CONJUGATION AND EXCRETION OF PHENOLIC COMPOUNDS BY THE LOBSTER, *HOMARUS AMERICANUS*

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Many foreign organic chemicals are very slowly excreted from crustaceans, even after biotransformation to more polar metabolites (Little et al Toxicol. Appl. Pharmacol. 77, 325-333, 1985 and Little et al. J. Environ. Pathol. Toxicol. Oncol. 6, 13-29, 1986). This may mean that mechanisms of excretion of foreign chemicals and their metabolites are poorly developed in crustacea. In vertebrates formation of a phenolic metabolite by cytochrome P-450 is a common first step in the biotransformation of xenobiotics containing aromatic rings: the phenolic group then combines with sulfate or glucuronic acid to form a polar, water-soluble metabolite which is well excreted by the vertebrate kidney. Although it has been demonstrated that cytochrome P-450 in the crustacean hepatopancreas will catalyze hydroxylation of aromatic rings, there have been few studies of the subsequent metabolism and excretion of the phenolic metabolites in crustacea, either *in vivo* or *in vitro* (James, Env. Hlth. Persp. 71, 97-103, 1987). In the present study, we investigated the occurrence and tissue distribution of glucose and sulfate conjugation pathways *in vitro*, with p-nitrophenol as model substrate, and the *in vivo* disposition of phenol in the Maine lobster, *Homarus americanus*. Our long-term goal is to understand the role of conjugation pathways in determining the rate of excretion of phenolic compounds in the Maine lobster, *Homarus americanus*.

The *in vivo* fate of ^{14}C-phenol (0.1 mg + 10 μCi/kg) was determined after intrapericardial administration to male or female lobsters, body weight 400 to 600 g. Hemolymph samples (1-2 ml) were withdrawn serially from appendage joints and placed in vials containing N-ethylmaleimide to prevent clotting. Portions of hemolymph were analyzed for radioactivity by scintillation counting, and for chemical composition (parent phenol separated from polar metabolites) by chromatography on XAD-2 resin minicolumns. Some isolated metabolite fractions from hemolymph were treated with arylsulfatase or alkaline phosphatase. Two groups of 3 male and 3 female lobsters were sacrificed at 24 hr and one group of 3 female lobsters was sacrificed at 4 hr after the dose for determination of the complete tissue distribution of radioactivity. The binding of ^{14}C-phenol and its metabolite to hemolymph was assessed by injecting lobsters with varying doses of ^{14}C-phenol and collecting hemolymph at 2-4 min, and also 30 min after the dose. The hemolymph was immediately ultrafiltered and portions of whole hemolymph, ultrafiltrate and concentrate were analyzed for radiolabel.

Studies of sulfate and glucose conjugation were conducted spectrophotometrically with p-nitrophenol as substrate. PAPS dependent sulfotransferase activity was measured at room temperature and pH 6.5 in hepatopancreas or green gland cytosol or 13,000 g supernatant. UDP-glucosyl transferase activity was measured at pH 7.6 in detergent-solubilized preparations of hepatopancreas or green gland microsomes. Sulfatase activity was measured in hepatopancreas cytosol with p-nitrophenylsulfate as substrate.

TABLE 1

Distribution of ^{14}C after intrapericardial injection of Phenol (0.1 mg/kg) to Lobsters, Homarus americanus

	Female - 4 hr		Female - 24 hr		Male - 24 hr	
	ng/g	%/dose	ng/g	%/dose	ng/g	%/dose
Hemolymph	95 ± 23	22.2 ± 5.4	33 ± 11	8.1 ± 3.7	8 ± 2	2.0 ± 0.2
Muscle	23 ± 4	8.6 ± 0.3	8 ± 1	3.7 ± 0.5	3 ± 1	1.9 ± 0.2
Shell	31 ± 4	9.4 ± 1.5	13 ± 2	4.4 ± 0.5	5 ± 0.1	1.7 ± 0.05
Green Gland	1006 ± 672	1.8 ± 1.0	251 ± 49	0.3 ± 0.1	90 ± 25	0.1 ± 0.0
Urine	1234 ± 746	12.3 ± 9.2	1751 ± 150	11.9 ± 0.9	1300 ± 270	4.3 ± 0.3
Hepatopancreas	45 ± 9	2.0 ± 0.4	33 ± 3	2.0 ± 0.1	8 ± 1	0.5 ± 0.1
Gill	113 ± 57	2.2 ± 1.1	36 ± 30	0.9 ± 0.7	8 ± 1	0.2 ± 0.05
Whole animal excluding urine		46.9 ± 5.0		21.0 ± 3.5		6.5 ± 0.5

Tissue concentrations are ng of phenol equivalents/g tissue.

Following intrapericardial administration of ^{14}C-phenol, hemolymph concentrations of ^{14}C were constant for 2 hr (males) or 4 hr (females) and thereafter decreased in a monoexponential manner, with terminal phase half lives of 6 hr (males) and 9 hr (females). Analysis of hemolymph showed that even as early as 20 min after dosing, >90% of the hemolymph ^{14}C was not parent phenol, but was a more polar metabolite which did not bind to XAD-2 resin. This metabolite was resistant to hydrolysis by arylsulfatase, β-glucosidase and alkaline phosphatase but was hydrolysed to phenol under acidic (pH 0) conditions. Phenol itself was highly (95%) bound to hemolymph protein, but the metabolite was less than 5% bound. For both males and females, hemolymph contained most of the dose remaining in the animal at 4 hr and 24 hr (Table 1). The only organ containing substantially higher concentrations of radioactivity than hemolymph was the green gland. Hepatopancreas and gill contained concentrations of radioactivity similar to those of hemolymph. Intestine and intestinal contents contained lower concentrations of ^{14}C than hemolymph (data not shown) indicating that "feces" was not a route of phenol excretion. Urine was an important route of excretion (Table 1). For both males and females, 93-95% of the urinary ^{14}C was a single polar metabolite with identical properties to the metabolite found in hemolymph. Phenylglucoside accounted for 0.5 to 0.7% of the urinary ^{14}C, while 3.1 to 6.4% of the urinary ^{14}C was another unknown polar metabolite. These data indicate that phenol is rapidly metabolized, probably in hemolymph or green gland, to a polar metabolite which is slowly excreted from the animal. Since less than 60% of the dose is accounted for at 4 hr after treatment, it is possible that phenol is eliminated by another route of excretion in addition to urine.

Studies of the *in vitro* conjugation of p-nitrophenol showed that UDP-glucosyltransferase activity was present in both green gland and hepatopancreas microsomes. The K_m for p-nitrophenol was 1 mM in hepatopancreas microsomes and V_{max} was 7 nmol/min/mg protein. The pH optimum was 8.5 and the reaction was linear with time for up to 30 min and with protein up to 2 mg/0.5 ml assay volume. The K_m for p-nitrophenol in green gland microsomes was not determined, but was less than 0.5 mM. At 0.5 mM or 1 mM p-nitrophenol, UDP-glucosyltransferase activity in green gland microsomes was 9 nmol/min/mg protein. Sulfotransferase activity was found in green gland cytosol and 13,000 g supernatant but not in hepatopancreas cytosol. Indeed, hepatopancreas cytosol hydrolysed p-nitrophenylsulfate to p-nitrophenol, indicating that hepatopancreas cytosol contained sulfatase activity. Green gland sulfotransferase activity was linear with time up to 20 min, had a pH optimum of 6.5, a K_m for p-nitrophenol of 0.7 mM and V_{max} of 4 nmole/min/mg protein. Thus, green gland can catalyze the conjugation of phenolic compounds with either sulfate or glucose, whereas hepatopancreas has only glucosyltransferase activity.

In summary, the fate of phenol in the lobster was very different from the fate of phenol in several vertebrate species, in which phenylsulfate or phenylglucuronide were the major urinary metabolites (Capel et al Xenobiotica 2, 25-34, 1972). Hepatopancreas, the major organ of formation of phenolic metabolites from aromatic xenobiotics, contained similar concentrations of radioactivity to hemolymph, and thus administration of phenol by intrapericardial injection may not be a good model for determination of the fate of phenolic compounds formed in hepatopancreas. Injected phenol was rapidly eliminated from hemolymph, in part by conversion to a polar metabolite which could be hydrolysed under acid conditions, but surprisingly, the polar conjugate had a much longer elimination half life from hemolymph than unmetabolized phenol. These finding support the hypothesis that lobsters cannot readily eliminate polar metabolites formed *in vivo* from foreign chemicals.

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