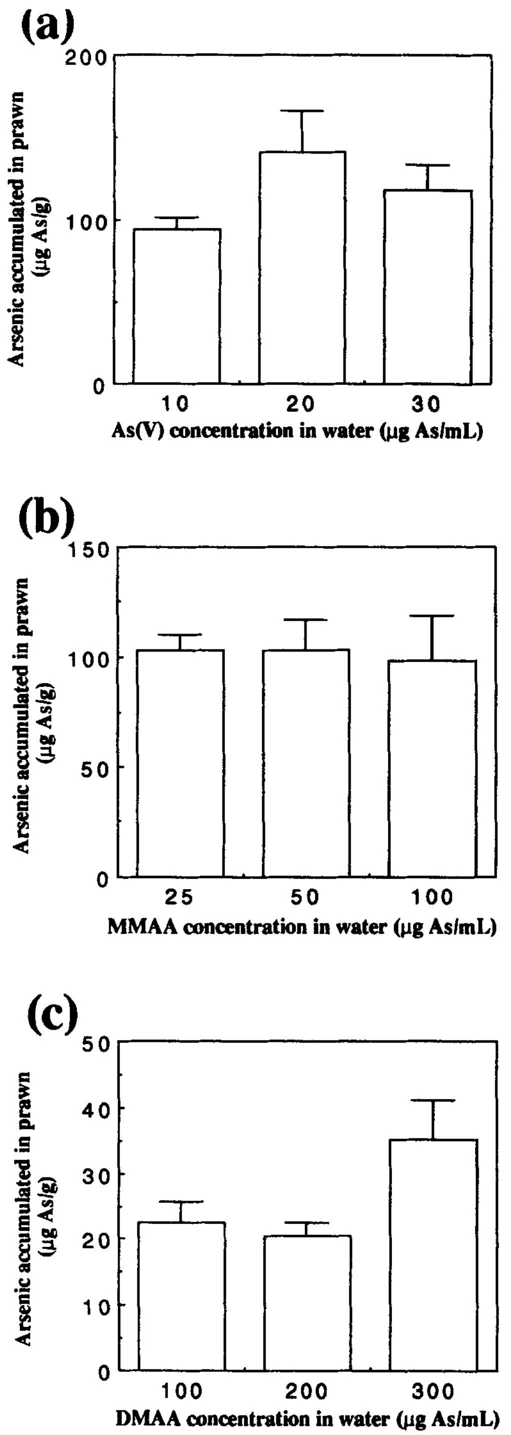

Figure 1 Total arsenic concentration accumulated by prawn (*M. rosenbergii*) from a water phase containing (a) disodium arsenate [As(V)]; (b) methylarsonic acid (MMAA); (c) dimethylarsinic acid (DMAA). Average of data from three independent series of experiments ±SD.

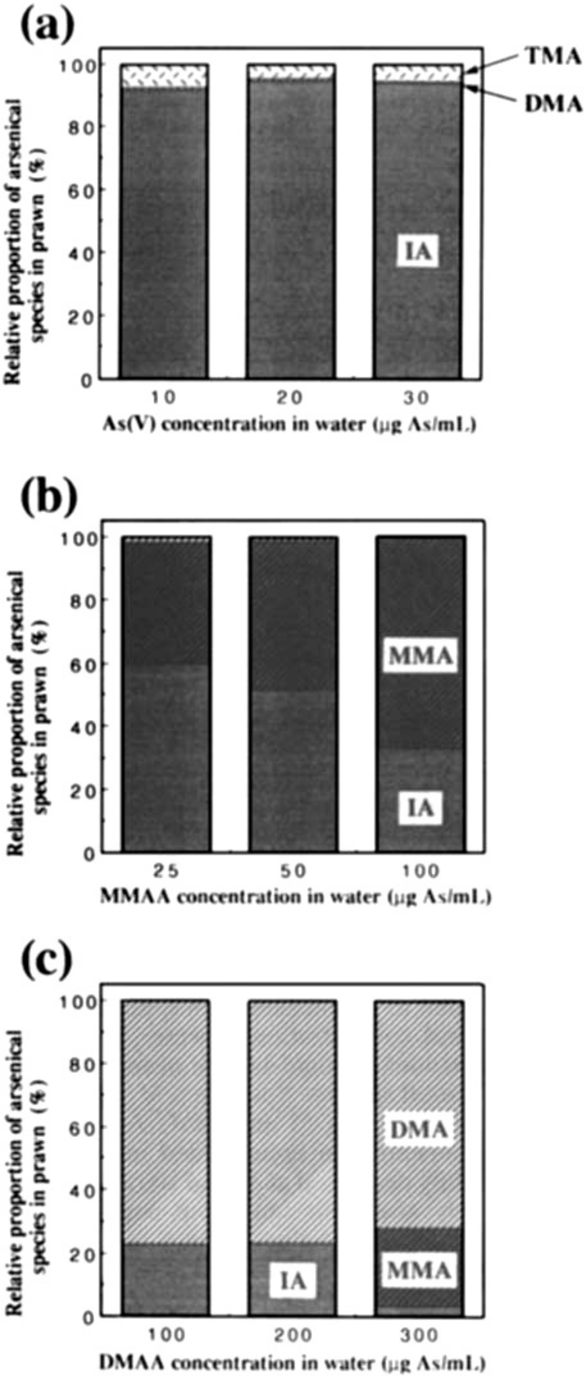

Figure 2 Relative proportion of arsenical species accumulated by prawn (*M. rosenbergii*) from a water phase containing (a) disodium arsenate [As(V)]; (b) methylarsonic acid (MMAA); (c) dimethylarsinic acid (DMAA):IA, MMA, DMA, TMA. The relative proportion is illustrated by average values of the data obtained from three independent series of experiments.

MMAA, and 350 μg As cm^{-3} for DMAA. From the experimental data shown in Table 4, tolerances (LC_{50}) of the prawn against As(V), MMAA and DMAA were evaluated to be 30, 100 and 300 μg As cm^{-3}, respectively. LC_{50} is defined here as the arsenic concentration at which about half of the prawns did not survive after 5–7 days' exposure. These results mean that the toxicity of the arsenicals for *M. rosenbergii* decreases with an increase in the number of methyl groups bonded to the arsenic atom. Similar results were reported for freshwater shrimp.[16]

Bioaccumulation and biotransformation of arsenicals by prawn (*M. rosenbergii*) from the water phase

The arsenic-dosed and survivor prawns in the above tolerance experiment were harvested, washed with pure water, dried at 60 °C to constant weight, ground into powder and analyzed for total arsenic and methylated arsenic. Experimental results are summarized in Tables 5–7 and total arsenic and relative proportions of methylated arsenic in prawn are illustrated in Figs 1 and 2, respectively.

Figure 1 shows that total arsenic concentration accumulated in *M. rosenbergii* tended to increase slightly with an increase in the arsenical concentration in water, when *M. rosenbergii* was exposed to As(V) and DMAA. However, when *M. rosenbergii* was exposed to MMAA, the total arsenic concentration accumulated in *M. rosenbergii* tended to be unchanged in spite of increasing arsenical concentration in water, and a pronounced tendency could not be observed in the three arsenical species. Yet, when *M. rosenbergii* was exposed to As(V) and MMAA, the total arsenic concentration accumulated in *M. rosenbergii* slightly decreased at 30 and 100 μg As cm^{-3}, respectively: these concentrations correspond to the respective LC_{50} values. These results imply that *M. rosenbergii* have the ability to accumulate arsenic directly from the water phase containing arsenicals at concentrations below the LC_{50}. When higher methylated arsenicals were dosed to *M. rosenbergii*, the total arsenic concentration accumulated in the organism was lower. This result implies that highly methylated and less toxic arsenicals are not much accumulated in *M. rosenbergii*. This is probably caused by the highly methylated arsenicals being excreted rapidly from the cell tissues, even if these were preferably taken up by the cell, compared with less methylated and more toxic arsenicals.

Experimental results for the relative proportion of arsenical species accumulated in the prawns are illustrated in Fig. 2, which shows average values of the data obtained from three independent series of experiments.

When the prawns were exposed to As(V) (Fig. 2a), the relative concentration of non-methylated arsenicals (IA) was within 90–95%: about 5–10% of arsenic accumulated was biomethylated. The predominant methylated arsenicals were dimethylarsenic (DMA) and trimethylarsenic (TMA) compounds. When exposed to MMAA (Fig. 2b), MMA in prawn was within 40–70% of accumulated arsenic: only a little accumulated MMAA was further methylated to DMA and 35–60% of that was demethylated to IA. When exposed to DMAA (Fig. 2c), no further methylation occurred, but 20–25% of accumulated DMAA was demethylated to IA at 100 and 200 μg As cm^{-3} exposure, and to IA and MMA at 300 μg As cm^{-3} exposure.

These experimental results show that *M. rosenbergii* carries out both methylation and demethylation of arsenic accumulated from the water phase, and monomethyl- and dimethyl-arsenic species would be the preferable form to the prawn.

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